TITLE: DIFFRENTIATION OF BONE SARCOMA AND OSTEOMYELITIS WITH FERUMOXYTOL-ENHANCED MRI

Coordinating Center
Stanford Cancer Center
875 Blake Wilbur Drive
Stanford, CA 94305

Protocol Director:
Dr.Heike Daldrup-Link
725 Welch Road, Rm 1679, MC 5913
Stanford, CA 94305-5654
Telephone: E-mail address: heiked@stanford.edu

Co-Investigators
Dr. Neyssa Maria Marina
Department of Pediatrics-Hematology/oncology
1000 Welch Road, Suite 300
Palo Alto, California 94604-1812
Telephone
Fax:

Biostatistician
Dr. Alex McMillan
Health Research and Policy
Redwood Building
Rm T160A
Stanford, CA
Biostatistics MC 5405
Telephone
Study Coordinator
Jennifer Vancil (Research Administrator)
Dr. Rakhee Gawande (Research Fellow)
Lucile Packard Children's Hospital
Radiology, MC 5913
725 Welch Road, Rm 1681
Stanford, CA 94305-5913
TelephoneFax:

SRC Approved Protocol / Version # 1/ Version Date: 4/04/2011

NCT01336803

TABLE OF CONTENTS

**Please include page numbers for each section below*

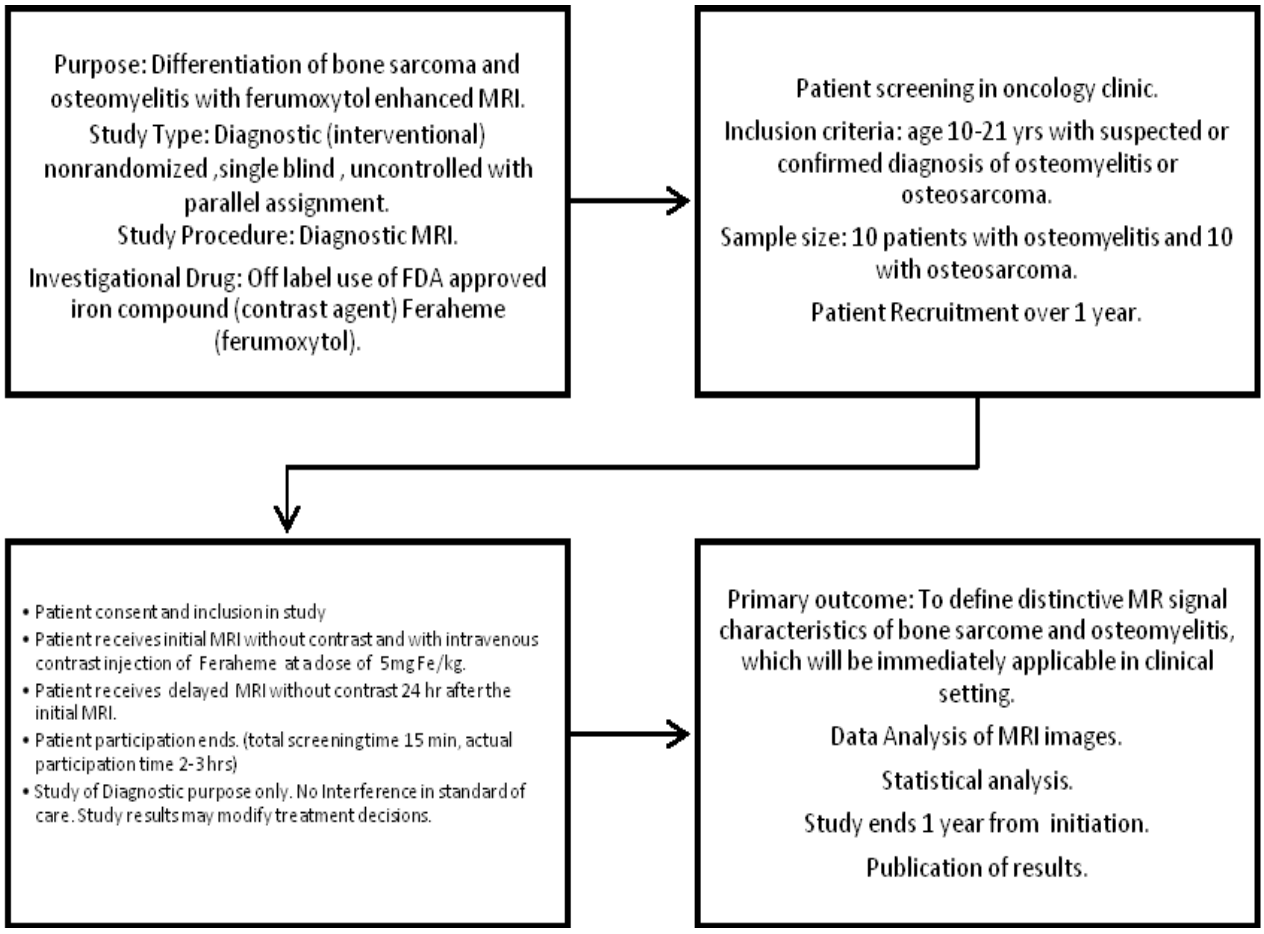
PROTOCOL SYNOPSIS

In the table below summarize the basic aspects of this research. This is to be used as a quick reference guide. Remove any section that is not relevant to the research.

TITLE	DIFFRENTIATION OF BONE SARCOMA AND OSTEOMYELITIS WITH FERUMOXYTOL-ENHANCED MRI
STUDY PHASE	Pilot
INDICATION	Pediatric patients of 10-21 years of age with bone sarcoma or osteomyelitis
INVESTIGATIONAL PRODUCT OR PROCEDURE	Diagnostic MRI with intravenous contrast agent Ferumoxytol (off label use of FDA approved iron compound Ferumoxytol).
PRIMARY OBJECTVE	Establish MR imaging characteristics of bone sarcomas and osteomyelitis based on their ferumoxytol-enhancement on relatively early and delayed post-contrast MR images
TREATMENT SUMMARY	Single Intravenous injection of MR contrast agent Ferumoxytol, administered during the initial MR exam
SAMPLE SIZE	20
STATISTICAL CONSIDERATIONS	Descriptive statistics will be used to characterize contrast enhancement; the key estimates will be the standard deviation and the coefficient of variation. The area under the ROC curve will be calculated as a measure of separation and the significance of the separation will be assessed using the Wilcoxon rank sum test.

SCHEMA

Provide an outline of the trial in graphical form. Show essential inclusion criteria, major decision branches (including randomization/stratification) and the primary outcome.



LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

Include additional abbreviations as needed. Remove any unnecessary abbreviations.

AE	Adverse event
CI	Confidence interval
CKD	Chronic renal disease
CRF	Case report/Record form
CTCAE	Common Terminology Criteria for Adverse Events
CT	Computed Tomography
ECG	Electrocardiogram
Gd	Gadolinium
GI	Gastrointestinal
IRB	Institutional Review Board
IV	Intravenous
MRI	Magnetic resonance Imaging
PET	Positron emission tomography
SAE	Serious adverse event
USPIO	Ultra small superparamagnetic iron oxide
VE-MRI	Vascular enhanced MRI
WHO	World Health Organization

1. OBJECTIVES

1.1. Primary Objective

The differentiation between malignant bone sarcomas, especially Ewing sarcomas, and a subacute osteomyelitis can be difficult based on imaging tests (1-4). Biopsy, curettage and/or cultures of the lesion may be necessary to establish a diagnosis. These procedures are invasive and time-consuming, causing delay in treatment and significant anxiety of the patients and their parents. **The goal of our project is to establish a novel, non-invasive, immediately clinically applicable imaging test for the differentiation of bone sarcomas and osteomyelitis.** Our approach relies on the FDA-approved iron supplement drug ferumoxytol (Feraheme), which is used in patients for intravenous treatment of iron deficiency (5-8). Ferumoxytol is composed of iron oxide nanoparticles, which provide a strong T1- and T2-signal on magnetic resonance (MR) images and, thus, can be used as an MR contrast agent (9, 10). *We hypothesize, that MR images after intravenous injection of ferumoxytol will demonstrate a significantly stronger T1- and T2-enhancement of osteomyelitis when compared to malignant bone sarcomas.* The T1-effect of ferumoxytol is dependent on its ability to interact with protons in the target tissue (9, 14, and 21). Thus, we expect to find a significantly stronger T1-enhancement of edematous inflammations as opposed to highly cellular tumors. In addition, ferumoxytol is phagocytosed by local macrophages, which leads to a persistent MR signal defect on 24 h delayed T2-weighted MR images (9). Thus, we expect to find a significantly stronger T2-enhancement of macrophage-rich inflammations as opposed to bone sarcomas with relatively few tumor-associated macrophages in their stroma.
We will investigate the following specific aims:

1) Establish MR imaging characteristics of bone sarcomas and osteomyelitis based on their ferumoxytol-enhancement on relatively early postcontrast T1-weighted images.
2) Establish MR imaging characteristics of bone sarcomas and osteomyelitis based on their ferumoxytol-enhancement on delayed postcontrast T2-weighted images.

