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MR, Histologic and EM Imaging Of Intravenous Ferumoxytol In Central Nervous System (CNS) Inflammation

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Multi-Disciplinary Study: MR, Histologic And EM Imaging Of Intravenous Superparamagnetic Crystalline Particles (ferumoxytol) In CNS and Head and Neck Lesions

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1 ABSTRACT

This exploratory study utilizes ferumoxytol, an iron oxide nanoparticle MR contrast agent for imaging various inflammatory processes in the head and neck region, spine, including the central nervous system. The protocol enrolls subjects with radiological or histological diagnosis of unknown, dural, or parenchymal CNS lesions, multiple sclerosis, TIA or stroke, vasculitis, or other vascular lesions; arterial vasculopathy and venous thrombosis; or enlarged cervical lymph nodes. The main purpose of this study is to better understand the underlying cellular mechanisms, contrast agent extravasation, uptake into macrophages and to assess its value in clinical MR imaging.

Three subject groups will be studied.

1. Group 1: (n= up to 260) subjects will include those with dural, central nervous system (CNS) parenchymal based inflammatory, vascular or demyelinating lesions. This includes but is not limited to unknown lesions, meningioma, neuroma, stroke, multiple sclerosis (MS) or related diseases such as acute disseminated encephalomyelitis (ADEM). In these subjects the brain or the spine will be imaged.
2. Group 2: (n=up to 25) subjects will include those with vascular CNS lesions such as ischemic stroke, TIA with suspected carotid embolic origin, or atherosclerotic or inflammatory vasculopathy involving the carotids (including diagnosed carotid stenosis >50%), aorta and the arteries of the extremities or thrombosis of the intraabdominal, pelvic or extremity veins. Carotid and/or aortic and/or extremity MRI and MRA or magnetic resonance venography (MRV) will be performed in these subjects.
3. Group 3: (n=up to 10) will include subjects with enlarged cervical lymph nodes in which inflammatory processes (reactive lymph nodes) is part of the differentials. MRI of the cervical lymph nodes will be performed.
4. Body imaging subgroup: will include any subject from the above 3 groups who agrees to participate in additional abdominal MR imaging to assess ferumoxytol uptake and clearance from body organs and bone marrow.

<u>The primary objectives are</u>:

1. To establish optimal sequencing parameters and compare ferumoxytol MR with gadolinium enhanced MR in adults
    1. with dural, parenchymal based, demyelinating or vascular CNS disease. This will involve imaging of the brain or the spine. (Group 1)
    2. with vasculopathy of carotids, aorta, or arteries of the extremities or thrombosis of the intraabdominal, pelvic or extremity veins. This will include carotid MRA as well as high resolution carotid plaque imaging and analysis, aortic/extremity MRA as well as high resolution aortic/extremity artery wall imaging and analysis or MRV as well as high resolution wall imaging of the vena cava, pelvic and extremity veins. (Group 2)

3. with cervical lymphadenopathy, to identify imaging characteristics of the various etiologies (e.g. reactive vs. metastatic lymph nodes). (Group 3)

1. To assess the utility of ferumoxytol in subjects who should not receive gadolinium-based contrast agents, this includes subjects with renal disease (GFR < 50), acute kidney injury, prior renal transplant, or an allergy to gadolinium.

The secondary objectives are:

1. To localize ferumoxytol particles with histology and electron microscopy in biopsy samples from a small subset of subjects in which a biopsy is needed. Results will also be compared to a companion study of brain tumor imaging entitled "NCI-Sponsored multi-disciplinary study for MR imaging of intravenous superparamagnetic crystalline particle ferumoxytol in primary high-grade brain tumors and/or cerebral metastases".

2. To assess side effects/safety of ferumoxytol when given during MRI.

3. To assess the time course of iron uptake and clearance (of the first ferumoxytol injection) in abdominal organs, such as the liver, spleen, pancreas and bone marrow by applying usual abdominal MR sequences at multiple time points (up to 6 months) after ferumoxytol injection in a subgroup of subjects. For this purpose a subgroup of 50 subjects will be recruited from primary groups 1, 2 and 3.

Future directions:

This exploratory study enrolls subjects with various pathologies, to help design further focused clinical trials. Based on the knowledge of this current study, there have been two multicenter imaging protocols being developed. One is to support FDA market approval of ferumoxytol as an MR imaging agent, and another analyzing steady state blood volume maps in glioblastoma patients.

2 BACKGROUND / RATIONALE

2.1 Multiple Sclerosis

2.1.1 Epidemiology

Prevalence and epidemiology: current estimates of the prevalence of MS are 400,000 [81] cases of MS in the United States, making it the leading cause of neurological disability among young people and recent data from the National Health Interview Survey suggest that the prevalence is increasing [93, 116]. Substantial variation in the prevalence of MS occurs even in areas with populations of similar ethnic origin, however the prevalence seems to be the highest among Caucasians. The prevalence seems to increase at higher latitudes [82-84].

2.1.2 Etiology

MS is believed to be an immune mediated disorder. What triggers the immune response has not yet been clarified. Viruses have been hypothesized as the initial culprits and associations between MS and several different viral infections have been found. Evidence of different viral infections has been shown in MS brains not present in controls, however, the results have not been reproducible and the viral hypothesis remains unconfirmed [85-89]. Molecular mimicry between sequences of the myelin constituents and other viral or bacterial antigens is another possibility [88]. In animal models of MS, CD-4 cells that infiltrate the CNS and reactive myelin components drive the autoimmune response to antigens presented by either microglial cells, astrocytes, or macrophages. The activation of CD-4 cells leads to the production of cytokines that trigger macrophages. CD-4 lymphocytes mediate the specificity of the immune response in the animal model of MS and may be directly responsible for some of the demyelination and axonal damage through the release of cytokines [90]. Macrophages however, are the main effector cells that mediate demyelination in animal models. In electron microscopy studies, macrophages have been documented stripping the axons of the affected neurons of their myelin sheaths [91] so the ability to image phagocytic cells *in vivo* may provide valuable information

Histopathologic hallmarks of the disease include multifocal lesions showing inflammation, lymphocytes and macrophages, demyelination, gliosis, attempts at remyelination, and relative sparing of axons [5]. Active histologic lesions show loss of myelin, infiltration with lipid-laden macrophages (Gitter cells), perivascular inflammatory cuffing with T- and B-cell lymphocytes, and plasma cells associated with perivascular demyelination. The histopathologic correlates of the various clinical courses, relapsing remitting, secondary progressive or primary progressive, are still not fully understood. [93] Trapp et al [6] challenged the relative sparing of axons by finding transected axons to be common in acute lesions and hypothesized that these could be responsible for permanent neurologic impairment. Since effective treatments are now available and irreversible damage to the axons may be an integral part of the disease, diagnosis needs to be made as early as possible. The current available therapies are not curative and are expensive so improving the specificity in the diagnosis is crucial.

2.1.3 Imaging/Diagnosis

MS is diagnosed clinically by demonstrating central nervous system lesions that are separated in time and space. MRI has been shown to be a valuable tool in the diagnosis of MS as it can show lesions that are asymptomatic. Several authors have proposed MRI criteria for the diagnosis of MS that would allow earlier diagnosis based on T2 weighted abnormalities in MRI. Paty et al [7] proposed that four or more lesions (Paty A criteria) or at least three lesions with one lesion bordering the lateral ventricles (Paty B criteria) are strongly suggestive of MS. The criteria suggested by Fazekas et al [8] include three or more lesions with the presence of at least two of the following characteristics: size greater than 5 mm, periventricular, and infratentorial. Although lesions in the centrum semiovale tend to occur with aging and other processes,

periventricular lesions appear to be more specific for MS. Lesions around the temporal horn and fourth ventricle in the cerebellar peduncle, as well as in the midbrain, also appear more specific for MS but have a low rate of occurrence. Yetkin et al [9] reported that 2% to 4% of healthy subjects had high-intensity periventricular abnormalities that could not be distinguished from MS. Barkhof et al [10, 96] proposed that the presence of juxtacortical lesions in subjects with monosymptomatic neurologic disease was a highly specific prognosticator of progression to MS.

An international panel organized by the National MS Society, chaired by W.I. McDonald, has recently updated the diagnostic criteria for MS to incorporate MRI information from both T2 weighted images and gadolinium enhanced images to complement clinical assessment and spinal fluid analysis. MRI is considered to provide evidence of dissemination in space if three of the following four criteria are present: one Gd enhancing lesion or 9 T2 hyperintense lesions either in the brain or spinal cord, one or more infratentorial lesions, one or more juxtacortical lesions, three or more periventricular lesions. MRI can provide evidence of dissemination in time if there is a Gd enhancing lesion in a scan done three months or more after an attack at a site different from the attack, or if the first scan is negative a new T2 lesion or Gd enhancing lesion in a second scan done three months or more after the first scan [16, 97].

The use of gadolinium as a contrast material depicts abnormalities of the BBB in subjects with MS and has become a valuable tool in the diagnosis as well as follow up of patients with MS [91, 94, 99, 101, 102, 113]. Contrast-enhanced examinations are more sensitive than either clinical examination or T2-weighted MR imaging in detecting disease activity, and can potentially separate clinical groups [4, 12-14, 94, 95, 98, 100, 110, 111, 113]. The normal window of enhancement is from 2 to 8 weeks; however, plaques can enhance for 6 months or more [17].

Several modifications of the traditional gadolinium enhanced MRI have been proposed to further improve the ability to detect active lesions. Enhancement cannot be viewed as an all-or-none phenomenon; rather, it is dependent on the time from injection to imaging, the dosage of contrast agent, the magnitude of the BBB abnormality, and the volume of the accumulation space [18-20]. Delayed imaging (usually 15 to 60 minutes after injection) increases the detection of enhancing MS lesions [21, 22]. Triple doses of contrast agent (0.3 mmol/kg) [21, 23, 24, 104] or a single dose (0.1 mmol/kg) with magnetization transfer (103) to suppress normal brain tissue can increase the number of detectable MS lesions. Silver et al. [25] found that a triple-dose of contrast material increased lesion detection by 75% as compared with a single dose. Magnetization transfer combined with a 20- to 40-minute scan delay increased the number of enhancing lesions detected with single-dose contrast agent by 47% and with triple-dose injection by only 27%. Magnetization transfer with a 40- to 60-minute delay after triple-dose contrast injection however, resulted in the detection of 126% more enhancing lesions than in standard single-dose imaging.

Excellent correlation has been reported between contrast enhancement and macrophage infiltration, but the association is weaker with perivascular lymphocyte infiltration [2]. Blood-brain barrier (BBB) disruption [44, 45] and demyelination appear to be linked, but how strongly remains open to debate. Enhancement may precede the development of high T2 signal intensity and clinical symptoms, which suggests that the BBB abnormality is the seminal event in the inflammatory cascade [18, 26]. Yet there is disease in the normal-appearing white matter that is unlikely to be detected with the available resolution and standard imaging and contrast techniques. [16, 27-30].

It is also unlikely that all the inflammatory changes occur in the 2- to 8-week window of enhancement. Miki et al [31] found a negative correlation between enhancing lesion volume and duration of disease, suggesting that the BBB abnormalities are less important over time. Furthermore, when the disease evolves to the secondary-progressive stage, decreasing levels of enhancement are observed in spite of increasing neurologic deficits, again suggesting a diminished role of the BBB abnormality. Recent experimental work by Dousset et al (V. Dousset, University of Bordeaux, France; personal communication), who used ultrasmall iron oxide to image macrophages in experimental autoimmune encephalomyelitis in rats, suggests that inflammatory cells may cross an intact BBB by diapedesis. This result is consistent with the reports of “abnormal” normal-appearing white matter without concomitant enhancement and of progressive disease with diminishing levels of enhancement. BBB abnormalities are associated in many macroscopic lesions with inflammatory change. However, inflammatory changes in MS are not always detected by enhancement, particularly when the level of inflammation is low. Lastly, primary-progressive MS displays little enhancement despite progressive neurologic decline, suggesting an alternative pathologic process most likely dissociated from BBB abnormality, such as primary neuronal loss.

A phase II study using USPIO on ten MS patients during acute exacerbations revealed enhancement in nine out of ten patients with USPIO vs. seven out of ten patients showing enhancement with gadolinium. Of the total number of lesions, 31 enhanced with both contrast agents, 24 enhanced only with gadolinium, and 2 enhanced with USPIO only. In the 2 patients that the lesions enhanced with USPIO only, there were no other enhancing lesions. This study suggests that the enhancement with USPIO is not superimposed on the enhancement with gadolinium and that the two contrast agents would complement each other [115]. The nature of the difference remains to be elucidated.

In the animal models of MS, experimental autoimmune encephalomyelitis (EAE), the cells infiltrating the central nervous system actively engage in the uptake of ferumoxytol and can be imaged *in vivo*. The areas of uptake correspond to acute plaques. USPIO particles have shown a high sensitivity for the detection of EAE lesions in animal models [92].

Ferumoxytol is attractive to use as a contrast agent because of its specific uptake by phagocytic cells [114]. This characteristic may help delineate plaques that have actively been infiltrated by inflammatory cells providing a more sensitive contrast media than gadolinium. Ferumoxytol may be taken up only by a certain population of plaques that enhance with gadolinium suggesting lesions with higher inflammation. It is also possible that ferumoxytol enhances areas of previously normal appearing white matter. Any of these findings would be very important for clinical use, as it would facilitate the diagnostic process leading to earlier treatment. The burden of gadolinium enhancing lesions has been incorporated as a quantitative outcome in phase III trials. MRI has been shown in trials of disease modifying drugs to be sensitive to treatment effects and to show changes before clinical measures do [3, 11, 105-109, 112]. It would also be possible to incorporate ferumoxytol in the evaluation of future treatment protocols. A more sensitive or more specific contrast media may result in protocols that require smaller sample sizes and a more rapid process of development of new therapies.

Clinically the differential diagnosis of MS is broad. Infectious diseases that cause multifocal involvement of the CNS could be confused with MS. These include Lyme disease, syphilis, PML, HIV and HTLV-1. Other inflammatory diseases can cause similar clinical pictures and include SLE, Sjögren's syndrome, vasculitis, sarcoidosis and Behçet disease. Certain metabolic and genetic conditions can cause diffuse involvement of the white matter and include B12 deficiency, lysosomal disorders, adrenoleukodystrophy, mitochondrial disorders and other genetic disorders. CNS lymphoma is the main neoplastic disease that can have a similar presentation to MS. When analyzing the MRI many other lesions exhibit an appearance similar to that of MS on conventional MR images. [1] Agents such as ferumoxytol that may be selective for areas of inflammation or wider openings in the blood brain barrier may facilitate the correct diagnosis. Preliminary data obtained with *Combidex* in OHSU protocol #644 suggest that iron particles may help differentiate lymphoma from inflammatory lesions. This would be of great clinical utility as differentiating MS from lymphoma is a frequent clinical problem and currently cannot be accomplished without brain biopsy.

