SPONSOR: Oregon Health & Science University
OHSU eIRB #16709 **NCT#: NCT03325166**

TITLE: The use of perfusion MRI using ferumoxytol and small molecular weight gadolinium (Gd) agents to assess response to pembrolizumab in brain metastases and systemic lesions in NSCLC: A comparison of imaging modalities to address brain metastases, pseudoprogression and systemic lesion tumor flare (neuro-check pilot)

Principal Investigator:
Edward Neuwelt, MD
Professor
Department of Neurology
Oregon Health & Science University
(503) 494-5626

Co-Investigators:

Guang Fang, MD, PhD
Director of Clinical Flow Cytometry Laboratory
Director of Hematopathology Fellowship Program
Department of Pathology
Oregon Health and Science University
(503)-494-9439

Biostatistician:
Rongwei (Rochelle) Fu, PhD
Professor of Biostatistics
Department of Public Health and Preventive Medicine
Oregon Health & Science University
(503) 494-6069
(503) 494-4981 (FAX)

Data Management/

Study Coordinators:

Lisa Muir, MPA, CCRP
Department of Neurology
Oregon Health & Science University
(503) 494-5626
(503) 494-5627 (FAX)

Amy Huddleston, MPA, CCRP
Oregon Health & Science University
(503) 494-5626
(503) 494-5627 (FAX)

TABLE OF CONTENTS

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Version 1	initial submission 01/20/2017; approved 7/27/17
Version 2	Submitted to IRB 12/14/17; approved 2/21/18
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Version 7	Submitted to IRB 3/31/22;

1.0 TRIAL SUMMARY

Abbreviated Title	A comparison of imaging modalities to address brain metastases, pseudoprogression and systemic lesion tumor flare in brain metastasis after radiation and pembrolizumab
Trial Phase	Pilot study
Clinical Indication	Brain metastases non-small cell lung carcinoma (NSCLC)
Trial Type	Pilot study
Type of control	None
Route of administration	Intravenous
Trial Blinding	None
Treatment Groups	None
Number of trial subjects	20
Estimated enrollment period	3 years
Estimated Duration of Trial	5 years
Duration of Participation	5 years

2.0 TRIAL DESIGN

A total of twenty (20) subjects scheduled to receive stereotactic radiosurgery (SRS) for brain metastases will be enrolled in this single institution pilot study. Subjects with up to ten measurable target metastatic brain lesions secondary to non-small cell lung carcinoma (NSCLC), with adequate performance status, baseline hematological and metabolic parameters will receive pembrolizumab (200mg intravenous every 3 weeks) in addition to standard of care SRS, until persistent radiographic progression is confirmed. Subjects may receive standard of care cytotoxic chemotherapy while on trial, at the treating physician's discretion. Subjects may undergo surgery to one or more of the target lesions if clinically indicated prior to enrollment. Subjects will be allowed to continue on pembrolizumab 200mg every 3 weeks until suspected radiographic progression. At suspected radiographic progression, subjects will be allowed to continue on Pembrolizumab for 2 more doses until follow up clinical and ferumoxytol steady state magnetic resonance imaging (MRI) in 6 weeks (+/- 5 days) confirms persistent radiographic progression.

Upon confirmation of persistent radiographic progression, subjects will be evaluated for repeat surgery or biopsy. After surgery, if histopathology confirms true progression, subjects will be taken off study; patients who are not candidates for surgery will also be taken off the study.

Subjects whose biopsy suggests pseudoprogression will be allowed to continue on pembrolizumab 200 mg Q3 weeks until further radiographic progression is confirmed.