2. BACKGROUND

2.1 Study Disease

Sarcomas arise from transformed mesoderm (connective tissue which forms bone, cartilage, and soft tissues) and represent approximately 5-10 % of adult and childhood tumors (1, 25). Bone sarcomas, such as osteosarcomas and Ewing sarcomas, occur in approximately 650 new cases annually in children and adolescents (cancernetwork.com). The patients typically present with pain and/or a palpable swelling of the extremities or the bones of the pelvis. An accurate diagnosis is essential in order to refer these patients to appropriate treatment by oncologists and orthopedic surgeons. However, conventional clinical, laboratory and imaging signs of bone sarcomas, especially Ewing sarcomas, can show significant overlap with a subacute osteomyelitis (2-4). On conventional radiographs or CT scans, a Ewing's sarcoma and an osteomyelitis may present as an ill defined, lytic diaphyseal lesion that aggressively destroys the bone, often with an onion-skin or sunburst pattern periosteal reaction. MRI is currently the clinical standard technique to further evaluate the extent of either a sarcoma or an osteomyelitis, but has also demonstrated significant overlap between the imaging signs of these two pathologies (26, 27). Bone scans and PET scans show overlap between these two entities as well (28,29). As a result, there are numerous reports of misdiagnoses of sarcomas as inflammations with inappropriate antibiotic treatment leading to tumor progression or inadequate biopsies leading to limb amputation (30-33). Thus, more accurate diagnostic techniques that can reliably differentiate these different pathologies are critically needed. We propose to utilize differences in the cellular composition of sarcomas and inflammations to generate a more specific diagnostic test. Interestingly, 99mTc sulphur colloid, which is phagocytosed by macrophages, demonstrated a marked uptake in osteomyelitis, but not malignant tumors, supporting our concept (34, 41, and 42). Sulphur colloid scans are associated with considerable radiation exposure and low anatomical resolution, thereby limiting its utility in children. Ultra small superparamagnetic iron oxide nanoparticles (USPIO) provide a radiation free alternative to sulphur colloid and can be depicted with MR imaging, which provides 3D information, high soft tissue contrast and sub-millimeter anatomical resolution. We and others have utilized preclinical USPIO compounds for MR imaging of inflammations and tumors (9, 11, 13, 14, 19, 20, and 35). However, to the best of our knowledge, the value of FDA-approved USPIO for the differentiation of malignant bone sarcomas and osteomyelitis has not been investigated.

2.2 Study Agent/Device/Procedure

Study Agent:
Ferumoxytol (Feraheme™) represents a novel USPIO compound that has been recently FDA-approved for intravenous treatment of iron deficiencies in patients with renal failure (6).
Indication and Usage: Ferumoxytol is used as an iron replacement product indicated for the treatment of iron deficiency anemia in adult patients with chronic kidney disease.
We propose an off label application of ferumoxytol as a MRI contrast agent.
Mechanism of Action: Ferumoxytol consists of a superparamagnetic iron oxide that is coated with a carbohydrate shell, which helps to isolate the bioactive iron from plasma components until the iron-carbohydrate complex enters the reticuloendothelial system macrophages of the liver, spleen and bone marrow. The iron is released from the iron-carbohydrate complex within vesicles in the macrophages. Iron then either enters the intracellular storage iron pool (e.g., ferritin) or is transferred to plasma transferrin for transport to erythroid precursor cells for incorporation into hemoglobin.
Animal Studies: Animal studies demonstrate that the plasma half-life of ferumoxytol increased with increasing dose. The highest tissue concentration of ferumoxytol was found in the liver, spleen and central lymphnode pool. Studies with radio-labeled drug product demonstrated that the renal elimination of iron in ferumoxytol was insignificant, while the carbohydrate coating was significantly excreted in urine and feces. Repeat dose

toxicity studies of ferumoxytol in rats and dogs demonstrated dose dependent decreases in body weight gain and food consumption, and increases in pigmentation intensity. No systemic toxicity or immunotoxicity was observed at relevant clinical doses.
Clinical Studies: The safety and efficacy of ferumoxytol for the episodic treatment of iron deficiency anemia in patients with CKD were assessed in three randomized, open-label, controlled clinical trials.
Ferumoxytol is not currently approved for diagnostic imaging applications. It is being evaluated as an agent for vascular enhanced MRI (VE-MRI). In August 2008 the FDA granted Fast track designation to ferumoxytol for its development as a diagnostic agent for VE-MRI to improve assessment of peripheral arterial disease in patients with known or suspected chronic renal disease (CKD). The fast track process is designed to facilitate the development and expedite the FDA's review of products.
Clinical Pharmacokinetics: Ferumoxytol exhibits dose-dependent, capacity-limited elimination from plasma with a half life of approximately 15 hours in humans.
Major route of elimination: Ferumoxytol is slowly cleared from blood by tissue macrophages (liver, spleen, lymph node and active bone marrow) over 2-3 months. The macrophages release the iron to body iron stores where it can be incorporated into hemoglobin.
Drug Interactions: Ferumoxytol may reduce the absorption of concomitantly administered oral iron preparations.

Study Device: MR Scanner, 1.5T and 3 T, GE healthcare. MRI with and without contrast is a non-significant risk exam, as defined by the FDA and IRB guidelines.

For clinicaltrials.gov compliance:
Ferumoxytol has been approved by The FDA for the treatment of iron deficiency anemia in adult patients with chronic kidney disease.
We propose an off label use of an FDA approved iron compound Ferumoxytol (contrast agent) for pediatric patients.

2.3 **Rationale**

Realization of our goal to develop an immediately clinically applicable MR imaging test for non-invasive differentiation of osteomyelitis and bone sarcomas will enable us to provide a quick and non-invasive diagnosis of these pathologies, accelerate patient referral to appropriate pediatric subspecialities and prevent inappropriate anti-inflammatory treatment or delay of cytotoxic treatment of sarcomas. Accelerated specific therapy of these very different pathologies based on a more accurate diagnostic test may ultimately improve and accelerate positive treatment-outcomes, reduce hospitalizations and reduce associated direct and indirect costs to our society. Since ferumoxytol is safe in patients with renal insufficiency and not associated with any risk of nephrogenic sclerosis (6), it can be used as an alternative contrast agent to gadolinium chelates in patients with renal insufficiency or in patients in whom creatinine lab values are not or not yet available. This aspect might be particularly significant for patients with a suspected osteomyelitis, in whom urgent MR scans may be needed and evaluation of serum creatinine values would delay imaging diagnosis and treatment.
The current alternative diagnostic methods are: biopsy, which is invasive, painful, and frequently inadequate; MRI, with significant overlap/difficulty in distinguishing between the two entities without an iron nanoparticle contrast; PET/CT/X-ray, which are even more difficult to distinguish and include exposure to ionizing radiation. The current standard of imaging is an MRI; the addition of an iron nanoparticle contrast agent is expected to make this a much more efficacious diagnostic tool. No standard treatment will be withheld, and the standard of care diagnostic test is included in our research.

2.4 Study Design

Diagnostic: The goal of our project is to establish a novel, non-invasive, immediately clinically applicable imaging test for the differentiation of bone sarcomas (osteosarcomas and Ewing sarcomas) and osteomyelitis. Our approach relies on the FDA-approved iron supplement drug ferumoxytol (Feraheme), which is used in patients for intravenous treatment of iron deficiency. Ferumoxytol is composed of iron oxide nanoparticles, which provide a strong T1- and T2-signal on magnetic resonance (MR) images and, thus, can be used as an MR contrast agent. Based on our extensive experience with pre-clinical and clinical imaging applications of iron oxide nanoparticles, we hypothesize that MR image after intravenous injection of ferumoxytol will demonstrate a significantly stronger T1- and T2- enhancement of osteomyelitis when compared to malignant bone sarcomas.