The ability to image the vessels with the bolus infusions of ferumoxytol may provide additional information. Demyelinating lesions tend to follow a specific pattern localizing around the venules, this gives them their periventricular location and their characteristic shapes. A prior study using gadolinium as a contrast agent done in 17 subjects with multiple sclerosis showed that all periventricular lesions and all but one deep white matter lesion had a central visible vein [121]. Using ferumoxytol as a contrast agent instead of gadolinium may provide better imaging of the blood vessels showing perivenular lesions more clearly. Showing lesions localized around the venules may help differentiate demyelinating lesions from white matter changes related to vascular disease. The need to distinguish ischemic from demyelinating lesions frequently comes up in clinical practice and is relevant as the treatments for each condition are very different.

2.2 Vascular diseases

2.2.1 Stroke and atherosclerosis of carotid arteries

2.2.1.1 Epidemiology

Cerebrovascular disease is the third leading cause of death in developed countries after heart disease and cancer; the overall prevalence is 794 per 100,000. It is estimated that more than 400,000 patients are discharged each year from hospitals in the United States after a stroke. The loss of these patients from the work force and the extended hospitalization they require during recovery make the economic impact of the disease one of the most devastating in medicine [32, 33, 117, 118].

The prevalence of stroke is rising. Stroke is a major medical problem because of the high rate of morbidity and mortality as illustrated by the following observations:

1. The mortality from the acute event is about 20 percent
2. Approximately 50 percent of patients are alive after five years
3. **There is substantial morbidity among the survivors; data from the Framingham study reveal that 18 percent are unable to return to work while up to 4 percent require total custodial care [118] and**
4. Approximately 25 percent of survivors will have a second neurologic event, leading to death in over one-half.

2.2.1.2 Etiology

Stroke may result from hemorrhage, either subarachnoid (e.g., ruptured berry aneurysm) or intracerebral (e.g., due to uncontrolled hypertension), or more often from ischemic infarction, which is usually due to embolization (primarily from the carotid artery or the heart) or thrombosis but can result from a low flow state. Cardiac-derived strokes are typically embolic and result from atrial fibrillation, a ventricular mural thrombus, cardiac surgery or valvular lesions (see below neurological deficit from cardiac surgery) [48].

Silent strokes or lacunar events were defined by Fisher as “infarcts caused by occlusion of small penetrating cerebral arteries”. The underlying vascular pathology, he suggested, was either intracranial atherosclerosis or a specific form of hypertensive cerebral small-vessel disease that he termed “segmental arterial wall disorganization” (and that others have referred to as lipohyalinosis), characterized in the acute stage as fibrinoid necrosis. Few further pathological studies have found occluded small vessels, yet the occlusion mechanism has been vigorously debated [34]. Others suggest that lacunar infarction results from the same factors responsible for cortical ischemic stroke, i.e., emboli, atherosclerosis occluding the mouth of the perforating artery, or hypoperfusion. A small embolus could enter and occlude a perforating artery, but the available evidence suggests that cardiac or artery-to-artery embolism from carotid or middle cerebral artery atheroma is unlikely to be a frequent cause of lacunar stroke.

In carotid artery atherosclerosis the proximal internal carotid artery and the bifurcation are most frequently involved. However, the origin of the middle cerebral artery, the distal

carotid artery, and the carotid siphon may also be affected. Progression of atheromatous plaque at the carotid bifurcation results in luminal narrowing and eventual hemodynamic compromise. Ulceration frequently occurs, placing the patient at higher risk for embolization or thrombosis. Risk factors for carotid artery atherosclerosis include hypertension, diabetes mellitus, hypercholesterolemia, obesity, smoking, and a family history of stroke [119,120]. The risk of stroke from carotid disease is highest in patients who have recently sustained a reversible neurologic event, such as a transient ischemic attack. Carotid disease is usually associated with coronary and small vessel atherosclerosis too; therefore ischemic events due to artery compromise may simultaneously occur on other organs.

2.2.1.3 Pathophysiology

Ischemic stroke is an injury to the brain due to the interruption in blood flow due to embolization or atherosclerosis of the cerebral arteries. The resulting damage leads to further chemical and cellular events that lead to injury of additional areas of tissue. Aborting this process of secondary damage may be possible in the minutes to hours following the initial interruption of blood flow. The following gross changes have been described in brains with strokes:

1. Laminar necrosis: deeper levels of cortex are more affected than superficial.
2. Focal cerebral ischemia followed by encephalomalacia ending in liquification necrosis.
3. Microscopically these changes may be seen:

    Cystic spaces from the resolved liquefactive necrosis. There can be hemosiderin pigment from hemorrhage as well.

    Cerebral infarction demonstrates the presence of many macrophages which are cleaning up the lipid debris from the liquefactive necrosis.

    Cerebral infarctions can be accompanied by Wallerian degeneration of descending tracts, as shown here at high power in the brainstem.

Hemorrhagic stroke results from rupture of a vessel instead of occlusion. Cytotoxic and vasogenic edema develop in the brain parenchyma surrounding the hematoma. Macrophages resorb the blood at the periphery of the hemorrhage over weeks to months. These changes result in a cavity lined by hemosiderin-laden macrophages and surrounded by an area of necrotic tissue.

2.2.1.4 Imaging/Diagnosis

Parenchymal ischemic injury can only be detected 6 to 12 hours after the onset of symptoms and signs on standard MR and thus it seems to be unsuited for quantifying acute ischemic lesions [36]. Diffusion weighted imaging and perfusion-weighted MR imaging [38, 39, 42] with the use of contrast agents however, may allow identification of ischemic lesions earlier and permit the monitoring of the effects of therapeutic strategies

[37]. Contrast agents that cause a regional signal loss because of magnetic susceptibility–induced T2* shortening have been shown to provide a substantial contrast between ischemic and normally perfused brain areas [40,41]. In the present study, we will use superparamagnetic iron oxide particles as an intravascular contrast agent; as well as gadopentetate dimeglumine, which readily crosses the vascular wall in tissues different from brain but cannot penetrate the intact blood-brain barrier. To our knowledge, the direct comparison of an intravascular contrast agent which targets inflammatory cells, such as super paramagnetic iron oxide particles, with gadopentetate dimeglumine using MRI and MRA imaging, has not been made.

2.2.1.5 Histology of Atherosclerosis Disease of The Carotid And Vertebral System

Rupture of atherosclerotic plaque is the main cause of acute coronary syndromes and carotid territory ischemic stroke. Hemodynamic stress is important in early plaque formation and may affect the stability of mature plaques. There is some evidence that macrophage infiltration and plaque rupture tend to localize to the proximal part of the plaque where shear stress is highest. A carotid plaque can be imaged relatively easily by a variety of radiological techniques, of which intra-arterial contrast angiography is considered to be the gold standard. Angiographic plaque surface morphology has good inter- and intra-observer reliability, good pathological validity and is a strong predictor of ischemic stroke distal to carotid stenosis. It is therefore a useful model for studies of plaque stability. Data from pathological studies suggest that plaque rupture is most common in the proximal region of the plaque, where shear stress is highest [122]. More than half of the embolic events in cardiac and cerebral ischemia are caused by rupture of a vulnerable plaque (soft plaque). A vulnerable plaque can be described as a large, soft lipid pool covered by a thin fibrous cap. Plaque material composition, geometry, and inflammation caused by infiltration of macrophages are considered as major determinants for plaque rupture [123].

Preliminary results in MRI detection of macrophage-rich atherosclerotic plaques has been reported with *Combidex* [124, 125] and other iron oxide nanoparticles[127].

We hypothesize that ferumoxytol is capable of providing information regarding the presence of inflammatory cells in the plaque. The plaque will be studied both radiologically and pathologically in subjects who may undergo endarterectomy for clinical purposes; enhancement on the MRA study with a corresponding iron accumulation in the inflammatory cells of the plaque will prove the usefulness of ferumoxytol in determining soft from hard plaques.

2.2.1.6 Treatment of stroke

The treatment of acute ischemic stroke has radically changed from being merely supportive to active intervention since the development of intravenous thrombolytic therapy to restore flow to the affected area. More recently intra-arterial thrombolytic therapy and mechanical interventions for the removal of the clot as well as stenting of stenotic vessels have also been used successfully. These therapies have a short time

window of 3 to 6 hours in which they are beneficial. This time window has been determined clinically by the onset of symptoms. However many patients present after this window and do not benefit from available therapies. Some of these patients may still have salvageable tissue and this cannot be determined clinically. Great interest has been raised for developing new imaging techniques that would be able to predict which patients that are outside the current treatment window would still benefit from treatment.

Magnetic contrast agents such as the intravascular super paramagnetic iron particles or the lanthanide chelate gadopentetate dimeglumine, provide a sensitive imaging method for the early differentiation of ischemic from normally perfused brain tissue [43]. This combined with new methods for determining the extent of early cellular changes that suggest areas of irreversible damage can help delineate the extent of salvageable tissue and extend the window of intervention.

Atherosclerosis is a process that involves macrophages and T cells. Histologic evaluation has implicated macrophages to the risk of fibrous cap rupture, and are found with greater frequency in symptomatic patient specimens. A non-invasive means of identifying high-risk atherosclerotic plaque is needed. Ferumoxytol, as a macrophage imaging agent, could improve detection of “at risk” atherosclerotic plaque in the internal carotid artery and/or other blood vessels.

2.2.2 Atherosclerotic diseases of aorta and other arteries of the extremities

2.2.2.1 Histology of aortic wall diseases

Changes of the vessel wall (for example: inflammation) play a key role in the pathophysiology of aortic disease. Pathologies of the aorta (aneurysm, dissection, thrombosis, and occlusion) can be easily visualized in detail with MRA with or without contrast agents. In the ascending aorta and the aortic arch, atherosclerotic changes can also cause stroke by plaque embolization. However, intraplaque or wall inflammation cannot be visualized with gadolinium based contrasts. Because ferumoxytol is taken up by the macrophages, we may be able visualize the intraplaque and wall inflammation to predict the occurrence of complications, such as rupture [1].

We hypothesize that ferumoxytol is an appropriate contrast agent for visualizing aortic wall changes, which will allow us to better understand the causes of many different vessel pathologies.

2.2.2.2 Atherosclerosis and inflammation in the arteries of the extremities

Peripheral arterial disease (PAD) is a worldwide common disease, which can lead to severe muscle pain on walking or later even in rest and can cause tissue loss, gangrene and limb loss if left untreated. Currently 2 million people in the USA have a diagnosis of

PAD, this is projected to be as high as 2.8 million by 2020 [2, 3]. The gold standard of treatment is endovascular or surgical revascularization. Surgical repair often means performing a bypass. Traditionally these bypasses improve overall perfusion to the distal lower extremity without necessarily targeting the treatment to a specific region of the foot where an ulceration or gangrene is present, generally through a lower extremity bypass procedure. With indirect revascularization, wound healing depends upon collateral pedal arteries to deliver blood flow to the ulcers or gangrenous tissue. Pre-surgical or interventional vessel imaging plays a key role in treatment planning. Contrast-enhanced (CE-MRA) angiography with ferumoxytol-enhanced MRI has the potential to visualize even the smaller arterioles and with steady state blood volume measurements we are able to quantify tissue perfusion. Unlike the rapid extravasation of more common gadolinium-based contrast agents after IV injection, ferumoxytol is a larger molecule and remains intravascular for a longer period, which allows the acquisition of higher resolution images, necessary for mapping arterioles. With ferumoxytol steady-state MRI we will measure the local tissue blood volumes. This approach will allow us to establish an optimal treatment strategy which may lead to increased long-term patency and limb salvage.

Another notable benefit of using ferumoxytol in this context is epidemiological; patients with renal insufficiency will not be excluded [4, 5]. Chronic kidney disease (CKD) is more common in patients with PAD, the incidence ranging 29-40 % [5]. Use of conventional contrast agents, both iodinated and gadolinium based in this population, are limited by the risks for additional acute kidney injury and nephrogenic systemic fibrosis. In addition, in the case of acute renal failure, there is no need to delay imaging until dialysis therapy is initiated.

Ferumoxytol is taken up by macrophages in the perivascular space, which allows the measurement of vascular inflammation. After endovascular or surgical revascularization there is an activation of the inflammatory system, as a critical component of the early response to injury. There is a negative correlation between the level of inflammation (measured by biomarkers) after endovascular repair and the success of the revascularization (measured by patency) [6-8]. By obtaining a delayed MRI we will measure inflammation with an imaging biomarker, which has never been used before in such cases. There is no published study about how inflammation affects patency and limb salvage.

2.2.3 Deep venous thrombosis

Deep vein thrombosis (DVT) is a disease where blood thrombi develop in the deep veins of the body, most commonly in the legs. Venous thrombi cause leg edema, pain, inflammation, skin warmth or erythema overlying affected areas and can embolize to the lungs and cause life-threatening pulmonary emboli (PE). After DVT, healing occurs through a process termed DVT resolution wherein a thrombus is resorbed and the vein re-establishes blood flow and function. Impaired DVT resolution leads to post thrombotic syndrome (PTS) [9]. PTS is a sequelae of DVT where signs and symptoms of DVT persist or worsen months to years after initial DVT [10] [11]. Signs and symptoms of

PTS include persistent leg edema, chronic pain and dramatic skin changes including skin pigmentation, pruritus, cellulitis, dermatitis and ulcers. These manifestations of PTS can cause long-term morbidity and disability for Veterans and active military personnel [12].

After acute DVT, DVT resolution and vein wall healing are mediated by leukocytes and associated chemokines, plasminogen activators, metalloproteinases (MMPs) and pro-inflammatory cytokines. The initial phases of DVT resolution elicits innate immune reactions characterized by neutrophil and monocyte influx and then subsequently thrombus resolution and fibrosis. Fibrinogen and its degradation products are abundant in thrombi and stimulate recruitment and activation of leukocytes [13] [14]. We and others have shown that DVT resolution involves influx of neutrophils (day 4) and monocytes (day 8-12) that release uPa and MMP [15] [14] [16]. Reduction of peripheral blood mononuclear cells (including neutrophils and monocytes) impaired DVT resolution in rats, while injection of macrophages (activated monocytes) resulted in accelerated DVT resolution and smaller venous thrombi in rats [17].