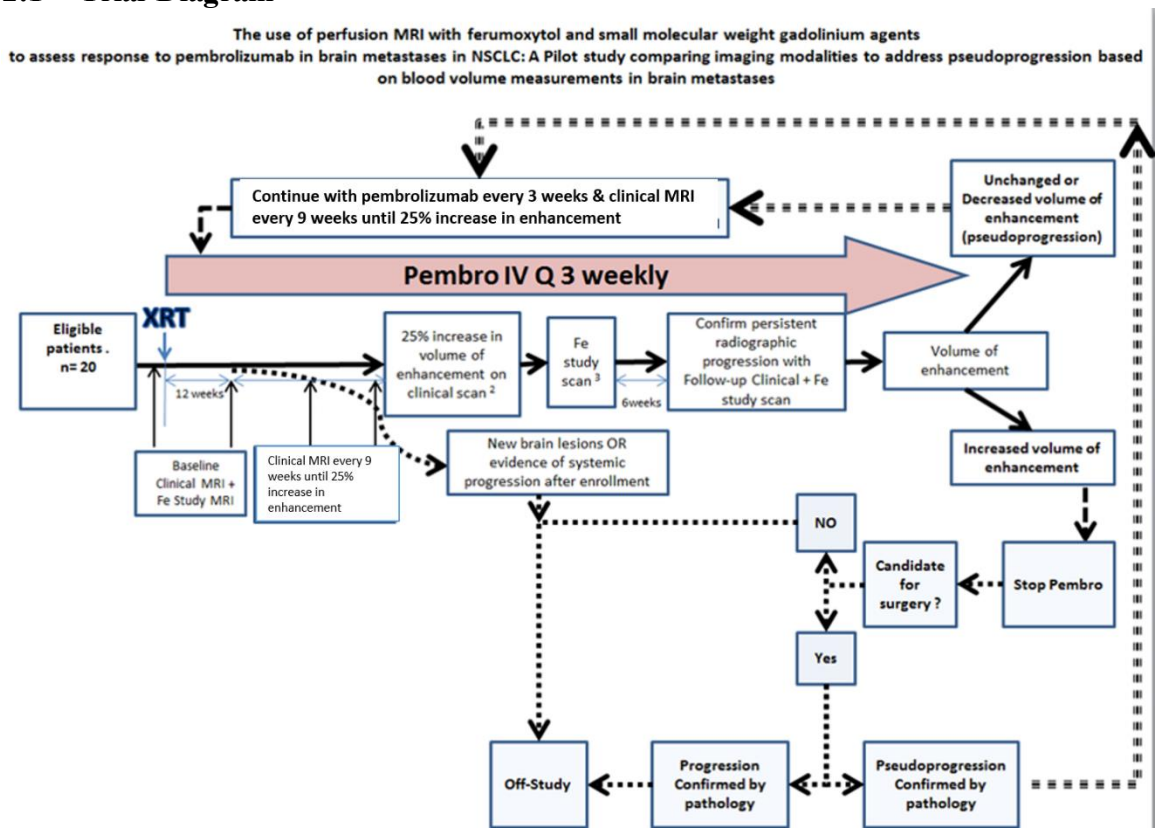
If, after initial suspected radiographic progression, persistent radiographic progression is not confirmed at follow up, subjects will continue on pembrolizumab until further persistent radiographic progression is detected and confirmed. Subjects with evidence of new lesions (not present at entry to the study) in the CNS or systemically that fulfil criteria for progression by immune related response criteria (irRC) [1]will be taken off study drug, but will be followed with serial clinical standard of care MRI scans until death.

In addition, subjects that are already enrolled in the study and have measurable systemic disease by RECIST 1.1 Criteria [2] at screening may undergo additional MRI sequences of the systemic disease to evaluate the immunological response to pembrolizumab outside the CNS (systemic disease). Patients who are taken off study due to persistent radiographic progression, who are not candidates for biopsy, refuse biopsy or have stable brain disease but have systemic disease progression may be continued on pembrolizumab at the discretion of the treating physician, if there is evidence of clinical benefit from continuation of therapy. They will be followed up with clinical standard of care MRI scans and evaluated for survival, but will not be included in the analysis for primary end-point.

Blood for biomarker evaluation is optional, and will be drawn as described in section 8.5. These markers will be correlated with imaging findings and survival. Analysis will be performed by the OHSU clinical flow cytometry laboratory.

Any archival biopsy, fresh tissue, as well as tissue obtained at persistent radiographic progression will be obtained for clinical pathology review at OHSU. Tissue samples will be sent to QualTek Molecular Laboratories for PD-L1 biomarker assay as described in section 8.5.

2.1 Trial Diagram



3.0 OBJECTIVES & RATIONALE

Primary Objective

1. Determine the sensitivity and specificity of relative cerebral blood volume (rCBV) measured by steady state MRI with ferumoxytol in predicting true vs pseudoprogression after SRS and intravenous (IV) pembrolizumab in subjects with brain metastases from NSCLC.

Rationale: This study is not powered to test any formal hypothesis and we aim to collect preliminary data on the sensitivity and specificity of rCBV measurements with ferumoxytol in the primary objective.

Secondary Objectives

1. Evaluate the safety and tolerability of pembrolizumab when given with SRS in subjects with brain metastasis.
2. Evaluate progression free survival, overall survival, best response in brain disease, best response in systemic disease, and duration of best responses of brain and systematic diseases.

Rationale: Given the small sample size, we aim to collect preliminary data on survival, safety, tolerability and efficacy of pembrolizumab when given with SRS in subjects with brain metastasis. No additional toxicity profile is expected when pembrolizumab is used in combination with SRS and ferumoxytol. Toxicities will be evaluated using CTCAE version

4.0. Subjects will have ongoing toxicity evaluation, with documentation of infusion related reactions (grade 3, 4), hematologic toxicities (grade 3, 4) and all other grade 3, 4 toxicities. Each toxicity will be attributed to pembrolizumab and ferumoxytol or neither/both.