Hypotheses:

(1) *Bone sarcomas and osteomyelitis show a different T1-enhancement at 1 h post injection (p.i.) of ferumoxytol*

(2) *Bone sarcomas and osteomyelitis demonstrate differences in T2-enhancement at 24 h p.i. of ferumoxytol*

We plan to enroll 20 patients in this study, 10 patients with bone sarcomas and 10 patients with an osteomyelitis. The sample size has been determined based on our preliminary data and power calculations. We will screen all patients with proven or suspected bone sarcoma or osteomyelitis for potential participation in this study. Inclusion criteria will comprise an age of 10-21 years (we do not include younger patients in order to exclude need of sedation) and a suspected or confirmed diagnosis of a bone sarcoma or osteomyelitis. Exclusion criteria comprise MR-incompatible metal implants, need of sedation (since an anesthesia is not supported by this), claustrophobia or hemosiderosis/hemochromatosis. All patients will undergo two subsequent imaging tests on a 3T MR scanner, using dedicated surface coils for high resolution MR imaging. During the first MR examination, ferumoxytol will be injected intravenously at a dose of 5 mg Fe/kg:

(A) MRI before and up to 1 h after injection of ferumoxytol for evaluation of lesion perfusion, blood volume, microvascular permeability and interstitial retention (enhanced permeability and retention effect of macromolecules)
(B) 24h after (A): Follow up MRI for evaluation of macrophage phagocytosis

The following pulse sequences will be applied: Long axis T1-spin echo (SE) images and short TI inversion recovery (STIR) images, covering the whole bone from joint to joint, axial T2-weighted fat-saturated fast spin echo (FSE) images focusing on the bone lesion, dynamic contrast-enhanced T1-weighted 3D spoiled gradient recalled echo (SPGR) images, and axial multi-echo T2*-weighted gradient echo (GE) images. The pulse and blood pressure of the patients will be measured before and after each MR exam and the patients and parents will be asked for any objective or subjective adverse events.

- Interventional model: Parallel
- Single Blind
- Nonrandomized study
- Primary outcome: Efficacy.
  Primary outcome of these examinations is to define distinctive MR signal characteristics of bone sarcomas and osteomyelitis, which will be immediately applicable in a clinical setting.

2.5 Correlative Studies Background

Our group has worked on MR imaging techniques with iron oxide nanoparticles since 1995 (11-24). We and others have found that intravenously injected USPIO with diameters in the order of 20-50 nm cause a long lasting, positive “blood pool” or vascular enhancement on T1-weighted MR images (9,14,19). In pathologies with an increased microvascular permeability, USPIO extravasate into the interstitium and cause a T1-effect of

the target tissue, which is strongly dependent on the proton content of the interstitial space (among other technical factors). We have thoroughly investigated various factors that lead to an optimized tissue T1-enhancement of USPIO (9, 14, and 19). We have shown that malignant lesions in the bone marrow of patients show nearly no USPIO T1-enhancement, presumably due to their high cellularity, small intercellular space and low proton content (Fig. 1). Inflammations, on the other hand, show a strong T1-enhancement, presumably due to the edematous, proton rich environment (Fig. 2). These data suggest that tumors and inflammations may demonstrate differences in T1-enhancement.

USPIO in the interstitium are subsequently slowly phagocytosed by macrophages in various target tissues, such as liver, spleen, bone marrow or macrophage containing pathologies, where they primarily cause a negative (dark) signal effect on T2-weighted MR images (11,13,18,19,24). Focal malignant tumor lesions in organs of the RES (reticulo-endothelial system) contain no or very few macrophages, thus do not phagocytose USPIO, and stand out as bright lesions (Fig. 1). However, macrophage rich inflammations cause a marked signal loss on delayed T2-weighted MR images (Fig. 2). Thus, we have evidence from a variety of pre-clinical and clinical investigations, that tumors and inflammations may show distinct T2-enhancement patterns.

Of note, the PI of this proposed study has applied USPIO as MR contrast agents in phase II and III clinical trials in adult patients (11, 13, 14, and 20). These contrast agents are very well tolerated and show excellent safety profiles (5-8). The delivered iron dose via a typical ferumoxytol administration is in the order of 150-500 mg iron oxides (note that these are coated iron particles, not free iron), which is equivalent to or lower than the iron dose administered with one blood sample. USPIO are slowly metabolized in the liver and not excreted via the kidneys. Thus, they are safe to use in patients with renal insufficiencies and are not associated with any risk of nephrogenic sclerosis (a potential adverse event after injections of certain gadolinium chelates). Anaphylaxis or anaphylactoid reactions were reported in 0.2% of a subject, which is in the order of or lower compared to other MR contrast agents (see FDA report, ref. 6).

Figure 1: MR signal characteristics of bone marrow tumors with USPIO, as demonstrated in a patient with Non-Hodgkin's lymphoma after chemotherapy and GCSF therapy. (A) T1-weighted MR images before contrast media administration show a hypercellular reconverted bone marrow, which demonstrates diffusely decreased signal due to its high cellularity. Two focal lesions are noted (arrows). (B) T1-weighted MR images after intravenous administration of the USPIO ferumoxtran-10 (Sinerem / Combidex) do not show any T1-enhancement (positive contrast) of these lesions. (C) Precontrast STIR images show a relatively high signal of the hypercellular reconverted bone marrow with focal, more hyperintense lesions (arrows). (D) STIR images after intravenous administration of ferumoxtran-10 show a marked, diffuse T2-enhancement (negative contrast, signal loss) of the normal bone marrow due to phagocytosis of the agent by macrophages in normal bone marrow. Focal neoplastic lesions (arrows) contain no or few macrophages and stand our as bright lesions (arrows).

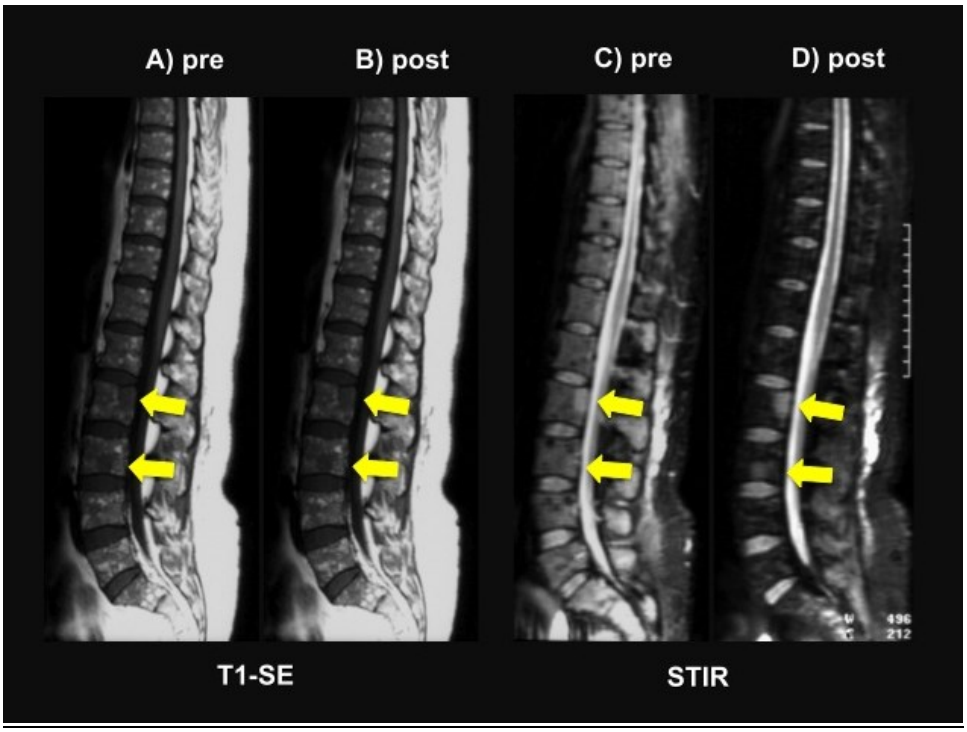
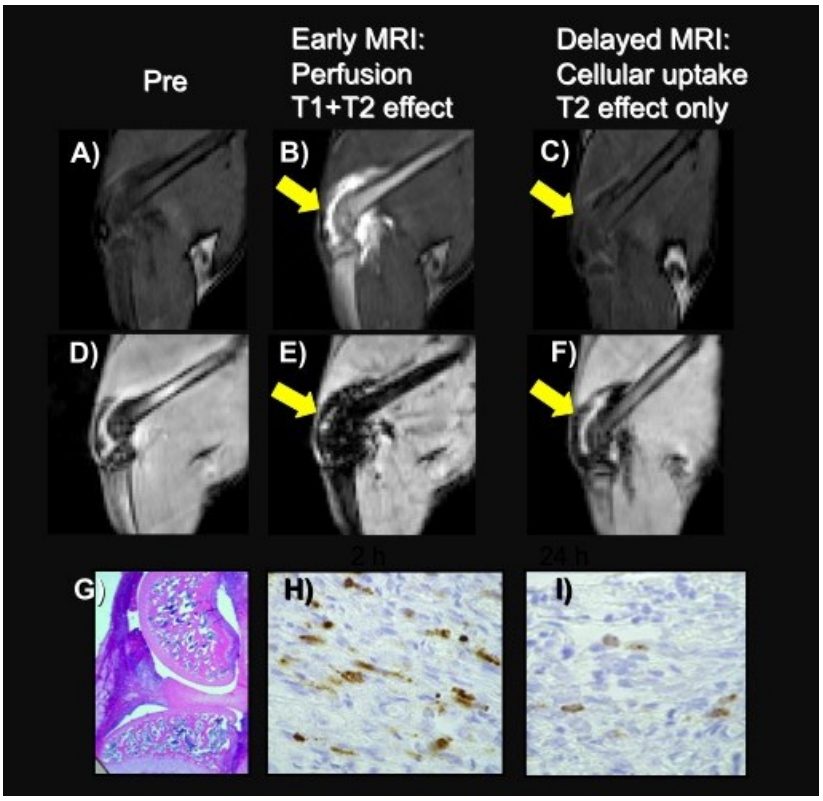