Ferumoxytol-enhanced MRI (Fe-MRI) has the potential to accurately and reliability diagnose the severity and progression of PTS, due to its ability to characterize macrophage activity, and post-thrombotic inflammatory responses in thrombi and vein walls. Fe-MRI will be evaluated as a novel imaging modality to diagnose subjects with acute leg DVT and characterize the anatomical and physiological changes in the thrombi and vein walls associated with PTS.

2.3 Cervical lymphadenopathy

Lymph node size is one of the most frequently used criteria for discriminating metastatic from nonmetastatic (benign inflammatory, reactive) nodes in the neck [128-130]. Furthermore, in CT and sonography, internal architectural evaluation was found to be beneficial for the detection of metastatic nodes [131]. In MR imaging, the use of conventional imaging sequences does not seem to significantly exceed that of CT [132]. However, the use of specific techniques, such as diffusion- weighted imaging [133] and magnetization transfer imaging [134], may improve the performance of MR imaging in discriminating metastatic nodes in the neck. Using superparamagnetic iron oxide nanoparticles as contrast agent for lymph node imaging is very promising, it has been tested earlier using ferumoxtran-10 [135].It allows differentiation between reactive cells (accumulating the nanoparticles intracellularly) and metastatic cells (showing no intracellular enhancement), providing a allowing noninvasively detect inflammatory vs. metastatic lymphadenopathy. For this reason the study will enroll subjects with enlarged cervical lymph nodes unknown etiology.

2.4 Iron oxide nanoparticle contrast agents – Ferumoxytol

Ultra-small superparamagnetic iron oxide (USPIO) monocrystalline nanoparticles were developed as MR imaging agents for the reticulo-endothelial system [35, 49]. They have also shown excellent potential for brain tumor imaging [50-52] since brain tumors often have an abnormal BBB (46, 47). These agents include the laboratory drug MION, and from the Advanced Magnetics Inc drug, ferumoxytol, which has a carbohydrate coating.

These virus sized particles (MION, and ferumoxytol) not only serve as contrast agents for magnetic resonance imaging (MRI), but the localization of the iron particles is easily identified histologically and ultrastructurally by standard and electron microscopy [53, 54, 55]. This clinical study will investigate the ferumoxytol in subjects where improved imaging of CNS lesions or atherosclerotic plaques in blood vessels may be a critical step in improving patient management.

Ferumoxytol is an iron oxide monocrystalline nanoparticle coated with a semi-synthetic modified carbohydrate with low molecular weight and formulated with mannitol. The modified carbohydrate design makes ferumoxytol a well-tolerated substance for bolus injections or fast infusions. This type of USPIO did not show significant adverse reactions, even using extremely high doses in preclinical trials with animals and *in vitro* using human blood cells. In clinical studies, serious hypersensitivity reactions were reported in 0.2% (3/1,726) of subjects receiving Feraheme® (the trade name of ferumoxytol, FDA approved in 2009 for iron replacement in patients with chronic kidney failure)

2.5 Preclinical USPIO Imaging Studies

Pilot experiments were performed to investigate the in vivo imaging characteristics of ischemic blood-brain barrier (BBB) breakdown using high molecular weight (HMW) ultrasmall superparamagnetic iron oxide particles (ferumoxtran-10, *Combidex* by Advanced Magnetics) in comparison to the standard low molecular weight (LMW) gadolinium in a rat model of stroke. Ischemic lesions were induced in Long-Evans rats (n=33) using the standard endovascular occlusion technique allowing temporary (30`, 60` and 120`) occlusion and subsequent reperfusion of the middle cerebral artery. The following day, the animals underwent contrast enhanced MRI using SE T1, GRET2* sequences in a 1.5T unit. Ferumoxtran-10 (10mg/kg) was administered intravenously at two time points: immediately or 6h after the reperfusion in order to test the penetration of HMW particles in the early and later phase of the ischemic BBB opening. High dose gadolinium (3mmol/kg) was administered intravenously the standard way, 5 minutes prior to MRI at 20-22h after reperfusion. Animals were sacrificed after MRI and ischemic lesions were confirmed with TTC staining.

Ultrasmall superparamagnetic iron oxide particles cause increased T1 and decreased GRET2* signals and therefore can be used as MR contrast agents. Our studies showed that HMW iron particles could penetrate the parenchyma through the ischemic BBB and could be detected in the basal ganglia at 24 hr by both MRI and histology. The extent of signal changes correlated with the occlusion time. More signal changes were detected

when ferumoxtran-10 was administered immediately after reperfusion than after 6h, suggesting transient penetration of HMW molecules at the early phase of BBB opening. Iron oxide particles given at reperfusion provided different, and a more intense pattern of enhancement in comparison to high dose gadolinium, given just before MRI. Our experiments suggest that iron oxide particles have significant potential in the in vivo imaging of ischemic BBB changes. (Bago, unpublished data 2003)

2.6 Safety and Preliminary Studies with Ferumoxytol

Ferumoxytol has provided improved imaging with minimal safety concerns in pre-clinical studies and IND sponsored studies. Ferumoxytol has few systemic reactions and can be given as a bolus for MRA analysis of vasculature. This agent did not show significant preclinical toxicity even using extremely high doses in preclinical trials with animals and *in vitro* using human blood cells.

2.6.1 Preclinical studies

Three animal studies evaluated the potential cardiovascular effects of ferumoxytol. Ferumoxytol and the vehicle control material, mannitol, were administered in escalating doses of 4, 40, and 200 mg Fe/kg intravenously to male and female beagle dogs. Each animal received all three doses. No effect on cardiovascular and respiratory parameters and no cardiac electrocardiographic abnormalities were detected. (Ferumoxytol Investigator's Drug Brochure, 2005). Additionally, there was no effect on arterial blood pressure, heart rate, femoral artery blood flow, left ventricular function, or respiratory function. No treatment related electrocardiographic abnormalities were found. There was an increase in urinary flow rate with both the vehicle control and ferumoxytol, this increase in diuresis was considered attributable to the mannitol. (Ferumoxytol Investigator's Drug Brochure, 2005). In a hemodynamic study in anesthetized rats, ferumoxytol caused no change in blood pressure parameters (mean arterial, systolic, diastolic, and pulse pressures) in three animals following intravenous administration of 120 mg Fe/kg. In a third hemodynamic study, guinea pigs received i.v. ferumoxytol at a dose level of 120 mg Fe/kg or a saline control (n = 9). One animal had a reduction in mean arterial blood pressure graded as mild. One animal appeared to be unstable post injection with fluctuating blood pressure over a period of 60 minutes. There were no hemodynamic changes in the other seven animals administered ferumoxytol or in two animals administered saline. A full panel of toxicity studies has been performed, which include: evaluation of acute and repeat dose administration, mutagenicity, reproduction, and immunotoxicity. Additional information can be found in the investigator's brochure. (Ferumoxytol Investigator's Drug Brochure, 2005).

Non-clinical toxicology-pathology studies are described in the investigator's brochure and include the following:

1. Single dose studies in small and large animals
2. Repeat dose studies in small and large animals

3. Special studies: acute intravenous irritation study in rabbits, compatibility with human erythrocytes, compatibility with human plasma or serum, effect on *in vitro* clotting times, effect on coagulation in rat plasma, effect on rat paw edema, evaluation of the formation of immune complexes with Dextran Reactive Antibodies
4. Genotoxicity studies: mutagenic activity, ability to induce chromosomal aberrations, in vivo clastogenic activity and/or disruption of the miotic apparatus
5. Maternal and developmental toxicity in small animals

2.6.2 Clinical studies of Ferumoxytol

2.6.2.1 On June 20, 2009, the FDA approved ferumoxytol (Feraheme™) to treat Iron Deficiency Anemia and Adult Chronic Kidney Disease Patients. The recommended dose is an initial 510 mg IV injection followed by a second 510 mg IV injection three to eight days later. Ferumoxytol is now commercially available in the U.S.A. Ferumoxytol has provided improved imaging with minimal safety concerns even using extremely high doses in preclinical trials with animals, in vitro using human blood cells, and in multiple IND sponsored studies.From the Investigational brochure 2009

The ferumoxytol clinical development program has been comprised of eleven studies. These included three Phase 1 studies (two dose-escalation studies and a study of the electrocardiographic/QTC effects of ferumoxytol), two Phase 2 studies that evaluated the safety and efficacy of ferumoxytol for iron replacement in patients with Chronic Kidney Disease (CKD), and two Phase 2 imaging studies that assessed the feasibility of ferumoxytol as an MRI contrast agent. There were three open-label Phase 3 studies that examined the efficacy and safety of ferumoxytol relative to oral iron in patients with CKD Stages 1-5 either on or not on dialysis. A fourth Phase 3 study in patients with any stage of CKD evaluated the safety of ferumoxytol relative to placebo in a cross-over, double-blind manner. Ferumoxytol has been evaluated at exposures up to 2 x 510 mg, including two courses of 2 x 510 mg in three of the four Phase 3 studies. Specifically, the following regimens of ferumoxytol were given during the clinical program:

1. ≤4 mg Fe/kg
2. 1 x 125 mg
3. 1 x 250 mg
4. 1 x 510 mg
5. 8 x 128 mg
6. 4 x 255 mg
7. 2 x 510 mg

Approximately 1,740 patients have been exposed to ferumoxytol in the entire clinical program, and approximately 1,509 patients have been exposed to ferumoxytol in the Phase 3 studies. To date, only one patient (0.06% of all patients exposed to ferumoxytol) has experienced an anaphylacoid reaction following treatment with ferumoxytol; this patient had a history of multiple drug allergies and experienced an anaphylactoid

reaction (hot flashes and itching, with no respiratory compromise) and severe hypotension a few minutes after receiving ferumoxytol. There have been no deaths that were considered to be related to ferumoxytol treatment. There were 28 deaths in the clinical program, all of which have occurred in Phase 3 studies, with 16 deaths among the 1,740 patients exposed to ferumoxytol (0.9%) and 8 deaths among the 296 patients exposed to oral iron (2.7%). Four deaths occurred in patients who had signed an informed consent for enrollment but did not receive any test article. All deaths in the clinical program have been in patients with CKD, who have a high risk of death due to cardiovascular disease and other causes. In completed studies that used oral iron as a comparator, there was a lower rate of SAEs among patients exposed to ferumoxytol than among patients exposed to oral iron: 4.4% of ferumoxytol treated patients (64 of 1,451 patients) vs. 10.7% of oral iron-treated patients (17 of 159 patients). Patients exposed to placebo had the lowest rate of SAEs (1.7%; 13 of 780 patients). One patient each following ferumoxytol (0.1%), oral iron (0.6%), and placebo (0.1%) experienced an SAE that was considered by the investigator to be related to treatment. In Phase 3 Study 62,745-5, which enrolled CKD patients undergoing hemodialysis, 31 of 199 (15.6%) patients experienced an SAE following ferumoxytol administration and 8 of 70 (11.4%) patients experienced an SAE following oral iron administration. These data were taken from an unlocked database and therefore are preliminary and patient to final verification. In the Phase 3 studies in patients with CKD (62,745-5, 62,745-6, 62,745-7, and 62,745-8, in which approximately 1,509 patients were exposed to ferumoxytol), the most common adverse events following ferumoxytol administration included nausea, diarrhea, dizziness, headache, and peripheral edema. These adverse events were usually more common in the oral iron group than the ferumoxytol group (in Studies 62,745-5, 62,745-6, and 62,745-7, which utilized oral iron as a comparator). Hypotension was one of the more common adverse events in Study 62,745-7 and occurred more frequently in the ferumoxytol group (5.0%) than the oral iron group (1.4%). By contrast, preliminary data show that there have been lower rates of hypotension in Study 62,745-5 (1.8%, 1.7%, and 0% in the ferumoxytol 2x510 mg, ferumoxytol 4x255 mg, and oral iron groups, respectively); hypotension rates were similar between the ferumoxytol and placebo groups (1.3% vs. 0.8%) in the large safety study, Study 62,745-8; and there were no hypotension adverse events in Study 62,745-6.

An independent Data Monitoring Committee met to review the clinical safety data for all Phase 3 studies in October 2005, February 2006, June 2006, October 2006, and March 2007, and they identified no safety concerns.

The dose of 510mg ferumoxytol has not been used in imaging studies before. The highest dose used was 4mg/kg (280mg for a 70kg patient). However 2 x 510mg dose was studied for iron replacement therapy in large number of patients without significant adverse effects. (Besarab et al 2007). If there is no history of iron over load, there is no need to screen for iron over load since 0.5 g will not hurt patients, even if they have asymptomatic iron over load. Phase 3 data by Besarab using two doses of 510 mg in a phase 3 trial showed no significant toxicity. A higher dose of ferumoxytol will result in a better contrast enhancement 24 hours after injection due to a higher plasma level.

2.6.2.2 Post-Marketing Program

Investigator's Brochure Released on February 15, 2018:

Since approval, AMAG has conducted two post-marketing clinical trials (Protocol Number FER-CKD-201 and AMAG-FER-CKD-401). FER-CKD-201 was a randomized, open-label trial that compared the safety and efficacy of ferumoxytol to iron sucrose for the treatment of IDA in CKD subjects either on or not on dialysis. In this trial, 162 patients were randomized in a 1:1 ratio to either ferumoxytol or iron sucrose. Ferumoxytol was administered as a 1.02 g course given as a regimen of 2 x 510 mg within 2 to 8 (5±3) days. The most common AEs among ferumoxytol treated subjects were nausea (7.5%) and muscle spasms (5.0%). Adverse events occurring in ≥2.0% of subjects treated with ferumoxytol included: nasopharyngitis, URI, headache, hyperkalemia, and cough (3.8%); peripheral edema, constipation, diarrhea, hypotension, hypoglycemia, and anemia (2.5%). There was only one ferumoxytol related SAE in the FER-CKD-201 study, 1 event of anaphylactic reaction in 1 subject occurred on the same day as the subject's first dose of ferumoxytol.

The AMAG-FER-CKD-401 study was an international Phase IV, randomized, open-label, active controlled multicenter trial of the safety and efficacy of ferumoxytol (2x510 mg) compared with IV iron sucrose (10x100 mg) in the treatment of iron deficiency anemia in 293 patients with CKD on hemodialysis. Overall related AEs, related SAEs and protocol-defined AEs of special interest were reported 4.4%, 0% and 17.4%.