Figure 2: MR signal characteristics of inflammation following intravenous injection of the USPIO ferumoxtran-10 in a rat model of antigen-induced arthritis of the knee joint. (A) A precontrast T1-weighted MR scan shows a swollen knee joint. (B) An early post-contrast T1-weighted scan demonstrates marked T1-enhancement of the inflamed joint (arrow). (C) On delayed MR images, the T1-contrast has diminished. (D) Precontrast T2-weighted scan. (E) Early postcontrast T2-scans demonstrate negative perfusion effect of the inflamed synovium (arrow). (F) Delayed T2-weighted MR scans demonstrate persistent T2-contrast enhancement of the synovium (compare D). (G) AN H&H stain of a sagittal histologic slice through the knee joint show massive inflammation of the synovium with thickening and cellular infiltration. (H) Antidextran stains of the arthritic synovium (100-fold magnification) show positive contrast agent particles mostly in the interstitium at 2 hours p.i. (I) and exclusively within macrophages (arrows) at 24 hours p.i. As can be seen from these images, USPIO exert both T1- and t2-effect when the nanoparticles are located extracellular. USPIO exert T2-effects only and loose their T1-effect when they are phagocytosed and located intracellulary.



3. PARTICIPANT SELECTION AND ENROLLMENT PROCEDURES

Refer to the Participant Eligibility Checklist in Appendix A.

3.1 Inclusion Criteria

3.1.1 Inclusion criteria will comprise:
a) An age of 10-21 yrs
b) Suspected or confirmed diagnosis of a bone sarcoma or osteomyelitis.

3.1.2 We do not include younger patients (age less than 10 yrs) in order to exclude need of sedation. There will be no gender/race-ethnic restrictions

3.1.3 In this pediatric & adult study, the participant or parent/guardian is consented, and the patient when a minor is given an assent form and involved in the discussion as appropriate.

3.2 Exclusion Criteria

3.2.1 Exclusion criteria will comprise:
a) Contraindication to MRI
b) Presence of metal implants
c) Need for sedation or anesthesia
d) Claustrophobia
e) Hemosiderosis/hemochromatosis

3.2.2 There will be no restrictions regarding use of other Investigational Agents.

3.2.3 Patients with evidence of iron overload, hemosiderosis/hemochromatosis will be excluded.

3.2.4 History of allergic reactions to similar compounds will be obtained and patients with positive history of allergic reactions will be excluded from the study.

3.2.5 Pregnancy or nursing patients will be excluded from the study. A pregnancy test will be done prior to the MR examination for postmenarchal teenage girls, in whom pregnancy may be possible. Only patients with a negative pregnancy test will be included in the study.

3.3 Informed Consent Process

All participants or parents/guardians will be provided a consent form describing the study with sufficient information for participants or parents/guardians to make an informed decision regarding their participation. Participants or parents/guardians will sign the IRB approved informed consent prior to participation in any study specific procedure. The participant or parents/guardians will receive a copy of the signed and dated consent document. The original signed copy of the consent document will be retained in the medical record or research file.

3.4 Randomization Procedures

This is a non-randomized study.

3.5 Study Timeline

Anticipated Start Date: 4/1/2011
Anticipated End Date: 4/1/2012

4. TREATMENT PLAN

STUDY PLAN:
All potential participants will be patients seen through regular referral methods in Dr. Marina's clinic. During the course of a normal clinic visit, if Dr. Marina notes a patient who meets the inclusion/exclusion criteria, they will be offered an opportunity to enroll in the study.

We will screen all patients with proven or suspected bone sarcoma or osteomyelitis for potential participation in this study.

Following screening parameters will be recorded within 30 days prior to enrollment:

i) Complete medical history

ii)Physical Examination

iii)Vital Signs

iv) Height

v) Weight

iv) ECG

Once enrolled, each participant will undergo an initial MRI without contrast and with ferumoxytol injection for anatomic detailing, and then another MRI without contrast (delayed imaging) 24 hours later to assess the metabolic reaction to the contrast agent (macrophage phagocytosis, the difference in how the two different diseases react to the contrast agent).

Screening of the patient will take 15-30 minutes. Active participation involves two MRI scans for a total of approximately 2-3 hours. Data analysis will continue until publication in approximately 12 months from initiation of the study.

Audio recording, video recording and photography will not be used. Tissue samples will not be retained.

DRUG ADMINISTRATION:

The study requires one intravenous injection of Feraheme during the first MRI scan for diagnostic purpose, at a dose of 5 mg Fe/kg. Considering a Ferumoxytol/Feraheme dose of 5 mg/kg and a concentration of 30 mg Fe/ml, we anticipate to inject 250-400 mg Fe in a teenager with an average body weight of 50-80 kg. This will translate into 8.3 – 13.3 ml volume of contrast agent to be injected. An M.D. will administer the contrast agent slowly via a hand injection, with continuous and direct feedback from the patient, over a time period of 1-2 minutes, i.e. with a maximum injection rate of 10 ml / min. According to continuous feedback from the patient during the contrast media administration, we will inject slower as deemed appropriate.

Since ferumoxytol is a “blood pool agent” (i.e. providing long lasting vascular enhancement), it is not crucial for the quality of the imaging scan to administer the contrast agent while the patient is in the scanner or to start a post-contrast scan immediately after contrast media administration. Thus, we will hand-inject ferumoxytol slowly into a peripheral vein while the patient is outside of the MR scanner and under direct supervision. We will observe the patient for a few minutes, acquire vital signs and ask him/her for any subjective adverse effects before we continue the imaging scan. We will continue to observe the patient closely and record heart rate and respiratory rate continuously during the MR scan. Blood pressure and temperature will be monitored before, directly after contrast media injection as well as at the end of the MR scan. The patient will be monitored up to 90 minutes after the contrast media administration. These data will be recorded according to the following table, which is part of the case report form:

Patient Name:	Patient weight:
Birth Date:	Ferumoxytol dose (5 mg/kg):
Medical Record #:	Ferumoxytol volume: = weight (kg) x dose (5 mg Fe/kg) / 30 mg Fe/ml
Ferumoxytol injection, date (dd/mm/yyyy) and start time (hh/min):	
Ferumoxytol injection end time (hh/min):	
Planned injection rate: 10 ml / min	Actual injection rate:
Contrast agent completely injected: yes / no	Actual injection volume:
Evidence of paravasal injection: yes / no	Local Reaction at injection site: yes / no

Vital signs:	pre	0 min	15 min	30 min	45 min	60 min	90 min
Heart Rate							
Respiratory rate							
Blood pressure							
Temperature							
Any objective or subjective adverse reaction (time):							
Any objective or subjective adverse reaction (describe):							

The MR exam will be carried out in the Pediatric CT/MR Imaging facility at Lucile Packard Children's Hospital or in the MR imaging suite at the Lucas Center. Both imaging facilities have resuscitation equipment for intervention in case of a rare, but possible event of an allergic contrast agent reaction. Oxygen supply, emergency medications, a "drug box" and CODE cart are available on both sites. A state licensed M.D will be present in the magnet suite during all contrast media injections. In addition, a pediatric nurse with special training in contrast agent interventions will be present in the imaging facility and available to assist with emergency interventions. The Emergency Department of Stanford Hospital is within walking distance to the imaging facilities. In case of serious AEs, physicians in the ED are also immediately available to help with emergency interventions.

Warnings: i) Ferumoxytol may cause serious hypersensitivity reactions including anaphylaxis and/or anaphylactoid reactions. In clinical studies, serious hypersensitivity reactions were reported in 0.2% of subjects receiving Ferumoxytol. This frequency of hypersensitivity reactions is not higher compared to standard MR contrast agents, used for routine clinical MR scans.
ii) Hypotension may follow Ferumoxytol administration. In clinical studies, hypotension was reported in 1.9 % of subjects.
iii) Iron Overload. Ferumoxytol should not be administered in patients with iron overload.

4.1 General Concomitant Medication and Supportive Care Guidelines

N/A**.** Our study is diagnostic only and will not limit the participants' treatment options or administration of concomitant medications or supportive care.

4.2 Criteria for Removal from Study

Criteria for removal from study:
i) Incomplete MR images,
ii) Image artifacts

iii) Adverse reaction to contrast agent,
iv) Upon request of Patient for exclusion from study
v) Suspected patients of bone sarcoma or osteomyelitis with incorrect assignment to a pathology.

Patients removed from the study will be replaced in order to reach a total of 10 subjects for each pathology.

4.3 **Alternatives**

The current alternative diagnostic methods are: biopsy, which is invasive, painful, and frequently inadequate; MRI, with significant overlap/difficulty in distinguishing between the two entities without an iron nanoparticle contrast; PET/CT/X-ray, which are even more difficult to distinguish and include exposure to ionizing radiation. The current standard of imaging is an MRI, which is included in our study; the addition of an iron nanoparticle contrast agent is expected to make this a much more efficacious diagnostic tool.
The conduct of our research should actually improve the speed with which the participants receive the most appropriate therapy, as we expect to be able to distinguish much more quickly between the differentials for the symptoms the patient's experience.

5. INVESTIGATIONAL AGENT/DEVICE/PROCEDURE INFORMATION

5.1 Investigational Agent/Device/Procedure

Study Agent:

Ferumoxytol (Feraheme™) represents a novel USPIO compound that has been recently FDA-approved for intravenous treatment of iron deficiencies in patients with renal failure (6). Ferumoxytol nanoparticles have a mean hydrodynamic diameter of 30 nm and are composed of an iron oxide core and a hydrophilic synthetic coating (6). Ferumoxytol exerts strong signal effects on MR images, as quantified by a high r1 relaxivity of 38 L $mmol^{-1}$ s^{-1} and a high r2 relativity of 83 L $mmol^{-1}$ s^{-1} at 20 mHz (9). Ferumoxytol has been applied as a contrast agent for MR imaging of arthritis in an animal model (9) and imaging of glioblastomas in patients (10). To the best of our knowledge, ferumoxytol has not been investigated for MR imaging of bone sarcomas or osteomyelitis. In MR imaging Ferumoxytol shortens the relaxation times for nearby hydrogen atoms. This effect can be imaged with appropriate MR pulse sequences.

Indication and Usage: Ferumoxytol is used as an iron replacement product indicated for the treatment of iron deficiency anemia in adult patients with chronic kidney disease.

We propose an off label application of Feraheme as a MRI contrast agent.

Mechanism of Action: Ferumoxytol consists of a superparamagnetic iron oxide that is coated with a carbohydrate shell, which helps to isolate the bioactive iron from plasma components until the iron-carbohydrate complex enters the reticuloendothelial system macrophages of the liver, spleen and bone marrow. The iron is released from the iron-carbohydrate complex within vesicles in the macrophages. Iron then either enters the intracellular storage iron pool (e.g., ferritin) or is transferred to plasma transferrin for transport to erythroid precursor cells for incorporation into hemoglobin. In MR imaging Ferumoxytol shortens the relaxation times for nearby hydrogen atoms. This effect can be imaged with appropriate MR pulse sequences.

Animal Studies: Animal studies demonstrate that the plasma half-life of Ferumoxytol increased with increasing dose. The highest tissue concentration of ferumoxytol was found in the liver, spleen and central lymphnode pool. Studies with radio-labeled drug product demonstrated that the renal elimination of iron in ferumoxytol was insignificant, while the carbohydrate coating was significantly excreted in urine and feces. Repeat dose toxicity studies of ferumoxytol in rats (cumulative exposure approximately 12 times the anticipated exposure of a human therapeutic course of 1.02 g of ferumoxytol on mg/m^2 basis) and dogs (cumulative exposure approximately 40 times the anticipated exposure of a human therapeutic course of 1.02 g of ferumoxytol on mg/m^2 basis) demonstrated dose dependent decreases in body weight gain and food consumption, and increases in pigmentation intensity. No systemic toxicity or immunotoxicity was observed at relevant clinical doses.

Clinical Studies: The safety and efficacy of Ferumoxytol for the episodic treatment of iron deficiency anemia in patients with CKD were assessed in three randomized, open-label, controlled clinical trials.

Ferumoxytol is not currently approved for diagnostic imaging applications. It is being evaluated as an agent for vascular enhanced MRI (VE-MRI). In August 2008 the FDA granted Fast track designation to ferumoxytol for its development as a diagnostic agent for VE-MRI to improve assessment of peripheral arterial disease in patients with known or suspected chronic renal disease (CKD). The fast track process is designed to facilitate the development and expedite the FDA's review of products. AMAG-sponsored Phase I/IIa studies have been completed and results published (36, 37, 38, and 39). Doses of 0.4-2 mg/kg in normal volunteers demonstrated arterial and venous vessels in several body regions (36). VE-MRI effectively identified aortic root incompetence in 6 subjects (37). In another reported study, 11 subjects with a variety of vascular disease who were scheduled for clinically indicated angiography, doses 1 to 4 mg/kg (38). In this study ferumoxytol imaging was done using a variety of pulse sequences. The study confirmed the ability to perform imaging of different arterial segments. An additional publication showed promise in the evaluation of deep venous thrombosis (39). Several additional studies on vascular imaging of the cerebral and pelvic arterial circulations have been conducted under physician-sponsored investigational new drug applications. In a study of patients with central

nervous system disease, Ferumoxytol in doses of 1 to 4 mg/kg was compared with non-contrast time-of-flight MRI, Gadolinium-enhanced MRI and VE-MRI (40). Ferumoxytol, with its persistence in vasculature due to its low vascular permeability and long intravascular half-life, provided better estimates of cerebral perfusion than gadolinium, and differences in biomarker properties were considered to be clinically advantageous in evaluating 12 patients with malignant brain tumors (40).

Clinical Pharmacokinetics: Ferumoxytol exhibits dose-dependent, capacity-limited elimination from plasma with a half life of approximately 15 hours in humans.

Major route of elimination: Ferumoxytol is slowly cleared from blood by tissue macrophages (liver, spleen, lymph node and active bone marrow) over 2-3 months. The macrophages release the iron to body iron stores where it can be incorporated into hemoglobin.

Drug Interactions: Ferumoxytol may reduce the absorption of concomitantly administered oral iron preparations.

Adverse Reactions: The most common adverse reactions (≥2%) following administration of Ferumoxytol are diarrhea, nausea, dizziness, hypotension, constipation and peripheral edema. Ferumoxytol may cause serious hypersensitivity reactions including anaphylaxis and/or anaphylactoid reactions. In clinical studies, serious hypersensitivity reactions were reported in 0.2% of subjects receiving Ferumoxytol. Hypotension may follow Ferumoxytol administration. In clinical studies, hypotension was reported in 1.9 % of subjects.

Dose: Single intravenous injection of Ferumoxytol during the first MRI scan, at a dose of 5 mg Fe/kg.

The delivered iron dose via a typical ferumoxytol administration is in the order of 150-500 mg iron oxides (note that these are coated iron particles, not free iron), which is equivalent to or lower than the iron dose administered with one blood sample.

Study Device:

MR Scanner, 1.5T and 3 T, GE healthcare. MRI with and without contrast is a non-significant risk exam, as defined by the FDA and IRB guidelines.

5.2 Availability

The study drug will be purchased from AMAG, Manufacturer

5.3 Agent Ordering

The agent will be ordered by research administrator Jennifer Vancil.

5.4 Agent Accountability

The investigational drug will be kept secure and locked with Jennifer Vancil.

6. DOSE MODIFICATIONS

Dose modification scheme:

(A) Serious anaphylactoid reactions are not dose dependent. Thus, in case we should observe an increased incidence of severe anaphylactoid reactions, i.e. more than one patient in our limited study population of 20 patients, or if any of our patients encounters a permanent impairment due to the ferumoxytol administration, the study will be terminated.

(B) In case we should observe an unexpected decrease in blood pressure of more than 10% in more than one patient via blood pressure measurements or in case we observe severe nausea in more than one patient, we will successively decrease the administered dose by 1 mg/kg until event-free tolerance is achieved. Of note, our colleagues from Oregon Health & Science University (see below) reported no such side effects of ferumoxytol in their population of children with brain tumors.

(C) Precontrast MR scans will serve as an internal standard. We will measure the signal intensity of sarcomas and osteomyelitis and divide this signal by the signal of normal muscle to normalize for potential MR spectrometer variations over time. We expect at least 20% tumor/muscle enhancement on post-contrast T2-weighted MR scans compared to precontrast scans. If this enhancement will not be achieved, but ferumoxytol is well tolerated, we will increase the administered dose to 6 mg Fe/kg with a maximum total amount of 510 mg Fe (FDA-approved dose for single injection).