Imaging Program:

The potential use of ferumoxytol as an MRI contrast agent was evaluated in open-label feasibility studies, including one Phase I study (Protocol Number 7228-01) in healthy volunteers and two Phase II studies (Protocol Numbers 58,254-2 and 58,254-5) in subjects undergoing a diagnostic imaging procedure. In the combined studies, 70 imaging subjects were exposed to a single administration of ≤4 mg Fe/kg ferumoxytol. Results from these studies demonstrated that ferumoxytol was useful in visualizing the arterial circulation. Ferumoxytol has a long blood half-life, unlike the majority of other MRI contrast agents, enabling arterial and venous imaging for a period of several hours following injection.

March of 2015 FDA Black Box Warning:In March of 2015 the FDA placed a black box warning on ferumoxytol stating:

WARNING: RISK FOR SERIOUS HYPERSENSITIVITY/ANAPHYLAXIS REACTIONS

Fatal and serious hypersensitivity reactions including anaphylaxis have occurred in patients receiving Feraheme. Initial symptoms may include hypotension, syncope, unresponsiveness, cardiac/cardiorespiratory arrest.

•Only administer Feraheme when personnel and therapies are immediately available for the treatment of anaphylaxis and other hypersensitivity reactions.

•Observe for signs or symptoms of hypersensitivity reactions during and for at least 30 minutes following Feraheme infusion including monitoring of blood pressure and pulse during and after Feraheme administration.

•Hypersensitivity reactions have occurred in patients in whom a previous Feraheme dose was tolerated.

In the black box warning the FDA updated their recommendations of how ferumoxytol should be given. The option to give ferumoxytol (510mg) as a fast undiluted injection in approximately 1 minute has been removed. It was recommended that ferumoxytol (510mg) be given over at least 15 minutes. To accommodate for this, the dose of ferumoxytol will be given in three separate, fractionated doses. The rate of administration of the second and third doses will be slowed down to no faster than 1 mL/s.

Post-Marketing Safety

AMAG's pharmacovigilance system proactively reviews spontaneous reports. Routine surveillance of events is performed daily and monthly signal detection and evaluation processes monitor and update the safety profile. To date the information received from the post-marketing setting is consistent with the known safety profile of ferumoxytol.

As shown in Table 1, cumulative postmarketing reporting rates for serious AESIs (adverse events of special interest) with ferumoxytol since product approval (30 June 2009) have remained low. These event reporting rates are rare and very rare using CIOMS standardized assessment, and no new safety trends or signals have been identified. The reporting rates for all AEs remained low and declined over time. In addition, the reporting rates for all serious events including HSRs have declined following the change in the prescribing information to administration with infusion instead of rapid injection (March 2015). Overall, there is no new safety signal arising from the review of the anaphylactic reactions/shock and hypersensitivity reaction events. The risk of anaphylaxis/hypersensitivity linked to iron agents is well recognized and the need for special caution when administrating ferumoxytol is adequately addressed in the US Prescribing Information. The available information from these events does not change the safety profile of ferumoxytol.

Table 1

Reporting Rates of Serious AESIs by Ferumoxytol Administration

AESI	Rapid Injection (2009-31 Mar 2015) Estimated Exposure: 1,216,518		Diluted infusion (1 Apr 2015-31 Mar 2017) Estimated Exposure: 556,117	
	No of Cases	Reporting Rate (Estimated)	No of Cases	Reporting Rate (Estimated)

Anapyhlaxis	95	0.0078	16	0.0029
Hypersensitivity (severe)	73	0.0060	5	0.00089
Cardiac disorders	37[1]	0.0030	4	0.00071
Hypotension (serious)	72	0.0059	2	0.00035
Syncope, loss of consciousness, unresponsive	44	0.0036	4[2]	0.00071
Fatal	48[1]	0.0040	4[3]	0.00071

[1]Includes cases received in April 2015 with event onset in March 2015 (AMAG201500330).
[2]Follow-up received on AEOI reported in Q2, per HCP due to underlying disease (epilepsy) and not ferumoxytol (AMAG201600940).
[3]Case AMAG201602875: As per the treating physician, the death is not related to ferumoxytol; Case AMAG201601453: Death due to metastasis of neoplasm after 6 months of discontinuation of ferumoxytol therapy.

Based on communication with AMAG Pharmaceuticals, manufacturer of ferumoxytol, all recommendations apply to iron replacement therapy where infusion rate and dilution do not impact efficacy. However, for an imaging indication certain infusion parameters are required to gain information, such as dynamic imaging. Increased infusion rate will only affect the first injection of 1 mg/kg, which is a small fraction of the full therapeutic dose, and therefore may minimally increase the risk of adverse reactions. The next two injections are given at a slower rate. The recommended 15 minutes has no scientific basis, it has been chosen arbitrarily. For MR imaging stopping the scanner for 15 minutes would be disadvantageous. Applying multiple injections, which is not the case with iron replacement therapy, may increase safety, compared to continuous injection. This may contribute to the fact that in our patient population only minor adverse reactions have been reported.

OHSU Toxicity Data

As of December 31st, 2015, a total of 671 infusions of ferumoxytol had been completed on 331 study subjects as part of OHSU's Blood Brain Barrier sponsored IRB approved protocols. The dose has ranged from 75 to 510 mg of ferumoxytol (0.5 to 11 mg/kg). In all these cases, ferumoxytol was given during MRI, using multiple IV bolus injections (1:1 diluted ferumoxytol, 3ml/s flow rate, with 20ml saline flush at the same flow rate.) In most studies the first 1mg/kg (or 75mg) was used for dynamic perfusion imaging; the remaining dose was injected in one or two subsequent bolus injections. The full 510mg was never given as a single injection. Within 42 days after ferumoxytol administration, there were 85 adverse events possibly, probably or definitely related to ferumoxytol. Among the 85 events, 43 patients had 1 event, 12 had 2 events, 3 had three events, one had 4 events and one had five events. 80% of the toxicity events occurred within 48 hours post ferumoxytol administration. There were no severe, grade 4 or 5 toxicities (CTCAE version 3 and 4) that were attributed to ferumoxytol. Table 2 shows the toxicities up to and beyond 48 hours. (Data has been published in AJNR in July of 2017).

	Overall (<42 days)		Within 48 hours		>48 hours	
Toxicity Category Attribute	**# of events**	**Freq of events**	**# of events**	**Freq of events**	**# of events**	**Freq of events**
Gastrointestinal	31	4.62%	23	3.43%	8	1.19%
Cardiac General	17	2.53%	17	2.53%	0	0.00%
Pain	17	2.53%	17	2.53%	0	0.00%
Dermatology/Skin	7	1.04%	5	0.75%	2	0.30%
Metabolic/Lab	5	0.75%	0	0.00%	5	0.75%
Occular/Visual	2	0.30%	1	0.15%	1	0.15%
Allergy/Immunology	1	0.15%	1	0.15%	0	0.00%
Constitutional	1	0.15%	1	0.15%	0	0.00%
Hemorrhage/Bleeding	1	0.15%	0	0.00%	1	0.15%
Other	1	0.15%	1	0.15%	0	0.00%
Pulmonary/Upper Resp	1	0.15%	1	0.15%	0	0.00%
Vascular	1	0.15%	1	0.15%	0	0.00%
Total	*85*	*12.67%*	*68*	*10.13%*	*17*	*2.53%*

Table 2. Toxicities possibly, probably or definitely related to ferumoxytol administration for MR imaging. Data includes 671 administrations at OHSU between 2004 and Dec 31st 2015.

2.7 Future Directions

Neuroprotection is one if not the most important topic in stroke matters. In an acute ischemic event, damage can extend beyond to areas neighboring the hypoxic tissue leading to a more extensive injury; hypoxia and hypoperfusion generates a series of abnormal metabolic events at different points within the neuron. As a consequence, there is a massive liberation of free radicals that will not only add more damage to the cells and will create a vasoactive effect.

Preclinical studies in rats have shown neuroprotective properties of n-acetylcysteine (NAC) in ischemic models [37b]. NAC was previously shown to be protective in several animal models of reperfusion injury, even in cerebral ischemia. The exact mechanism of action of NAC mediated neuroprotection is unknown. NAC is reported to have an intrinsic scavenging effect through direct reaction with hydroxyl radicals. In addition, NAC was shown to inhibit nitric oxide (NO) formation, which is thought to be due to the inhibition on nitric oxide synthetase [80]. NO production indirectly correlates with ischemic brain injury, since NOS inhibitors and nNOS deficient mice are reported to exhibit diminished neuronal damage after experimental stroke. We intend to use ferumoxytol and gadolinium to assess CNS inflammation and ischemia, with the use of NAC and other thiols.

3 OBJECTIVES

3.1 Primary objectives

3.1.1 Group 1: To compare ferumoxytol MR with gadolinium “gold standard” enhanced MR in adults with dural, parenchymal based, demyelinating or vascular CNS disease. This will involve imaging of the brain or the spine. Optimum sequencing parameters of ferumoxytol MRI will be established.

3.1.2 Group 2: To compare ferumoxytol MR with gadolinium enhanced MR in adults with any of the following (alone or in combination): vasculopathy of carotids, aorta, arteries of the extremities; or thrombosis of intraabdominal, pelvic or extremity veins. This will include carotid and/or aortic and/or extremity MRA or MRV as well as high resolution carotid plaque and vessel wall imaging and analysis. Optimum sequencing parameters of ferumoxytol MRI will be established.

3.1.3 Group 3: To compare ferumoxytol MR with gadolinium enhanced MR in adults with cervical lymphadenopathy, to identify imaging characteristics of the various etiologies (e.g. reactive vs. metastatic lymph nodes). Optimum sequencing parameters of ferumoxytol MRI will be established.

3.1.4 To assess the utility of ferumoxytol in subjects with renal disease, or with an allergy to gadolinium, who should not receive gadolinium-based contrast agents: this includes those with a chronic GFR < 50 ml/min/1.73m2, acute kidney injury, and prior renal or liver transplant.

3.2 Secondary Objectives

3.2.1. This study will also provide preliminary, descriptive data for the following secondary objective: To localize ferumoxytol particles with histology and electron microscopy in biopsy samples from a small subset of subjects in which biopsy is needed. Results will also be compared to a companion study of brain tumor imaging entitled “NCI-Sponsored multi-disciplinary study for MR imaging of intravenous superparamagnetic crystalline particle ferumoxytol in primary high-grade brain tumors and/or cerebral metastases

3.2.2. To assess side effects/safety of ferumoxytol when given during MRI.

3.2.3 To assess the time course of iron uptake and clearance (of the first ferumoxytol injection) in abdominal organs, such as the liver, spleen, pancreas and bone marrow by applying usual abdominal MR sequences at multiple time points (up to 6 months) after ferumoxytol injection in a subgroup of subjects. For this purpose a subgroup of 50 subjects will be recruited from primary groups 1, 2 and 3.

4 TRIAL DESIGN

4.1 Inclusion Criteria

4.1.1 Group specific inclusion criteria:

Group1: Subjects must have a radiological or histological diagnosis of dural or central nervous system (CNS) parenchymal based inflammatory, vascular or demyelinating lesions. This includes but is not limited to unknown lesions, meningioma, neuroma, stroke, multiple sclerosis (MS) or related diseases such as acute disseminated encephalomyelitis (ADEM). Subjects with a CNS inflammatory lesion that is suspicious for neoplasm or radiation induced inflammation (vasculitis) will also be included.

Group2: Subjects must have radiological suspected diagnosis of vascular CNS lesions such as ischemic stroke, TIA with suspected carotid embolic origin, or vasculopathy involving the carotids (including diagnosed carotid stenosis >50%), the aorta, the arteries of the extremities, or diagnosed thrombosis of the intraabdominal, pelvic or extremity veins.

Group3: subjects must have clinical or radiological diagnosis of enlarged cervical lymph nodes in which inflammatory processes (reactive lymph nodes) is part of the differentials.

Subgroup: any subject in a primary group who agrees to participate in the abdominal imaging portion of the study.

4.1.2 Subjects must be 18 years or older for inclusion in this study.

4.1.3 Subjects must have a Karnofsky of 30% or greater.

4.1.4 Subjects agree to follow up approximately 4-6 weeks after each infusion of ferumoxytol.

4.1.5 All subjects or their authorized representative must sign a written informed consent and give HIPAA authorization in accordance with institutional guidelines.

4.1.6 Sexually active women of child-bearing potential and men must agree to use adequate contraception (hormonal or barrier method of birth control; or abstinence) at the start of study treatment and for the duration of study treatment. Should a female become pregnant or suspect she is pregnant while participating in this study, she should inform her treating physician immediately.

4.2 Exclusion criteria

4.2.1 Subjects with clinically significant signs of uncal herniation, such as acute pupillary enlargement, rapidly developing motor changes (over hours), or rapidly decreasing level of consciousness.

4.2.2 Subjects who have a contraindication for MRI: metal in their bodies (a cardiac pacemaker or other incompatible device).

4.2.3 Subjects with known allergic or hypersensitivity reactions to parenteral iron, parenteral dextran, parenteral iron-dextran, or parenteral iron-polysaccharide preparations (Investigator's Drug Brochure, Combidex 2002; Advanced Magnetics Inc; Investigator's Drug Brochure, Code 7228; 2003 Advanced Magnetics Inc).

4.2.4 Subjects with known hepatic insufficiency or cirrhosis

4.2.5 Subjects with known or suspected iron overload (genetic hemochromatosis or history of multiple transfusions)

4.2.6 Subjects with symptoms or signs of hemodynamic instability.

4.2.7 HIV-positive patients on combination antiretroviral therapy are ineligible because of the potential for pharmacokinetic interactions with ferumoxytol.

4.2.8 Pregnant or lactating women are excluded from this study because of possible risk to the fetus or infant.

4.2.9 Subjects with three or more drug allergies from separate drug classes.

4.2.10 Subgroup only exclusion criteria: prior administration of ferumoxytol.

4.3 Pre-Screen

Subjects will be initially evaluated in a variety of OHSU clinics or the inpatient setting to determine their eligibility. Possible clinics include; neuro-oncology, stroke, multiple sclerosis, cardiology, cardiothoracic, vascular surgery, hematology clinics, or inpatient services to determine possible candidacy for the study by one or more of the clinical investigators. If study participation is appropriate and the subject fulfills the eligibility criteria, they will be invited to participate.

4.4 Subject Registration

Following appropriate pre-registration evaluation, a signed consent, HIPAA authorization, and completed eligibility checklist will be obtained. New subject registrations will be submitted to the study coordinator, who will record study data on all subjects entered into the study and complete subsequent forms.