(D) In the unlikely event, that the T1- and T2-enhancement of both pathologies exceeds 500%, we have reached a plateau of contrast agent accumulation for both pathologies rather than depicting an expected relative limited accumulation of ferumoxytol in sarcomas compared to osteomyelitis. Based on our experience with pre-clinical and clinical iron oxide applications, we expect that maximal difference between sarcomas and osteomyelitis occur with the highest dose. This is the reason why we limit our study population to this dose for this initial pilot study. In the unlikely event that contrast agent accumulation reaches a plateau in both of these pathologies or no enhancement is noted on MR scans, we will terminate the study after evaluation of n=6 patients (MR data from n=6 patients should allow evaluation of significant differences between quantitative pre- and post-contrast signal intensities via a t-test). We have submitted an R21 NIH grant proposal which would allow more extensive evaluation of patient subgroups (n=5), examined with decreasing ferumoxytol doses.

Stopping rules for an individual test:

(A) To avoid paravasal injections, the peripheral venous access will be checked with a saline flush prior to contrast media injection. In case of any swelling or discoloration at the ferumoxytol injection site (in the extremely rare event of a paravasal injection despite prior uncomplicated saline injection), the ferumoxytol injection will be stopped immediately, a new peripheral venous access will be placed at the contralateral extremity and the remaining ferumoxytol will be slowly administered. In case a local reaction is observed again, the ferumoxytol injection will be discontinued completely.

(B) If we observe a decline in blood pressure during the contrast media injection, we will discontinue the ferumoxytol injection immediately (in the experience of the PI, a transient hypotension represents a possible AE). In case we observe any signs of a potential anaphylactoid reaction (e.g. significant hypotension > 10% with associated tachycardia or bradycardia) during the contrast media injection, the injection will be discontinued immediately. Patients with mild symptoms such as urticaria, pruritus, rhinorrhea, nausea, diaphoresis and/or coughing will be observed for resolution of symptoms or progression. Patients, who show severe and/or progressive symptoms, such as arrhythmias, severe hypotension, breathing difficulties, dizziness or vomiting will be treated immediately by the M.D. on site and the Pediatric Emergency Care team will be

notified for immediate assistance.

Stopping rules for the study:

(A) In case of any serious AE, that leads to persistent impairment of a study patient, the study will be terminated.

(B) Anaphylactoid reactions are a possible, but rare side effect of any contrast agent, reported in 1% of patients receiving Gd-based contrast agents and 1% of patients receiving ferumoxytol. In case we observe an increased incidence of severe anaphylactoid reactions, i.e. more than one patient in our limited study population of 20 patients, the study will be terminated.

(C) According to our statistician, the study population of n=10 patients with sarcomas and n=10 patients with osteomyelitis is already very limited and considered a pilot population to demonstrate major differences in ferumoxytol enhancement of these two pathologies. In the unlikely event, that contrast enhancement reaches a plateau in both pathologies or no enhancement at all is noted in these pathologies on MR scans, neither on T2-weighted MR scans nor on T1-weighted MR scans, the study will be terminated after evaluation of n=6 patients (MR data from n=6 patients should allow evaluation of significant differences between quantitative pre- and post-contrast signal intensities via a t-test).

7. ADVERSE EVENTS AND REPORTING PROCEDURES

7.1 Potential Adverse Events

The most common adverse reactions (≥2%) following administration of Ferumoxytol are diarrhea, nausea, dizziness, hypotension, constipation and peripheral edema. Ferumoxytol may cause serious hypersensitivity reactions including anaphylaxis and/or anaphylactoid reactions. In clinical studies, serious hypersensitivity reactions were reported in 0.2% of subjects receiving Ferumoxytol. Hypotension may follow Ferumoxytol administration. In clinical studies, hypotension was reported in 1.9 % of subjects.

7.2 Adverse Event Reporting

In an unanticipated and unlikely event of any adverse event or anaphylactoid reaction to this contrast agent, appropriate actions will be taken to treat the reaction and the event will be reported to the IRB.

8. STUDY CALENDAR :

	Pre study	Initial MRI	24 hour delayed MRI
Complete medical history	**X**		
Physical Examination	**X**		
Vital Signs (Heart rate, respiratory rate, blood pressure, temperature)	**X**	**X**	
Height	**X**		
Weight	**X**	**X**	
ECG	**X**		
Pregnancy Test (B-HCG)	**X**		
Informed consent	**X**		
Investigational agent administration		**X**	
Adverse event evaluation		**X**	**X**
MRI exam evaluation		**X**	**X**

9. MEASUREMENT

9.1 **Primary Outcome measures**

Primary outcome: To determine contrast enhancement characters on T1 (immediate post contrast) and T2 (24 hours) weighted MR images, of bone sarcoma and osteomyelitis.

9.1.1 Relevant Subset

The outcome will be measured on all subjects included in the study.

9.1.2 Measurement Definition

To define distinctive MR signal characteristics of bone sarcomas and osteomyelitis.
Qualitative measurement: An initial precontrast scan of the lesion will be obtained to evaluate the lesion. This will serve as an internal control to define the postcontrast effects. Following administration of contrast agent ferumoxytol, post contrast MR images will be obtained and enhancement characteristics of the lesion will be assessed visually in comparison with the precontrast scan. The following pulse sequences will be applied: Long axis T1-spin echo (SE) images and short TI inversion recovery (STIR) images, covering the whole bone from joint to joint, axial T2-weighted fat-saturated fast spin echo (FSE) images focusing on the bone lesion, dynamic contrast-enhanced T1-weighted 3D spoiled gradient recalled echo (SPGR) images, and axial multi-echo T2*-weighted gradient echo (GE) images.
Quantitative measurement: Signal Intensity of the lesion and internal controls will be measured by operator defined ROI. Contrast enhancement and signal to noise ratios will be calculated. T2- and T2* relaxation times and R2 and R2* relaxation rates of the bone lesions will be calculated based on multiecho T2 weighted sequences.

9.1.3 Measurement Methods

Primary outcome of these examinations is to define distinctive MR signal characteristics of bone sarcomas and osteomyelitis, which will be immediately applicable in a clinical setting.
The MR signal intensity of the bone lesion, bone marrow, adjacent normal muscle, and background noise will be measured by operator defined regions of interest (ROI). The contrast enhancement of the bone lesions will be calculated as (SIpre-SIpost)/SIpre x 100%. The bone marrow will serve as a positive internal standard to confirm contrast accumulation at the target site and muscle will serve as a negative standard (no enhancement expected). Serial signal-to-noise ratios of the lesion on dynamic postcontrast T1-SPGR sequences will be used to calculate the blood volume of the lesion and microvascular permeability of ferumoxytol via kinetic analyses as described previously.

9.1.4 Measurement Time Points:

Data analysis of the initial MR scan and 24hr Delayed MR scans will be performed

9.1.5 Response Review

All MR images will be reviewed by two blinded readers.
The results of both readers will be included in data and statistical analysis. Inter-reader agreement will also be evaluated by K statistics.

10. REGULATORY CONSIDERATIONS

10.1 Monitoring plan

The Stanford Cancer Center Data and Safety Monitoring Committee (DSMC) will be the monitoring entity for this study. The DSMC will audit study-related activities approximately once per year to determine whether the study has been conducted in accordance with the protocol, local standard operating procedures, FDA regulations, and Good Clinical Practice (GCP). In addition, the DSMC will regularly review serious adverse events and protocol deviations associated with the research to ensure the protection of human subjects. Results of the DSMC audit will be communicated to the IRB and the appropriate regulatory authorities at the time of continuing review, or in an expedited fashion, as needed.

10.2 Protocol Review and Amendments

The protocol, the proposed informed consent and all forms of participant information related to the study (e.g. advertisements used to recruit participants) will be reviewed and approved by the Stanford IRB and Stanford Cancer Center Scientific Review Committee (SRC). Any changes made to the protocol will be submitted as a modification and will be approved by the IRB prior to implementation. The Protocol Director will disseminate the protocol amendment information to all participating investigators.

10.3 Data management

Patient data/demographics will be kept in the RedCap clinical research database tool provided by Stanford University's Clinical Informatics department (clinicalinformatics.stanford.edu). Images are kept in the clinically supported and secured PACs system, until a researcher exports de-identified images for publication. Identified information will only be kept in either a secure clinical system or the secure RedCap database; all exported data will be de-identified.

10.4 Study Documentation

The Protocol Director, or her designee, will prepare and maintain adequate and accurate participant case histories with observations and other data pertinent to the study. Original source documents should be transcribed to Case Report Forms (CRFs) and used to analyze the study data. Source documents include hospital records, clinical charts, laboratory and pharmacy records, and recorded electronic data

11. STATISTICAL CONSIDERATIONS

11.1 Statistical Design

11.1.1 Randomization

Non Randomized

11.2 Primary Analysis

11.2.1 Analysis Population

All MR images of the study population will be included in the analysis. In case of missing data or non-adherence to protocol, the subject will be excluded from analysis.

11.2.2 Analysis Plan

Descriptive statistics will be used to characterize contrast enhancement and relaxation times in both T1 and T2* settings, in each population; the key estimates will be the standard deviation and the coefficient of variation. The area under the ROC curve will be calculated as a measure of separation and the significance of the separation will be assessed using the Wilcoxon rank sum test.

11.3 Sample Size

11.3.1 Accrual estimates

We plan to enroll 20 patients in this pilot study, 10 patients with bone sarcomas and 10 patients with an osteomyelitis. Osteomyelitis is a relatively common disease, while bone sarcoma is relatively rare and we expect to enroll 1-2 subjects per month of both these pathologies.

11.3.2 Sample size justification

This is a pilot study to establish proof of concept and obtain data for future investigations. If applicable, the precision of the data could be summarized with a pooled standard deviation, which is expected to have 18 degrees of freedom. A future sample size calculated on that basis would have a 30% probability of underestimating a sample size by as much as 20%, and a 4% probability of underestimating a sample size by as much as 50%. For the record the sample sizes in the present study provide 80% power to detect a difference of 1.33 standard deviations, but we have no information on the magnitude of a clinically relevant difference.

11.4 Criteria for future studies

With this pilot study we want to determine the distinctive contrast enhanced MR signal characteristics of Bone Sarcoma and Osteomyelitis. Subsequent future studies will evaluate the actual macrophage quantity within the study lesions.

REFERENCES

Provide the citations for all publications referenced in the text.

(1) Ewing J. Classics in oncology. Diffuse endothelioma of bone. James Ewing. Proceedings of the New York Pathological Society, 1921. *CA Cancer J Clin* 1972;22(2):95-8
(2) Petrikowski CG, Pharoah MJ, Lee L, Grace MG. Radiographic differentiation of osteogenic sarcoma, osteomyelitis and fibrous dysplasia of the jaws. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 1995 Dec;80(6):744-50.
(3) Lyall H, Constant C, Wraight E. Case report: Ewing's sarcoma in distal tibial metaphysis mimicking osteomyelitis. *Clinical Radiology* 48(2): 140-142
(4) Mellado Santos, JM. Diagnostic imaging of pediatric hematogenous osteomyelitis: lessons learned from a multi-modality approach. *Eur Radiol* 2006, 16(9): 2109-2119
(5) Pai AB, Nielsen JC, Kausz A, Miller P, Owen JS. Plasma pharmacokinetics of two consecutive doses of ferumoxytol in healthy subjects. *Clin Pharmacol Ther*. 2010;88(2):237-42
(6) Lu M, Cohen MH, Rieves D, Pazdur R. FDA report: Ferumoxytol for intravenous iron therapy in adult patients with chronic kidney disease. Am J Hematol. 2010;85(5):315-9
(7) Provenzano R, Schiller B, Rao M, Coyne D, Brenner L, Pereira BJ. Ferumoxytol as an intravenous iron replacement therapy in hemodialysis patients. *Clin J Am Soc Nephrol.* 2009; 4(2):386-93
(8) Schwenk MH. Ferumoxytol: a new intravenous iron preparation for the treatment of iron deficiency anemia in patients with chronic kidney disease. *Pharmacotherapy.* 2010;30(1):70-9
(9) Simon GH, von Vopelius-Feldt J, Fu Y, Schlegel J, Piontek G, Wendland MF, Mei-Hsiu C, Daldrup-Link HE: Ultrasmall superparamagnetic iron oxide-enhanced magnetic resonance imaging of antigen-induced arthritis: a comparative study between SHU555C, Ferumoxtran-10 and Ferumoxytol. *Investigative Radiology* 2006**;** 41(1):45-51
(10) Gahramanov S, Raslan AM, Muldoon LL, Hamilton BE, Rooney WD, Varallyay CG, Njus JM, Haluska M, Neuwelt EA. Potential for Differentiation of Pseudoprogression from True Tumor Progression with Dynamic Susceptibility-weighted Contrast-enhanced Magnetic Resonance Imaging using Ferumoxytol vs. Gadoteridol: A Pilot Study. *Int J Radiat Oncol Biol Phys*. 2010 Apr 13. [Epub ahead of print]
(11) Reimer P, Rummeny EJ, Daldrup HE, Tombach B, Berns T, Balzer T, Peters PE: Clinical Results with Resovist: A Phase 2 Clinical Trial. *Radiology* 1995; 195: 489-496
(12) Daldrup HE, Link TM, Blasius S, Könemann S, Jürgens H, Rummeny EJ: Monitoring Radiation-Induced Changes in Bone Marrow Histopathology with Ultra-Small Superparamagnetic Iron Oxide (USPIO) Enhanced MRI. J Magn Reson Imaging 1999, 9: 643-652
(13) Daldrup-Link HE, Rummeny EJ, Ihßen B, Kienast K, Link TM: Iron oxide enhanced MR imaging of bone marrow in patients with non-Hodgkin`s lymphoma: differentiation of tumor infiltration and hypercellular bone marrow. *Europ Radiol* 2001; 11: 1276-1284
(14) Daldrup-Link HE, Rydland J, Helbich T, Turetschek K, Haraldseth O, Link TM, Brasch RC, Shames D, Rummeny EJ: Quantitative MRI-Estimates of breast tumor microvascular permeabilities to the macromolecular contrast agent Feruglose (Clariscan) correlate with histologic tumor grade: Initial Phase II Multicenter Trial. *Radiology* 2003; 229(3): 885-92
(15) Metz S, Bonaterra G, Rudelius M, Settles M, Rummeny EJ, Daldrup-Link HE: Capacity of Human Monocytes to phagocytose approved iron oxide MR contrast agents. Eur Radiol 2004; 14(10):1851-8
(16) Daldrup-Link HE, Rudelius M, Piontek G, Metz S, Bräuer R, Debus G, Corot C, Schlegel J, Link TM, Peschel C, Rummeny EJ, Oostendorp RAJ: Migration of iron oxide labeled human hematopoietic progenitor cells in a xenotransplant model: in vivo monitoring using clinical magnetic resonance imaging equipment. *Radiology* 2005; 234(1): 197-205
(17) Daldrup-Link HE, Meier R, Rudelius M, Piontek G, Piert M, Metz S, Settles S, Uherek C, Schlegel J, Rummeny EJ: In-vivo Tracking of genetically engineered anti-HER2/neu directed Natural Killer to HER2/neu positive Mammary Tumors with Magnetic Resonance Imaging. Eur Radiol 2005; 15(1): 4-13