4.5 Ferumoxytol Administration: Route and Dosing

Ferumoxytol will be injected intravenously in divided doses followed by a saline flush. The maximum total dose during the day 2 MRI will be 510mg Fe. Ferumoxytol will be diluted in normal saline (1:1 or 15mg/ml) to reduce T2* effects in the MR angiography. [126] The drug will be stored at room temperature (68-77° F) away from direct sunlight. The solution will be prepared and dispensed by the investigational drug pharmacist. It will be administered in an OHSU MR suite.

For acquisition of DSC-MRI data, ferumoxytol (5 ml=75 mg) will be injected at up to 3 ml/sec followed by a saline flush. This rate is required for adequate signal change during the dynamic imaging. When no DSC-MRI data is obtained (spine and Group 3, the rate of the first dose (5 ml=75mg) will be 1 ml/sec. The remainder of the dose will be given in a second (10 ml=150 mg) and third (19 ml=285 mg) injection at a lower flow rate, not faster than 1 ml/s. There will be a short break in time between the three injections to allow for assessment per sections 4.7.5 and 5.1. *The dilution and injection parameters of the ferumoxytol may be adjusted to obtain optimal signal changes on MRI/MRA and to decrease the possibility of an allergic reaction. Injection rate can be varied based on the participant's IV site, but the total dose will never exceed 510mg.*

4.6 Study Groups

1. Group1: (n=up to 260) subjects will include those with dural, central nervous system (CNS) parenchymal based inflammatory, vascular or demyelinating lesions. This includes but is not limited to unknown lesions, meningioma, neuroma, stroke, multiple sclerosis (MS) or related diseases such as acute disseminated encephalomyelitis (ADEM). In these subjects the brain or the spine will be imaged.
2. Group2: (n=up to 25) subjects will include those with vascular CNS lesions such as ischemic stroke, TIA with suspected carotid embolic origin, or vasculopathy involving the carotids, (including diagnosed carotid stenosis >50%) the aorta, or the arteries of the extremities, or diagnosed thrombosis of the intraabdominal, pelvic or extremity veins. Carotid and/or aortic and/or extremity MRI and MRA or MRV will be performed in these subjects.
3. Group3: (n= up to 10) will include subjects with enlarged cervical lymph nodes in which inflammatory processes (reactive lymph nodes) is part of the differentials.
4. Subgroup: (n=50) will be comprised of subjects from any of the primary groups who agree to participate in the abdominal imaging portion of the study.

4.7 Study Visits, Procedures and Schedule of Events

Subject's need for anti-anxiolytics for each MRI study visit will be evaluated and administered as needed. Common anti-anxiolytics include lorazepam and diazepam; diphenhydramine may also be used. The administration of a relaxing/anti-anxiolytic agent will be given at the treating physician or the PI's discretion and is considered routine prior to MRI and as such is not being evaluated on this study.

4.7.1 Study Visit 1

4.7.1.1 Day 1 (gadolinium MRI)

The subject will have an anatomical MRI (brain &/or spine [group1], carotids and/or aorta and/or other arteries or veins [group2] or cervical lymph nodes [group3]) with and without gadolinium, which may include DWI, MRA or perfusion study. This MRI will be done

within 7 days prior to the ferumoxytol infusion and may have been done off study as medically necessary; and may or may not be repeated at PI's discretion. Laboratory testing (creatinine/GFR) to check kidney function and a pregnancy test in women of child bearing potential will be done prior to GBCA administration done on study. Subjects with renal insufficiency (GFR <50ml/min/1.73m^2) and/or contraindication to GBCA, will not undergo imaging with gadolinium, they will proceed to "Day 2". Short abdominal MR imaging as a baseline will be performed in the subgroup prior to any ferumoxytol administration and may be done either day 1, or on day 2 prior to ferumoxytol administration).

4.7.1.2 Day 2 (early ferumoxytol MRI)

The subject will undergo an anatomical MRI of the CNS/body/extremities (brain &/or spine [group1], carotids and/or aorta and/or other arteries or veins [group2] or cervical lymph nodes [group3]) with and without ferumoxtyol, which may include DWI, MRA, MRV or perfusion study. Ferumoxytol will be administered intravenously, not exceeding the total dose of 510mg; see sections 4.5 and 5.1. Short abdominal MR imaging will be performed in the subgroup.

4.7.1.3 Day 3 (late ferumoxytol MRI)

Group1: Approximately 24 hours after the ferumoxytol infusion, the subject will have a post- ferumoxytol MRI brain and/or spine, Gadolinium MRI may be repeated at the investigator's discretion based on subject's suspected or known diagnosis. Subjects with renal insufficiency (GFR <50ml/min/1.73m^2) and/or contraindication to GBCA, will not receive Gadolinium. Group2 and 3 subjects will undergo MRI of the carotids, aorta or other arteries or veins or cervical lymph nodes respectively approximately 48h after ferumoxytol injection. Short abdominal MR imaging will be performed in the subgroup (24-48 hours after ferumoxytol administration).

4.7.1.4 Day 4 (2nd late ferumoxytol MRI) (at physician's discretion for groups 2 and 3)

Subjects in groups 2 and 3 who undergo vessel imaging or cervical lymph node imaging will repeat the post ferumoxytol MRI approximately 1 week (6-8 days) after the ferumoxytol infusion, at the physician's discretion. Any subject in the abdominal imaging subgroup from groups 1, 2 & 3 will undergo short abdominal MR imaging 1 week after ferumoxytol administration.

4.7.1.5 Body Imaging (to assess for body imaging clearance)

Subgroup subjects will undergo short abdominal MR imaging 1, 2 ,3 and 6 months after ferumoxytol infusion to test long term ferumoxytol uptake and clearance from the body. Once no ferumoxytol is detected on the abdominal MR images, no further scanning will be done. (i.e. if ferumoxytol is detected at 1 month, 3 month abdominal scans will be performed. If no ferumoxytol is detected at 3 months, the 6 month scans will not be performed). During this time, subjects that receive additional ferumoxytol will be removed from the subgroup portion of the study If subject comfort or logisitics do not allow for the completion of all post-ferumoxytol abdominal MRI timepoints, it will not exclude the patient from subsequent abdominal scans.

4.7.2 Histology

When indicated, tissue samples will be obtained in those subjects requiring surgery, which may occur up to 4 weeks after the ferumoxytol administration. The MR localization and histology will be compared. Only tissue that is routinely obtained will be used for this study.

4.7.3 Study Duration

Study duration is 2 years. If there are no adverse events, the already enrolled subjects may repeat the study visit (days 1, 2, 3 and 4). This may be repeated at least 30 days apart, for up to a total of 4 study visits. Laboratory testing (creatinine/GFR) to check kidney function (prior to GBCA on study) and a pregnancy test in women of child bearing potential will be done at each subsequent visit.

4.7.4 Follow up

To assess for adverse events, the subject will be seen in an OHSU outpatient clinic or a telephone interview will be conducted approximately 4-6 weeks after the study visit..

4.7.5 Data Collection

All subjects will be registered and sign a consent form which includes HIPAA authorization prior to receiving ferumoxytol.

Data, such as patient demographic information, survival times may be also collected.

In addition to the imaging data the following data collection will occur when the ferumoxytol is administered:

- Blood pressure and heart rate will be recorded on day 2 of each study visit
    - Before the first ferumoxytol infusion
    - within 10 minutes after each ferumoxytol injection
    - 30 (±5) minutes after the final ferumoxytol infusion.
- Monitoring for side effects including allergic reactions will be done during the day 2 ferumoxytol MRI session.
    - After the final ferumoxytol infusion, the subject will be monitored for adverse events for 30(±5) minutes and discharged if stable.
- Height, weight and lab values of the subjects will also be recorded.

Adverse events related to or potentially related to the administration of ferumoxytol will be recorded; adverse events related to the MRI's experience itself, such as claustrophobia/anxiety, nausea with GBCA injection, or discomfort due to positioning, is not being studied and will not be reported. The adverse events that will be monitored for during the infusion include localized discomfort at the IV injection site, pain, respiratory difficulties, flushing, dizziness, pruritis/rash, and any other symptoms that could be secondary to an anaphylactoid type reaction.

All data points will be flexible as subject safety while in the magnet is first priority. In addition to monitoring the above-mentioned safety parameters, an emergency code cart is located in the MR suite and can be utilized by the MR team, as well as accessed by the code team at the institution should an emergent situation arise.

4.8 Duration and Follow-up Care

Study duration is up to 2 years. Subjects will be followed for 4-6 weeks after each ferumoxytol administration. See section 4.7.4.

4.9 Evaluation of Organ Uptake and Clearance of Ferumoxytol

This additional part of the study will evaluate the time needed after ferumoxytol administration before the MR signal in certain organs including the liver, spleen, pancreas and bone marrow returns to a level which allows clinically satisfactory MR imaging of these organs. Various MR sequences will be tested to determine which is the most applicable to reduce confounding effect of prior ferumoxytol administration. Abdominal MR scans will be performed in 50 subjects before and at multiple time points after the first ferumoxytol administration. These subjects will be a subgroup from groups; 1, 2 and 3. The time points will be: before, shortly after, 24-48 h after, 1 week after , 1, 2, 3 and 6 months after ferumoxytol administration. Once signal changes are no longer present, subsequent imaging will not be necessary. The sequences will be short, without full anatomical coverage.

If a subject does not qualify for the abdominal imaging (because of previous iron injection) or chooses not to participate in the abdominal imaging, the subject can still be enrolled in and complete the primary group imaging sessions.

The MRI sequences are detailed in section 5.1.

5 MRI SEQUENCES, CRITERIA FOR EVALUATION, AND DISCIPLINE REVIEWS

5.1 MRI Sequences

For all subject groups images will be acquired on a 3.0 Tesla magnetic field strength scanner.

5.1.1 Brain MRI (Group1):

Subjects should be positioned according to the following protocol. Subjects are allowed to adopt a comfortable position within the head coil before being advanced within the magnet bore. Three slices are acquired in the axial plane by fast spin echo. A coronal slice is prescribed graphically from one of the axial slices showing the falx cerebri. The line cursor is then angled along the midsagittal plane and then rotated 90 degrees. One slice is acquired in this plane. This corrects for right left rotation. A sagittal oblique slice is then prescribed from the coronal oblique. The cursor is angled along the midsagittal plane and one slice is acquired in this plane by spin echo. This corrects for right left cant. From this last image contiguous interleaved slices are prescribed to cover the brain. The

line cursor is angled to pass along the anterior commissure-posterior commissure line (AC-PC line). The localizing scans are saved for reference.

Standard gadolinium contrast MRI will be performed with the following sequences using a SENSE (parallel image) head coil or equivalent. Subjects with renal insufficiency (GFR <50ml/min/1.73m2) or other GBCA contraindication will not undergo an MR with Gadolinium

5.1.1.1 Day 1

Scan type	Scan Time (min)
0. Setup/localizer/B_0 shimming/B_1 mapping	2
1. T1-w sagittal FFE (TR300NEX 4) 180mm x 240mm FOV, 192 x 256 matrix, 3 mm contiguous, interleaved	3
2. FLAIR coronal SE/IR (TE109/TR9140/TI2500) 180mm x 240mm FOV, 192 x 256 matrix, 5mm slice thickness, 0.5mm sp	3
3. T1-w axial SE (TE14/TR700 NEX 4) 180mm x 240mm FOV, 192 x 256 matrix, 3 mm contiguous, interleaved	4
4. T2-w axial TSE (TE72/TR9000) 180mm x 240mm FOV, 192 x 256 matrix, 3 mm contiguous, interleaved	2
5. T1-w MPRAGE sagittal 3D TFE (TE3.8/TR8.2/FA8) 230mm x 230mm FOV, 230 x 230 matrix, 1 mm contiguous, interleaved	6
6. Diffusion weighted MRI axial (TE90/TR6000 SE EPI b=0, 500, 1000 s/mm^2) 220mm x 220mm FOV, 128 x 128 matrix, 3 mm contiguous, interleaved	4
7. MRA 3D-TOF (TE3.2/TR18/FA20) 170mm x 200mm x 150 mm FOV 200 x 400 x 140 matrix	5
8. SWI (TE20/TR26/FA15) 198mm x 210mm x 127 mm FOV 248 x 300 x 116 matrix	4
9. PWI T_2*-w axial; GEPI (TE25/TR1500/FA30) x 100 volumes 220mm x 220mm FOV 128 x 128 matrix, 3 mm contiguous, interleaved Gadoteridol bolus injection 0.1mmol/kg, 3ml/s flow rate.	2
10. T1-w sagittal FFE (TR300NEX 4) 180mm x 240mm FOV, 192 x 256 matrix, 3 mm contiguous, interleaved	3
11. SWI (TE20/TR26/FA15) 198mm x 210mm x 127 mm FOV 248 x 300 x 116 matrix	4
12. T2-w axial TSE (TE72/TR9000) 180mm x 240mm FOV, 192 x 256 matrix, 3 mm contiguous, interleaved	2
13. T1-w axial SE (TE14/TR700 NEX 4) 180mm x 240mm FOV, 192 x 256 matrix, 3 mm contiguous, interleaved	4
14. T1-w MPRAGE sagittal 3D TFE (TE3.8/TR8.2/FA8) 230mm x 230mm FOV, 230 x 230 matrix, 1 mm contiguous, interleaved	6