(18) Simon GH, von Vopelius-Feldt J, Wendland M, Schlegel J, Mei-Hsiu C, Daldrup-Link HE: Ultrasmall superparamagnetic iron oxide enhanced MR imaging of normal bone marrow in rodents. *Academic Radiology* 2005; 12(9):1190-7
(19) Simon GH, von Vopelius-Feldt J, Wendland M, Fu Y, Piontek G, Schlegel J, Chen, MH, Daldrup-Link HE: MRI of arthritis: Comparison of ultrasmall superparamagnetic iron oxide vs. Gd-DTPA. *Journal of Magnetic Resonance Imaging* 2006; 23(5):720-7
(20) Metz S, Lohr S, Settles S, Beer A, Woertler M, Rummeny EJ, Daldrup-Link HE: Ferumoxtran-10 enhanced MR imaging of the bone marrow before and after conditioning therapy in patients with Non Hodgkins Lymphoma. *Eur Radiol*. 2006; 16(3):598-607, 2006
(21) Simon GH, Bauer J, Saborowski O, Fu Y, Corot C, Wendland MF, Daldrup-Link HE: T1 and T2 relaxivity of intracellular and extracellular USPIO at 1.5T and 3T clinical MR scanning. *Eur Radiol.* 2006; 16(3):738-45
(22) Henning TD, Wendland MF, Golovko D, Sutton EJ, Sennino B, Malek F, Bauer JS, McDonald DM, Daldrup-Link HE: Relaxation effects of ferucarbotran-labeled mesenchymal stem cells at 1.5T and 3T: discrimination of viable from lysed cells. *Magn Reson Med* 2009; 62(2):325-332
(23) Golovko D, Henning T, Bauer JS, Settles M, Frenzel T, Mayerhofer A, Rummeny E, Daldrup-Link HE: Accelerated stem cell labeling with ferucarbotran and protamine. *Eur Radiol*. 2010; 20(3): 640-8
(24) Meier R, Henning TD, Boddington S, Tavri S, Arora S, Corot C, Daldrup-Link HE: Breast cancers: MR imaging of folate receptor expression with the folate-specific nanoparticle P1133. *Radiology* 255(2):527-35
(25) Henry TD, Mary E. McCarville ME, Hoffer, FA. Diagnostic Imaging of Pediatric Bone and Soft Tissue Sarcomas. *Pediatric Oncology*, 2006, 35-69
(26) Gyoerke T. Impact of FDG PET for staging of Ewing sarcomas and primitive neuroectodermal tumours. *Nuclear Medicine Communications* 2006; 27(1): 17-24
(27) Kan JH. Major pitfalls in musculoskeletal imaging–MRI. Ped Radiol 2008, 38, Supplement 2, 251-255
(28) Mathur K, Nazir AA, Sumathi VP, Kumar T. Ewing's sarcoma masquerading as chronic osteomyelitis: a case report. *Eur J of Orthop Surgery & Traumatology* 2005; 16(2):175-177
(29) Kleis M, Daldrup-Link H, Matthay K, Goldsby R, Lu Y, Schuster T, et al. Diagnostic value of PET/CT for the staging and restaging of pediatric tumors. *Eur J Nucl Med Mol Imaging* 2009;36(1):23-36
Article I. (30) Metcalfe J, Grimer R. Ewing's sarcoma of the foot masquerading as osteomyelitis. *Foot and Ankle Surgery* 2004, 10(1) 29-33
(31) Tow BPB, Tan MH. Delayed diagnosis of Ewing's sarcoma of the right humerus initially treated as chronic osteomyelitis: A case report. *Journal of Orthopaedic Surgery 2005:13(1):88-92*
(32) Durbin M, Randall RL, James M, Sudilovsky D, Zoger S. Ewing's sarcoma masquerading as osteomyelitis. *Clin Orthop* 1998; (357):176–85
(33) Akeda K, Kasai Y, Kawakita E, Seto M, Kono T, Uchida A. Primary Ewing Sarcoma of the Spine Mimicking a Psoas Abscess Secondary to Spinal Infection. *Spine* 2009, 34(9): E337-E341
Section 1.01 *(34) Lichtenstein M, Andrews J, Scales R. Localization of osteomyelitis with ^{99m} technetium sulfur colloid.* Australian and New Zealand Journal of Surgery 1983; 53(4): 339–342
(35) Bierry G, Jehl F, Neuville A, Lefevre S, Robert P, Kremer S, Dietemann JL. MRI of macrophages in infectious knee synovitis. *AJR Am J Roentgenol.* 2010;194(6):W521-6
(36) Prince MR, Zhang HL, Chabra SG et al. A pilot investigation of new superparamagnetic iron oxide (ferumoxytol) as a contrast agent for cardiovascular MRI. J X-Ray Sci Tech 2003; 11:231-240.
(37) Ersoy H, Jacobs P, et al. (2004). Blood pool MR angiography of aortic stent-graft endoleak. AJR Am J Roentgenol 182(5): 1181-6.
(38) Li W, Tutton S, et al. (2005). First pass contrast enhanced magnetic resonance angiography in humans using ferumoxytol, a novel ultrasmall superparamagnetic iron oside (USPIO)-based blood pool agent. J Magn Reson Imaging 21(1): 46-52
(39) Li W, Salanitri J, et al (2007). Lower extremity deep venous thrombosis: evaluation with ferumoxytol-enhanced MR imaging and dual-contrast mechanism—preliminary experience. Radiology 242(3): 873-81.
(40) Neuwelt EA Varallyay CG, et al (2007). The potential of ferumoxytol nanoparticle magnetic resonance

imaging, perfusion and angiography in central nervous system malignancy: a pilot study. Neurosurgery 60(4):601-11; discussion 611-2.
41) Palestro CJ , Love C , Tronco GG , Tomas MB , Rini JN . Combined labeled leukocyte and technetium 99m sulfur colloid bone marrow imaging for diagnosing musculoskeletal infection. Radiographics. 2006 May-Jun;26(3):859-70.
42) Strobel K, Stumpe KD. PET/CT in musculoskeletal infection. Semin Musculoskelet Radiol . 2007 Dec;11(4):353-64. Review .

APPENDIX A: Participant Eligibility Checklist

A Participant Eligibility Checklist must be completed in its entirety for each subject prior to registration. The completed, signed, and dated checklist must be retained in the patient's study file and the study's Regulatory Binder.

Protocol Title:	**Differentiation of bone sarcomas and osteomyelitis with Ferumoxytol-Enhanced MRI**
Protocol Number:	**20253**
Principal Investigator:	**Dr.Heike Daldrup-Link**

II. Subject Information:

Subject Name/ID:
Gender: ☐ Male ☐ Female

III. Inclusion/Exclusion Criteria

Inclusion Criteria (From IRB approved protocol)	**Yes**	**No**	**Supporting Documentation***
1. 10-21 years in age	☐	☐	
2. Suspected or confirmed diagnosis of osteosarcoma or osteomyelitis	☐	☐	
Exclusion Criteria (From IRB approved protocol)			
1. Contraindication to MRI (metal implants)	☐	☐	
2. Need for sedation or anesthesia (claustrophobia)	☐	☐	
3. hemosiderosis/ hemochromatosis	☐	☐	

*All subject files must include supporting documentation to confirm subject eligibility. The method of confirmation can include, but is not limited to, laboratory test results, radiology test results, subject self-report, and medical record review.

IV. Statement of Eligibility

This subject is [☐ **eligible** / ☐ **ineligible**] for participation in the study.

Signature:	Date:
Printed Name:	

APPENDIX B: Case Report Forms
I. Patient History

Patient Name:	**Medical Record #:**	
Birth Date:	**Patient body weight:**	
Gender:	**Patient height:**	
Medications:	**Allergies:**	
Inclusion Criteria:		
Age: 10-21 years	yes	no
suspected or confirmed diagnosis of a bone sarcoma or osteomyelitis	yes	no
Exclusion Criteria:	yes	no
Hemosiderosis/Hemochromatosis	yes	no
History of any allergies to contrast agents or any severe allergies to other substances	yes	no
Claustrophobia	yes	no
MR-incompatible metal implants	yes	no
Any significant previous illnesses or injuries: (date / completed or ongoing)	**Any previous surgeries**: (date / completed or ongoing)	
CNS:	CNS:	
Head/Neck:	Neck:	
Chest:	Chest:	
Abdomen/Pelvis:	Abdomen/Pelvis:	
MSK:	MSK:	
Psychological / Emotional:	Psychological / Emotional:	
Other:	Other:	
Details:		

II. Physical Examination

Patient Name:	**Medical Record #:**
Patient body weight:	**Patient height:**
Blood Pressure:	**Pulse rate:**
ECG date (within 30 d prior to Ferumoxytol MR): **ECG report (summary):**	

Clinical Evaluation:	**normal**	**abnormal**	**Describe abnormal findings**
CNS			
Head/Nose/Mouth			
Ears			
Eyes			
Neck			
Lungs / Auscultation			
Heart / Auscultation			
Abdomen			
Pelvis			
Spine			
Upper Extremities			
Lower Extremities			
Skin			

Pregnancy test (within 24 h prior to contrast media injection):

Other:

III. Contrast Media Administration

Patient Name:	**Patient weight:**
Birth Date:	**Ferumoxytol dose (5 mg/kg):**
Medical Record #:	**Ferumoxytol volume:** **= weight (kg) x dose (5 mg Fe/kg) / 30 mg Fe/ml**
Ferumoxytol injection, date (dd/mm/yyyy) and start time (hh/min):	
Ferumoxytol injection end time (hh/min):	
Planned injection rate: 10 ml / min	Actual injection rate:
Contrast agent completely injected: yes / no	Actual injection volume:
Evidence of paravasal injection: yes / no	Local Reaction at injection site: yes / no

Vital signs:	pre	0 min	15 min	30 min	45 min	60 min	90 min
Heart Rate							
Respiratory rate							
Blood pressure							
Temperature							
Any objective or subjective adverse reaction (time):							
Any objective or subjective adverse reaction (describe):							

IV. MR Imaging

Acquired pulse sequences			
Date of MR 1 (dd/mm/yyyy): Date of MR 2 (dd/mm/yyyy):			
	precontrast	1-60 min p.i.	24 h p.i.
T1-SE			
STIR			
T2-TSE			
T2*-GE			
Other (specify)			
Other (specify)			

(add pulse sequence parameters: TR/TE/alpha/slice thickness/orientation)