5.1.1.2 Day 2

Scan type	Scan Time (min)
0. Setup/localizer/B0 shimming/B1 mapping	2
1. T1-w sagittal FFE (TR300NEX 4) 180mm x 240mm FOV, 192 x 256 matrix, 3 mm contiguous, interleaved	3
2. FLAIR coronal SE/IR (TE109/TR9140/TI2500) 180mm x 240mm FOV, 192 x 256 matrix, 5mm slice thickness, 0.5mm sp	3
3. T1-w axial SE (TE14/TR700 NEX 4) 180mm x 240mm FOV, 192 x 256 matrix, 3 mm contiguous, interleaved	4
4. T2-w axial TSE (TE72/TR9000) 180mm x 240mm FOV, 192 x 256 matrix, 3 mm contiguous, interleaved	2
5. T1-w MPRAGE sagittal 3D TFE (TE3.8/TR8.2/FA8) 230mm x 230mm FOV, 230 x 230 matrix, 1 mm contiguous, interleaved	6
6. Diffusion weighted MRI axial (TE90/TR6000 SE EPI b=0, 500, 1000 s/mm2) 220mm x 220mm FOV, 128 x 128 matrix, 3 mm contiguous, interleaved	4
7. MRA 3D-TOF (TE3.2/TR18/FA20) 170mm x 200mm x 150 mm FOV 200 x 400 x 140 matrix	5
8. SWI (TE20/TR26/FA15) 198mm x 210mm x 127 mm FOV 248 x 300 x 116 matrix	4
9. PWI T2*-w axial; GEPI (TE25/TR1500/FA30) x 100 volumes 220mm x 220mm FOV 128 x 128 matrix, 3 mm contiguous, interleaved **ferumoxytol bolus injection 75mg (5ml) 2 fold diluted, up to 3ml/s flow rate. ***	2
8. SWI (TE20/TR26/FA15) 198mm x 210mm x 127 mm FOV 248 x 300 x 116 matrix	4
9. PWI T2*-w axial; GEPI (TE25/TR1500/FA30) x 100 volumes 220mm x 220mm FOV 128 x 128 matrix, 3 mm contiguous, interleaved **ferumoxytol injection 150mg (10 ml) 2 fold diluted, at 1ml/s flow rate. ***	2
8. SWI (TE20/TR26/FA15) 198mm x 210mm x 127 mm FOV 248 x 300 x 116 matrix	4
9. PWI T2*-w axial; GEPI (TE25/TR1500/FA30) x 100 volumes 220mm x 220mm FOV 128 x 128 matrix, 3 mm contiguous, interleaved **ferumoxytol injection 285mg (19ml) 2 fold diluted, at 1ml/s flow rate. ***	2
10. T1-w sagittal FFE (TR300NEX 4) 180mm x 240mm FOV, 192 x 256 matrix, 3 mm contiguous, interleaved	3
11. SWI (TE20/TR26/FA15) 198mm x 210mm x 127 mm FOV 248 x 300 x 116 matrix	4
12. T2-w axial TSE (TE72/TR9000) 180mm x 240mm FOV, 192 x 256 matrix, 3 mm contiguous, interleaved	2
13. T1-w axial SE (TE14/TR700 NEX 4) 180mm x 240mm FOV, 192 x 256 matrix, 3 mm contiguous, interleaved	4
14. T1-w MPRAGE sagittal 3D TFE (TE3.8/TR8.2/FA8) 230mm x 230mm FOV, 230 x 230 matrix, 1 mm contiguous, interleaved	6

** BP and heart rate check prior to ferumoxytol and after each ferumoxytol injection and 30 minutes after the final ferumoxytol injection*

5.1.1.3 Day 3

Scan type	Scan Time (min)
0. Setup/localizer/B0 shimming/B1 mapping	2
1. T1-w sagittal FFE (TR300NEX 4) 180mm x 240mm FOV, 192 x 256 matrix, 3 mm contiguous, interleaved	3
2. FLAIR coronal SE/IR (TE109/TR9140/TI2500) 180mm x 240mm FOV, 192 x 256 matrix, 5mm slice thickness, 0.5mm sp	3
3. T1-w axial SE (TE14/TR700 NEX 4) 180mm x 240mm FOV, 192 x 256 matrix, 3 mm contiguous, interleaved	4
4. T2-w axial TSE (TE72/TR9000) 180mm x 240mm FOV, 192 x 256 matrix, 3 mm contiguous, interleaved	2
5. T1-w MPRAGE sagittal 3D TFE (TE3.8/TR8.2/FA8) 230mm x 230mm FOV, 230 x 230 matrix, 1 mm contiguous, interleaved	6
6. SWI (TE20/TR26/FA15) 198mm x 210mm x 127 mm FOV 248 x 300 x 116 matrix	4

5.1.2 Spine MRI (Group1)

will be performed on the 3T scanner using SENSE spine coil with the following sequences:

5.1.2.1 Day 1

Scan type	Scan Time (min)
0. Setup/localizer/B0 shimming/B1 mapping	2
1. T1-weighted image Sagittal – Turbo Spin Echo TR=700 msec/TE=10 msec, 3 mm slice thickness, 260mm x 260mm FOV, 320 x 240 matrix	4
2. T1-weighted image Axial – Turbo Spin Echo TR=700 msec/TE=10 msec, 3 mm slice thickness, 180mm x 180mm FOV, 512 x 512 matrix,	4
3. T2-weighted image Sagittal – Turbo Spin Echo TR=4000msec/TE=116 msec, 3 mm slice thickness, 260mm x 260mm FOV, 320 x 240 matrix,	3
4. T2-weighted image Axial – Turbo Spin Echo TR=4000 msec/TE=116 msec, 3 mm slice thickness, 180mm x 180mm FOV, 512 x 512 matrix	3
5. T2*- weighted image Sagittal - Gradient refocused echo (GRE) TR=100 msec/TE=20 msec, 3 mm slice thickness, 260mm x 260mm FOV, 320 x 320 matrix, Flip Angle 10 deg	4
Gadoteridol bolus injection 0.1mmol/kg	1
5. T2*- weighted image Sagittal - Gradient refocused echo (GRE) TR=100 msec/TE=20 msec, 3 mm slice thickness, 260mm x 260mm FOV, 320 x 320 matrix, Flip Angle 10 deg	4
3. T2-weighted image Sagittal – Turbo Spin Echo TR=4000msec/TE=116 msec, 3 mm slice thickness, 260mm x 260mm FOV, 320 x 240 matrix,	4
4. T2-weighted image Axial – Turbo Spin Echo TR=4000 msec/TE=116 msec, 3 mm slice thickness, 180mm x 180mm FOV, 512 x 512 matrix	4
1. T1-weighted image Sagittal – Turbo Spin Echo TR=700 msec/TE=10 msec, 3 mm slice thickness, 260mm x 260mm FOV, 320 x 240 matrix	3

2. T1-weighted image Axial – Turbo Spin Echo TR=700 msec/TE=10 msec, 3 mm slice thickness, 180mm x 180mm FOV, 512 x 512 matrix,	3
5. T2*- weighted image Sagittal - Gradient refocused echo (GRE) TR=100 msec/TE=20 msec, 3 mm slice thickness, 260mm x 260mm FOV, 320 x 320 matrix, Flip Angle 10 deg	4

5.1.2.2

5.1.2.3 Day 2

Scan type	Scan Time (min)
0. Setup/localizer/B0 shimming/B1 mapping	2
1. T1-weighted image Sagittal – Turbo Spin Echo TR=700 msec/TE=10 msec, 3 mm slice thickness, 260mm x 260mm FOV, 320 x 240 matrix	4
2. T1-weighted image Axial – Turbo Spin Echo TR=700 msec/TE=10 msec, 3 mm slice thickness, 180mm x 180mm FOV, 512 x 512 matrix,	4
3. T2-weighted image Sagittal – Turbo Spin Echo TR=4000msec/TE=116 msec, 3 mm slice thickness, 260mm x 260mm FOV, 320 x 240 matrix,	3
4. T2-weighted image Axial – Turbo Spin Echo TR=4000 msec/TE=116 msec, 3 mm slice thickness, 180mm x 180mm FOV, 512 x 512 matrix	3
5. T2*- weighted image Sagittal - Gradient refocused echo (GRE) TR=100 msec/TE=20 msec, 3 mm slice thickness, 260mm x 260mm FOV, 320 x 320 matrix, Flip Angle 10 deg	4
Ferumoxytol : total dose 510mg, diluted 2 fold, given in divided injections: • **5 ml (75mg) at 1ml/sec *** • **10 ml (150 mg) at 1ml/sec*** • **18 ml (285 mg) at 1 ml/sec***	1
5. T2*- weighted image Sagittal - Gradient refocused echo (GRE) TR=100 msec/TE=20 msec, 3 mm slice thickness, 260mm x 260mm FOV, 320 x 320 matrix, Flip Angle 10 deg	4
3. T2-weighted image Sagittal – Turbo Spin Echo TR=4000msec/TE=116 msec, 3 mm slice thickness, 260mm x 260mm FOV, 320 x 240 matrix,	4
4. T2-weighted image Axial – Turbo Spin Echo TR=4000 msec/TE=116 msec, 3 mm slice thickness, 180mm x 180mm FOV, 512 x 512 matrix	4
1. T1-weighted image Sagittal – Turbo Spin Echo TR=700 msec/TE=10 msec, 3 mm slice thickness, 260mm x 260mm FOV, 320 x 240 matrix	3
2. T1-weighted image Axial – Turbo Spin Echo TR=700 msec/TE=10 msec, 3 mm slice thickness, 180mm x 180mm FOV, 512 x 512 matrix,	3
5. T2*- weighted image Sagittal - Gradient refocused echo (GRE) TR=100 msec/TE=20 msec, 3 mm slice thickness, 260mm x 260mm FOV, 320 x 320 matrix, Flip Angle 10 deg	4

** BP and heart rate check prior to ferumoxytol and after each ferumoxytol injection and 30 minutes after the final ferumoxytol injection*

5.1.2.4 Day 3

Scan type	Scan Time (min)
0. Setup/localizer/B0 shimming/B1 mapping	2
1. T1-weighted image Sagittal – Turbo Spin Echo TR=700 msec/TE=10 msec, 3 mm slice thickness, 260mm x 260mm FOV, 320 x 240 matrix	4
2. T1-weighted image Axial – Turbo Spin Echo TR=700 msec/TE=10 msec, 3 mm slice thickness, 180mm x 180mm FOV, 512 x 512 matrix,	4
3. T2-weighted image Sagittal – Turbo Spin Echo TR=4000msec/TE=116 msec, 3 mm slice thickness, 260mm x 260mm FOV, 320 x 240 matrix,	3
4. T2-weighted image Axial – Turbo Spin Echo TR=4000 msec/TE=116 msec, 3 mm slice thickness, 180mm x 180mm FOV, 512 x 512 matrix	3
5. T2*- weighted image Sagittal - Gradient refocused echo (GRE) TR=100 msec/TE=20 msec, 3 mm slice thickness, 260mm x 260mm FOV, 320 x 320 matrix, Flip Angle 10 deg	4

5.1.3 Carotid MRA and plaque imaging (Group2)

Carotid imaging will be performed using a SENSE spine coil with activation of the cervical segments or a surface coil to take advantage of maximal signal received in the proximity of the carotids. To reduce pulsation artifacts, imaging of carotid plaques will be acquired using a cardiac gating technique. Since there have been no established imaging sequences for ferumoxytol for this indication, the sequences will be tested by repeating various imaging sequences by altering imaging parameters and acquiring images multiple times. MR sequences are subject to change.

5.1.3.1 Day1

Scan type	Scan Time (min)
0. Setup/localizer/B0 shimming/B1 mapping	2
1. Axial T1-weighted (T1w) gradient-recalled-echo (GRE)	4
2. Axial and/or oblique sagittal T2*-weighted gradient-echo (GRE) Frequency-selective prepulse for fat suppression	4
3. Axial proton-density-weighted (PDw) fast spin-echo (PDw FSE) Frequency-selective prepulse for fat suppression	3
4. Cervical MRA: 3D T1 Fast Field Echo, 25° Flip Angle, TR=5.5 msec/TE=1.3 msec 1-1.5 mm slice thickness in the coronal plane **Gadoteridol bolus injection 0.1 mmol/kg 3ml/s flow rate**	3
5. Axial T1-weighted (T1w) gradient-recalled-echo (GRE)	4
6. Axial and/or oblique sagittal T2*-weighted gradient-echo (GRE) Frequency-selective prepulse for fat suppression	4
7. Axial proton-density-weighted (PDw) fast spin-echo (PDw FSE) Frequency-selective prepulse for fat suppression	3

5.1.3.2 Day 2

Scan type	Scan Time (min)
0. Setup/localizer/B0 shimming/B1 mapping	2
1. Axial T1-weighted (T1w) gradient-recalled-echo (GRE)	4
2. Axial and/or oblique sagittal T2*-weighted gradient-echo (GRE) Frequency-selective prepulse for fat suppression	4
3. Axial proton-density-weighted (PDw) fast spin-echo (PDw FSE) Frequency-selective prepulse for fat suppression	3
4. Cervical MRA: 3D T1 Fast Field Echo, 25° Flip Angle, TR=5.5 msec/TE=1.3 msec 1-1.5 mm slice thickness in the coronal plane **Ferumoxytol injection 75mg (5ml) up to 3ml/s flow rate**	3
5. Cervical MRA: 3D T1 Fast Field Echo, 25° Flip Angle, TR=5.5 msec/TE=1.3 msec 1-1.5 mm slice thickness in the coronal plane **Ferumoxytol injection 150mg (10ml) at 1ml/s flow rate**	3
6. Cervical MRA: 3D T1 Fast Field Echo, 25° Flip Angle, TR=5.5 msec/TE=1.3 msec 1-1.5 mm slice thickness in the coronal plane **Ferumoxytol injection 285mg (19 ml) at 1ml/s flow rate**	3
7. Axial T1-weighted (T1w) gradient-recalled-echo (GRE)	4
8. Axial and/or oblique sagittal T2*-weighted gradient-echo (GRE) Frequency-selective prepulse for fat suppression	4
9. Axial proton-density-weighted (PDw) fast spin-echo (PDw FSE) Frequency-selective prepulse for fat suppression	3

** BP and heart rate check prior to ferumoxytol and after each ferumoxytol injection and 30 minutes after the final ferumoxytol injection*

5.1.3.3 Day 3 (48h post ferumoxytol)

Scan type	Scan Time (min)
0. Setup/localizer/B0 shimming/B1 mapping	2
1. Axial T1-weighted (T1w) gradient-recalled-echo (GRE)	4
2. Axial and/or oblique sagittal T2*-weighted gradient-echo (GRE) Frequency-selective prepulse for fat suppression	4
3. Axial proton-density-weighted (PDw) fast spin-echo (PDw FSE) Frequency-selective prepulse for fat suppression	3

5.1.3.4 Day 4 (1week post ferumoxytol) (at physician's discretion)

Scan type	Scan Time (min)
0. Setup/localizer/B0 shimming/B1 mapping	2
1. Axial T1-weighted (T1w) gradient-recalled-echo (GRE)	4
2. Axial and/or oblique sagittal T2*-weighted gradient-echo (GRE) Frequency-selective prepulse for fat suppression	4
3. Axial proton-density-weighted (PDw) fast spin-echo (PDw FSE) Frequency-selective prepulse for fat suppression	3

5.1.4 Aortic MRA and wall imaging

Aortic imaging will be performed using a cardiac surface coil (receiver) and a body coil (transmission). To reduce pulsation artifacts, imaging will be acquired using a cardiac gating technique. Since there have been no established imaging sequences for ferumoxytol for this indication, the sequences will be tested by repeating various imaging sequences by altering imaging parameters and acquiring images multiple times. MR sequences are subject to change.

5.1.4.1 Day1

Scan type	Scan Time (min)
0. Setup/localizer/B0 shimming/B1 mapping	2
1. Axial T1-weighted (T1w) gradient-recalled-echo (GRE)	4
2. Axial and/or oblique sagittal T2*-weighted gradient-echo (GRE) Frequency-selective prepulse for fat suppression	4
3. Axial proton-density-weighted (PDw) fast spin-echo (PDw FSE) Frequency-selective prepulse for fat suppression	3
4. CE-MRA: 3D T1 Fast Field Echo, 25° Flip Angle, TR=5.5 msec/TE=1.3 msec 1-1.5 mm slice thickness in the coronal plane **Gadoteridol bolus injection 0.1 mmol/kg 3ml/s flow rate**	3
5. Axial T1-weighted (T1w) gradient-recalled-echo (GRE)	4
6. Axial and/or oblique sagittal T2*-weighted gradient-echo (GRE) Frequency-selective prepulse for fat suppression	4
7. Axial proton-density-weighted (PDw) fast spin-echo (PDw FSE) Frequency-selective prepulse for fat suppression	3

5.1.4.2 Day 2

Scan type	Scan Time (min)
0. Setup/localizer/B0 shimming/B1 mapping	2

1. Axial T1-weighted (T1w) gradient-recalled-echo (GRE)	4
2. Axial and/or oblique sagittal T2*-weighted gradient-echo (GRE) Frequency-selective prepulse for fat suppression	4
3. Axial proton-density-weighted (PDw) fast spin-echo (PDw FSE) Frequency-selective prepulse for fat suppression	3
4. CE-MRA: 3D T1 Fast Field Echo, 25° Flip Angle, TR=5.5 msec/TE=1.3 msec 1-1.5 mm slice thickness in the coronal plane **Ferumoxytol injection 75mg (5ml) up to 3ml/s flow rate**	3
5. CE-MRA: 3D T1 Fast Field Echo, 25° Flip Angle, TR=5.5 msec/TE=1.3 msec 1-1.5 mm slice thickness in the coronal plane **Ferumoxytol injection 150mg (10ml) at 1ml/s flow rate**	3
6. CE-MRA: 3D T1 Fast Field Echo, 25° Flip Angle, TR=5.5 msec/TE=1.3 msec 1-1.5 mm slice thickness in the coronal plane **Ferumoxytol injection 285mg (19 ml) at 1ml/s flow rate**	3
7. Axial T1-weighted (T1w) gradient-recalled-echo (GRE)	4
8. Axial and/or oblique sagittal T2*-weighted gradient-echo (GRE) Frequency-selective prepulse for fat suppression	4
9. Axial proton-density-weighted (PDw) fast spin-echo (PDw FSE) Frequency-selective prepulse for fat suppression	3

** BP and heart rate check prior to ferumoxytol and after each ferumoxytol injection and 30 minutes after the final ferumoxytol injection*

5.1.4.3 Day 3 (48h post ferumoxytol)

Scan type	Scan Time (min)
0. Setup/localizer/B0 shimming/B1 mapping	2
1. Axial T1-weighted (T1w) gradient-recalled-echo (GRE)	4
2. Axial and/or oblique sagittal T2*-weighted gradient-echo (GRE) Frequency-selective prepulse for fat suppression	4
3. Axial proton-density-weighted (PDw) fast spin-echo (PDw FSE) Frequency-selective prepulse for fat suppression	3

5.1.4.4 Day 4 (1week post ferumoxytol) (at physician's discretion)

Scan type	Scan Time (min)
0. Setup/localizer/B0 shimming/B1 mapping	2
1. Axial T1-weighted (T1w) gradient-recalled-echo (GRE)	4
2. Axial and/or oblique sagittal T2*-weighted gradient-echo (GRE) Frequency-selective prepulse for fat suppression	4
3. Axial proton-density-weighted (PDw) fast spin-echo (PDw FSE) Frequency-selective prepulse for fat suppression	3

5.1.5 Lower extremities MRA and MRV

Subjects should be positioned according to the following protocol. Subjects are allowed to adopt a comfortable position within the peripheral vascular/lower extremity and ankle and foot coil before being advanced within the magnet bore. Since there have been no established imaging sequences for ferumoxytol for this indication, the sequences will be tested by repeating various imaging sequences by altering imaging parameters and acquiring images multiple times. MR sequences are subject to change.

The localizing scans are saved for reference.

5.1.5.1 Day 1 (gadolinium MRI)

Scan type	Scan Time(min)
0. Setup/localizer/B_0 shimming/B_1 mapping (runoff coil)	0:50
1. Quick phase contrast MRA for localising major vessels (coronal, runoff coil Rx)	1:12
2. 3D T1w fat suppressed GRE (1.1 mm isotropic, FOV : 500x375, IM:448x336)	4 :35
3. 2D T2*w multiecho GRE, w/ fat saturation (coronal, runoff coil Rx) (0.4 mm inplane, 3 mm slice, 5 echoes)	4 :12
4. Setup/localizer/B_0 shimming/B_1 mapping (foot coil)	0:50
5. 2D T2*w multiecho GRE, w/ fat saturation (axial, foot coil Rx) (0.4 mm inplane, 2 mm slice, 5 echoes)	3 :50
6. First pass CE-MRA: 3D T1w (mask, bolus track and contrast) (coronal, runoff coil Rx) (1.1 mm inplane, 1.5 mm slice)	0 :11

7. Post-gadolium 3D T1w fat suppressed GRE (coronal, runoff coil Rx) (0.5 mm isotropic)	4 :35
8. Post-gadolinium 2D T2*w multiecho GRE, w/ fat saturation (coronal, runoff coil Rx) (0.4 mm inplane, 3 mm slice, 5 echoes)	4 :12
9. 2D T2*w multiecho GRE, w/ fat saturation (axial, foot coil Rx) (0.4 mm inplane, 2 mm slice, 5 echoes)	3 :50
Total gradient time :	28:17

5.1.5.2

5.1.5.3 Day 1 (ferumoxytol MRI-preoperative)

Scan type	Scan Time(min)
0. Setup/localizer/B_0 shimming/B_1 mapping (runoff coil)	0:50
1. Quick phase contrast MRA for localising major vessels (coronal, runoff coil Rx)	1:12
2. 3D T1w fat suppressed GRE (1.1 mm isotropic, FOV : 500x375, IM:448x336)	4 :35
3. 2D T2*w multiecho GRE, w/ fat saturation (coronal, runoff coil Rx) (0.4 mm inplane, 3 mm slice, 5 echoes)	4 :12
4. Setup/localizer/B_0 shimming/B_1 mapping (foot coil)	0:50
5. 2D T2*w multiecho GRE, w/ fat saturation (axial, foot coil Rx) (0.4 mm inplane, 2 mm slice, 5 echoes)	3 :50
6. First pass CE-MRA: 3D T1w (mask, bolus track and contrast) (coronal, runoff coil Rx) (1.1 mm inplane, 1.5 mm slice)	0 :11
7. Post-ferumoxytol 3D T1w fat suppressed GRE (coronal, runoff coil Rx) (“steady-state” angiography, 0.5 mm isotropic)	4 :35
8. Post-ferumoxytol 2D T2*w multiecho GRE, w/ fat saturation (coronal, runoff coil Rx) (0.4 mm inplane, 3 mm slice, 5 echoes)	4 :12

9. 2D T2*w multiecho GRE, w/ fat saturation (axial, foot coil Rx) (0.4 mm inplane, 2 mm slice, 5 echoes)	3 :50
Total gradient time :	28:17

** BP and heart rate check prior to ferumoxytol and after each ferumoxytol injection and 30 minutes after the final ferumoxytol injection*

5.1.5.4 Day 3 (late ferumoxytol MRI)

Scan type	Scan Time(min)
0. Setup/localizer/B0 shimming/B1 mapping (runoff coil)	0:50
1. Late-ferumoxytol 3D T1w fat suppressed GRE (coronal, runoff coil Rx) (1.1 mm isotropic, FOV : 500x375, IM:448x336)	4:35
2. Late-ferumoxytol 2D T2*w multiecho GRE, w/ fat saturation (coronal, runoff coil Rx) (0.4 mm inplane, 3 mm slice, 5 echoes)	4:12
3. Setup/localizer/B_0 shimming/B_1 mapping (foot coil)	0:50
4. Late-ferumoxytol 2D T2*w multiecho GRE, w/ fat saturation (axial, foot coil Rx) (0.4 mm inplane, 2 mm slice, 5 echoes)	3 :50
Total gradient time :	13 :23

5.1.6 Cervical lymph node imaging (Group3)

5.1.6.1 Day 1

Scan type	Scan Time (min)
0. Setup/localizer/B0 shimming/B1 mapping	2
1. Axial T1-weighted (T1w) Spin Echo (SE)	4
2. Axial T2-weighted (T2w) Fast Spin Echo (FSE)	4
3. Axial T2*-weighted gradient-echo (GRE)	4

Gadoteridol bolus injection 0.1 mmol/kg 3ml/s flow rate	1
4. Axial T1-weighted (T1w) Spin Echo (SE)	4
5. Axial T2-weighted (T2w) Fast Spin Echo (FSE)	4
6. Axial T2*-weighted gradient-echo (GRE)	4

5.1.6.2 Day 2

Scan type	Scan Time (min)
0. Setup/localizer/B0 shimming/B1 mapping	2
1. Axial T1-weighted (T1w) Spin Echo (SE)	4
2. Axial T2-weighted (T2w) Fast Spin Echo (FSE)	4
3. Axial T2*-weighted gradient-echo (GRE)	4
Ferumoxytol : total dose of not more than 510mg, diluted 2 fold, given in divided injections: • **5 ml (75mg) at 1ml/sec *** • **10 ml (150 mg) at 1ml/sec*** • **18 ml (285 mg) at 1 ml/sec***	5
4. Axial T1-weighted (T1w) Spin Echo (SE)	4
5. Axial T2-weighted (T2w) Fast Spin Echo (FSE)	4

** BP and heart rate prior to Ferumoxtyol and after each Ferumoxytol injection and 30 minutes after final injection*

5.1.6.3 Day 3 (48h post ferumoxytol injection)

Scan type	Scan Time (min)
0. Setup/localizer/B0 shimming/B1 mapping	2
1. Axial T1-weighted (T1w) Spin Echo (SE)	4
2. Axial T2-weighted (T2w) Fast Spin Echo (FSE)	4
3. Axial T2*-weighted gradient-echo (GRE)	4

5.1.6.4 Day 4 (1week post ferumoxytol injection) (at physician's discretion)

Scan type	Scan Time (min)
0. Setup/localizer/B0 shimming/B1 mapping	2
1. Axial T1-weighted (T1w) Spin Echo (SE)	4
2. Axial T2-weighted (T2w) Fast Spin Echo (FSE)	4
3. Axial T2*-weighted gradient-echo (GRE)	4

5.1.6.5 Subgroup - Abdominal Sequences: baseline, shortly after Fe, 24-28 hours after Fe, 1 week after Fe, 1, 2, 3 and 6 months after Fe

Additional abdominal sequences may be performed before and after ferumoxytol injection: single breath hold each.	
Setup/localizer/B0 shimming/B1 mapping abdomen	2
Axial T2-weighted single shot fast spin echo (SSFSE)	1
Axial T2-weighted fat saturated fast spin echo (BH FRFSE)	1
Axial diffusion weighted imaging (DWI) x 2. b value of 50 and 750	1
Axial T1-weighted spoiled gradient echo (in phase and out of phase)	1
Axial 3D T1 gradient echo for dynamic (LAVA) sequence	1
Coronal T2-weighted single shot fast spin echo (SSFSE)	1

The dose, dilution and injection parameters may be adjusted to obtain optimal signal changes, but will never exceed 510mg Fe.

The above detailed sequences are subject to change in order to acquire the best imaging results on the applied field strength.

5.2 Criteria for Image evaluation

Contrast-Enhanced MRI: All the superparamagnetic iron oxide agents display a much more effective MR relaxation than their paramagnetic counterparts. The following changes will be evaluated on ferumoxytol enhanced MRI when compared to non enhanced MRI images: increased or decreased signal on T1-weighted SE images, and decreased signal on T2-weighted SE images.

Image Analysis of Lesions: In order to determine the sensitivity of the gadolinium and ferumoxytol contrast agents in revealing abnormally enhancing brain regions, the gadolinium and ferumoxytol scans will be independently assessed on two measures: a) the number of intensity-enhanced abnormalities and b) the volume of enhancements.

Contrast enhancements in each scan will be identified by two radiologists who will review the gadolinium and ferumoxytol scans and identify regions of contrast enhancement using a drawing tool to identify voxels that are enhanced by the contrast agent. These measurements will yield a count of the number of lesions and the volume of each lesion detected for each scan. Differences in the number of lesions detected and the volume of each lesion will be resolved by a third neuroradiologist.

For carotid stenosis, degree of anatomical narrowing will additionally be described using standard NASCET criteria in addition to subjective and quantitative characterization of vessel wall presence or absence and thickness (where applicable), of enhancement.

The analysis of cervical lymph nodes will be performed by two radiologists, by analyzing the heterogeneity of the lymph nodes. Cellular ferumoxytol uptake results in a signal loss of the normal functioning lymphatic tissue, whereas the lack of ferumoxytol uptake will result in areas with higher signal, indicating etiologies different from reactive changes. Diagnostic accuracy will be compared between gadolinium and ferumoxytol, and confirmed with biopsy specimens in case lymph node sampling is performed.

MR analysis of abdominal organs and bone marrow with ferumoxytol uptake and clearance will be evaluated by comparing images at various time points with pre-ferumoxytol baseline scans.

Discipline reviews

5.2.1 Neuropathology

Tissue will be obtained after infusion with ferumoxytol for those subjects in who it is clinically indicated. Review of tissue may be done at OHSU.

Samples will be stained for iron using the Perl's reaction, which consists of incubation for 30 minutes at room temperature in equal parts of 2% HCL and 2% potassium ferrocyanide, followed by rinsing for 30 minutes in deionized water. Sections are then incubated in 0.5% diaminobenzidine in Tris buffer. This stain yields a brown reaction product.

Electron microscopy samples will be glutaraldehyde-fixed, postfixed in 2% osmium tetroxide, dehydrated in ascending concentration of ethanol, and embedded in Epon 812. Sections will be cut with a diamond knife, mounted on bare copper grids, and stained with uranyl acetate and lead citrate. Electron microscopy will be used to determine extracellular versus intracellular location and whether the iron is localized at the basement membrane. Localization to capillaries will be assessed by light and electron microscopy.

Histochemical staining of the biopsies for iron will correlate iron localization at the cellular level with MRI. Cell type specificity for iron uptake will be evaluated by co-localization for immunofluorescence staining of GFAP, olig 2, and S100 for astrocytes and oligodendroglia, CD56 or HAM57 for microglia, CD68 and CD163 for macrophages, NeuN or neurofilament for neurons, and CD3 and CD45 for lymphocytes. Demonstration of ferumoxytol vs. endogenous (blood-derived) iron will be evaluated with immunofluorescence staining for the dextran (carbohydrate) coating of ferumoxytol.

5.2.2 Neuroradiology

All MRIs and MRAs will be reviewed at OHSU by the neuroradiologists on the BBBD clinical team. In case of discordance between the two neuroradiologists, a third neuroradiologist will be involved. Gadolinium enhanced MR and ferumoxytol enhanced MR will be assessed for qualitative differential enhancement.

6 DATA ANALYSIS AND STATISTICS

The primary aim of this project is to qualitatively assess the adequacy of imaging and suitability for determination of permeability of the contrast agent into human inflammatory, dural or parenchymal based or ischemic or vascular lesions, carotid plaques, or enlarged cervical lymph nodes.

Subjects will be recruited into one of 3 study groups.

6.1 Statistical Summaries

Descriptive summaries will be provided for baseline characteristics (including demographic characteristics) and for all outcomes including side effects and adverse events. For group 1 patients, we will assess the number of lesions, T1 enhancement, relative cerebral blood volume and visualization of vascularity of lesions. Since group 1 includes heterogeneous pathologies, we will make qualitative comparisons between ferumoxytol and Gd, and quantitative comparisons if the sample size is large enough, stratified by pathology. The enhancing lesions will be classified as visible only with ferumoxytol, only with Gd, and visible with both to evaluate the differential values of the two images. For group 2 patients, we will assess vessel MR angiography, contrast imaging agent uptake by the plaques and vessel walls and the number of unstable plaques. Visualization of cervical lymph nodes, the number of pathological lymph nodes and differentiation between reactive vs. metastatic lymph nodes will be assessed for group 3 patients. Again we will make qualitative comparisons between ferumoxytol and Gd, and consider quantitative comparisons if the sample size is large enough. The enhancing plaques or lymph nodes will be classified as visible only with ferumoxytol, only with Gd, and visible with both.

6.2 Sample Size Justification.

This study is to explore the efficacy of ferumoxytol imaging in heterogeneous patient groups. The targeted number of subjects per group is designed to give a reasonable number of subjects to determine whether ferumoxytol detects more lesions than does Gd and whether ferumoxytol provides more information for better differential diagnosis of diseases.

7 ADMINISTRATIVE AND REGULATORY DETAILS

7.1 Retention of Records

U.S. FDA regulations (21 CFR §312.62[c]) and the ICH Guideline for GCP (see Section 4.9 of the guideline) require that records and documents pertaining to the conduct of clinical trials and the distribution of investigational drug, subject records, consent forms, laboratory test results, and medication inventory records, must be retained for 2 years after the last marketing application approval in an ICH region or after at least 2 years have elapsed since formal discontinuation of clinical development of the investigational product. All state and local laws for retention of records also apply.

7.2 Data Collection, Storage, Privacy, Confidentiality and Security

Research charts will be maintained for each subject and housed in the offices of the PI; only the study staff has access to the research charts and these offices are secure, non-public spaces that are locked after hours, ensuring that all records will remain confidential and secure. All study staff is trained on the subject privacy and confidentiality policies and is up to date on all required research staff training. This ensures subject privacy during recruitment, consent and all study procedures.

The Investigator is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported. All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data. The Investigator will maintain adequate case histories of study participants, and source documentation.

Data will not be released to any entity other than the IRB and study personnel. No information that would reveal the identity of the subject such as name, social security number, address, or phone number will be disclosed.

The information obtained during the conduct of this clinical study is confidential, and unless otherwise noted, disclosure to third parties is prohibited. Information contained within this study will be maintained in accordance with applicable laws protecting participant privacy, including the provisions of the Health Insurance Portability and Accountability Act (HIPAA).

Participant confidentiality is strictly held in trust by the participating Investigator(s) and study team. This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor.

The study monitor, other authorized representatives of the sponsor, representatives of the IRB may inspect all documents and records required to be maintained by the Investigator, including but not limited to, medical records (office, clinic, or hospital) and

pharmacy records for the participants in this study. The clinical study site will permit access to such records.

Upon enrollment, participants will be assigned a code that will be used instead of their name, medical record number or other personally identifying information. Electronic files for data analysis will contain only the participant code. Codes will not contain any part of the 18 HIPAA identifiers (e.g., initials, DOB, MRN). The key associating the codes and the participants' personally identifying information will be restricted to the Investigator and study staff. The key will be kept secure on a restricted OHSU network drive a in a limited access folder.

The study participant's contact information will be securely stored at each clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by local IRB and institutional regulations. Study participant research data, which is for purposes of statistical analysis and scientific reporting, will be stored at OHSU BBB Program per OHSU's Information Security Directives. Individual participants and their research data will be identified by a unique study identification number. The study data entry and study management systems used by clinical sites and by the research staff will be secured and password protected per OHSU's Information Security Directives. At the end of the study, all study databases will be de-identified and archived within the OHSU BBB Program.

7.3 Data Storage and disposition

The study team will maintain a spreadsheet and a database for subject enrollment and all data collection. The spreadsheet and the database will be maintained on an OHSU secured, password protected network, and only the study team will have access to the spreadsheet and the database, ensuring confidentiality and security of the data. Data will be kept indefinitely.

If the investigator relocates or for any reason withdraws from the study, the study records must be transferred to an agreed upon designee, such as another institution, or another investigator. Records must be maintained according to sponsor or FDA requirements.

Data collection is the responsibility of the clinical trial staff at the site under the supervision of the site Investigator. The Investigator is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported. Standard institutional practices will be followed as described in the OHSU's Information Security Directives to maintain the confidentiality and security of data collected in this study. Study staff will be trained with regard to these procedures.

Loss of participant confidentiality is a risk of participation. Efforts will be made to keep study participant identities confidential except as required by law. Participants' samples will be identified by code only. Specifically, each consenting participant will be assigned a unique coded identifier consisting of numbers. This identifier will be associated with the participant throughout the duration of their participation in the trial. The coded identifier will also be used to identify any participant specific samples.

Basic accrual tracking information (demographic, consent, visit information) will be captured in OHSU's electronic clinical information research system (eCRIS), hosted on OHSU secure servers and managed by OHSU's information technology group at their data center in downtown Portland, Oregon. Any additional printed documents containing participant identifiers, such as those from the medical record to confirm eligibility, will be filed in binders and kept in a locked, secure location.

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7.4 Clinical Data & Safety Monitoring

Monitoring visits will be performed by an OHSU designee monitor during the study to ensure that the rights and well-being of human participants are protected, that the reported trial data are accurate, and that the conduct of the trial is in compliance with the protocol, GCP, and applicable regulatory requirements.

Details of monitoring activities, including designation of assigned monitoring entities, scope of monitoring visits, timing, frequency, duration of visits, and visit reporting, will be included in a separate trial-specific monitoring plan (TSMP).

The Investigator agrees that the monitor will be permitted to conduct monitoring visits at appropriate intervals. The Investigator agrees to provide all relevant information and documentation as requested by the monitor, including access to all original study documents and source data, including access to electronic medical records and/or source documents if necessary.

The monitor will conduct source data review and verification as outlined in the TSMP, and following each visit will generate a report summarizing the visit findings.

Regardless of monitoring entity, the OHSU Sponsor-investigator is ultimately, singularly responsible for overseeing every aspect of the design, conduct, and final analysis of his/her investigation.

If at any time Investigator noncompliance is discovered at OHSU, the Sponsor-investigator shall promptly either secure compliance or end the Investigator's participation in the study.

Independent audits may be conducted by the Knight DSMC to verify that the rights and well-being of human participants are protected, that the reported trial data are accurate, that the conduct of the trial is in compliance with the protocol and applicable regulatory requirements, that monitoring practices are adequate and in compliance with the monitoring plan, and that evidence of ongoing investigator oversight is present.

Quality Control

The investigational site will provide direct access to all trial related source data/documents, and reports for the purpose of monitoring by the monitor and/or sponsor, and auditing by the Knight DSMC and/or regulatory authorities.

The Sponsor-investigator, or study monitor, will verify that the clinical trial is conducted and data are generated, documented (recorded), and reported in compliance with the protocol, GCP, and the applicable regulatory requirements (e.g., Good Laboratory Practices (GLP), Good Manufacturing Practices (GMP)).

8 ETHICAL AND REGULATORY CONSIDERATIONS

8.1 Monitoring Obligations

8.1.1 Institutional Review Board (IRB)

The protocol and informed consent form for this study must be approved in writing by the appropriate Institutional Review Board (IRB) prior to any subject being registered on this study. This study must be approved by the appropriate Institutional Review Board as defined by Federal Regulatory Guidelines (Federal Register Volume 46, No. 17, January 27, 1982, part 56) and the Office for Protection from Research Risks Reports: Protection of Human Subjects (Code of Federal Regulations 45 CFR 46).

8.2 Informed Consent

Written informed consent will be obtained from all subjects or their legally authorized representative participating in this trial, as stated in the Informed Consent section of the case of Federal Regulations, Title 21, Part 50. If a subjects signature cannot be obtained, the investigator must ensure that the informed consent is signed by the subjects legally authorized representative. The original shall be maintained in the subjects record. The principles of informed consent are described by Federal Regulatory Guidelines, (Federal Register Volume 46, No. 17, January 27, 1981, part 50) and the Office for Protection from Research Risks Reports: Protection of Human Subjects (Code of Federal Regulations 45 CFR 46). They must be followed to be in compliance with FDA and OPRR regulations for the conduct and monitoring of clinical investigations.

8.3 Changes to Protocol

8.3.1 Amendments

Changes in any portion of this protocol that affect subject safety or that alter efficacy parameters, must be documented in the form of an amendment and signed by the appropriate study personnel, including clinical scientist, statistician, and the investigator, and be approved by the IRB before the amendment may be implemented. The IRB chairpersons may approve minor changes or may designate one or more regulatory members to approve it. The only circumstance in which the amendment may be initiated without regulatory approval is for a change necessary to eliminate an apparent and immediate hazard to the subject. In that event, the investigator must notify the IRB in writing within 10 working days after the implementation.

8.3.2 Administrative Changes

Clarification or interpretation of the study protocol or changes in the methods of statistical analysis may be documented in the form of an administrative change. Administrative changes do not require the investigators signature or IRB approval. Administrative changes will be transmitted to the investigator and be provided to the IRB for completeness.

8.3.3 Maintenance of Records

The investigator must maintain all study records for fifteen years. If the investigator relocates or for any reason or withdraws from the study, the study records may be transferred to an agreeable designee, such as another institution or another investigator.

8.3.4 Early Termination

Criteria for early termination:

- Voluntary subject withdrawal
- Investigators decision that it is in the subject’s best interest to withdraw
- If the subject becomes pregnant
- If, in the opinion of the investigator, laboratory values become abnormal to a clinically significant degree during the course of the study
- Significant protocol violation
- At the Sponsor or Investigators discretion

9 ADVERSE EVENTS

9.1 Unanticipated Problems and Adverse Events

- Unanticipated Problems (UP) and Adverse Events (AE) will be reported to IRB according to the policies, procedures and guidelines posted on the OHSU IRB web site http://www.ohsu.edu/research/rda/irb/policies.shtml.
- Fatal and life-threatening UP will be reported to OHSU IRB within 7 days of notification of the event. All other UP reports will be submitted to OHSU IRB no later than 15 days of occurrence or notification of the event. Copies of the report documents will be kept in the study regulatory binder.
- In addition to the OHSU IRB, SAE reports and supporting documents will be sent to the following:

AMAG Pharmaceuticals, Inc.
61 Mooney Street
Cambridge, MA 02138
Tel (617) 497-2070 x 3009
Fax (617) 574-2445

Adverse events related to or potentially related to the administration of ferumoxytol will be recorded; adverse events related to the MRI's experience itself, such as claustrophobia/anxiety, nausea with GBCA injection, or discomfort due to positioning, is not being studied and will not be reported. The adverse events that will be monitored for during the infusion include localized discomfort at the IV injection site, pain, respiratory difficulties, flushing, dizziness, pruritis/rash, and any other symptoms that could be secondary to an anaphylactoid type reaction.

9.2 IND Safety Reporting

Adverse events will be graded according to the NCI Common Toxicity Criteria Per CFR 312.32 (c), the investigator-sponsor of the IND must notify the Food and Drug Administration (FDA) and all participating investigators in a written IND safety report of any adverse experience **associated with use of the drug** that is **both serious and unexpected.** Such notification shall be made as soon as possible, and in no event later than 10 working days after the investigator-sponsor's initial receipt of the information. Each written notification must bear prominent identification of its contents, i.e., "IND Safety Report". Follow-up information to a safety report should be submitted as soon as the relevant information is available.

The investigator-sponsor must also notify FDA by telephone of any **unexpected fatal or life-threatening experience associated with use of the drug** in the clinical studies conducted under the IND no later than 3 working days after receipt of the information. Each written IND safety report and telephone notification to FDA shall be transmitted to the FDA division of the Center for Drugs and Biologics which has responsibility for review of the IND; a specific contact person is assigned to each IND at the time the application is filed, and this will be included in the FDA's correspondence acknowledging

receipt of the IND application. For purposes of this protocol, the Adverse Reaction Form for Investigational Drugs (Appendix VI), along with FDA Form 1571, and a cover letter submitted to the appropriate FDA division, will serve as the written IND safety report.

9.3 Definitions:

The following terms are defined in Food and Drug Administration, HHS 21 CFR 312.32:

"Associated with the use of the drug" means that there is a reasonable possibility that the experience may have been caused by the drug.

"Serious adverse experience" means any experience that suggests a significant hazard, contraindication, side effect, or precaution. With respect to human clinical experience, a serious adverse drug experience includes any experience that is fatal or life-threatening, is permanently disabling, requires inpatient hospitalization, or is a congenital anomaly, cancer, or overdose.

"Unexpected adverse experience" means any adverse experience that is not identified in nature, severity, or frequency in the current investigator brochure; or, if an investigator brochure is not required, that is not identified in nature, severity, or frequency in the risk information described in the general investigational plan or elsewhere in the current application, as amended.

"Life-threatening" means that the subject was, in the view of the investigator, at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction that, had it occurred in a more serious form, might have caused death.

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APPENDICES:

APPENDIX A
CTCAE Website

APPENDIX B
Karnofsky and ECOG Performance Critieria

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