Exploratory Objectives

1. Compare the immune response as determined by the volume, pattern and intensity of delayed (24hr) ferumoxytol uptake between subjects who develop true vs pseudoprogression.
2. Investigate the serum immunological parameters and correlate clinical as well as radiological response with systemic immune response to pembrolizumab as measured by immunological panel.
3. Compare the changes percentage expression in PDL-1 in the biopsy tissue before and after therapy at the time of progression.
4. In subjects with measurable systemic lesions, investigate the feasibility of measuring vascular volume fraction (VVF), vessel size index (VSI) and vessel density index (VDI) as surrogate for response (true vs. pseudoprogression, as measured with RECIST 1.1 criteria).

Rationale for Exploratory Objectives: This study is not powered to test any formal hypothesis and we aim to collect preliminary data regarding the exploratory endpoints that will enable us to better understand the tumor biology of brain metastases and PD-1 expression and interaction with PD-1 inhibitors. In addition, this pilot study will enable us to collect preliminary data on efficacy of pembrolizumab on brain metastases.

Evidence of up-regulation of the immune system is expected in the tumor micro-environment as well as systemically in subjects that develop pseudoprogression and respond favorably to pembrolizumab.

1) Local immune up regulation in the tumor microenvironment will be characterized by increased delayed ferumoxytol uptake, the total volume of T1 intensity 24 hours after ferumoxytol administration will be measured and correlated with compared to response to therapy. Ferumoxytol is taken up by activated inflammatory cells including macrophages. An increased uptake on delayed scans suggests a robust inflammatory response. We expect greater intensity and magnitude of delayed ferumoxytol uptake signal in subjects who develop pseudoprogression and not in those that have true tumor progression.
2) PD-L1 expression and the type of immune cells present in the tumor microenvironment will be compared between archival biopsy tissue and brain tumor tissue obtained before initiation of pembrolizumab and at the time of progression.
3) Systemic immune up regulation will be measured by the blood immunological assay as described in (section 8.5). Clinical outcomes will correlate with T cell activation/phenotype, increases in systemic inflammatory markers will be associated with outcomes.
4) In a subset of subjects with measurable systemic disease, response to therapy in the systemic disease will be monitored by measuring changes in tumor microvasculature

by MRI imaging of the lesion of interest that will precede changes in tumor response as measured by traditional RECIST 1.1 and immune-related response criteria (irRC). We expect that changes in tumor microvascular will provide a more accurate determination of tumor immune flare versus true progression as determined by immune response criteria

4.0 BACKGROUND & RATIONALE

Refer to the Investigator's Brochure (IB)/approved labeling for detailed background information on pembrolizumab.

4.1 Burden of Disease: brain metastases from non-small cell lung carcinoma

Lung cancer is the leading cause of cancer related mortality in the United States with non-small cell lung carcinoma (NSCLC) accounting for approximately 85 % of lung cancers and for up to 20% of all new cases of brain metastases. The advent of newer chemotherapies and targeted agents have resulted in better control of systemic disease. However, management of metastases in the CNS continues to be a challenge. Metastatic brain tumors are known to be highly heterogeneous in terms of imaging characteristics, molecular markers and response to therapy. Current therapy for metastatic brain tumors includes surgery or radiation and has only shown modest improvements in survival from around 2 months to 3-6 months when compared to best supportive care [3, 4]. Novel immune modulatory therapies like pembrolizumab that can augment the body's own immune system to respond to cancer may significantly improve survival outcomes in subjects with CNS disease.

4.2 Immuno-therapy in the brain

Until recently, brain was believed to an immune privileged region devoid of lymphatic tissue and immune cells necessary to aid immune-surveillance and antitumor activity. Our understanding of the CNS immune microenvironment has drastically changed in the last few years. The presence of tumor infiltrating lymphocytes around primary and metastatic brain tumors and improved survival in patients with dense infiltrates of tumor infiltrating lymphocytes suggest that the immune system plays an antitumor important role [5]. More recent evidence indicates the presence of previously unidentified central nervous system lymphatic system that plays an important role in trafficking immune cells and antibodies in and out of the CNS [6]. Cancer cells evade this immune surveillance by various mechanisms including high expression of the immune checkpoints. Immune checkpoints are a group of inhibitory pathways that help maintain self-tolerance and minimize collateral tissue damage from an inflammatory response. However, this pathway is a major mechanism by which cancer cells evade immune-surveillance by T cells that are specific for tumor antigens. It is now believed that inhibiting the immune checkpoints is one of the most promising approaches to activating and maintaining therapeutic antitumor immunity [7].

Pembrolizumab is a checkpoint inhibitor that targets the programmed death 1 Protein (PD-1), a receptor on T lymphocytes. Pembrolizumab augments the anti-tumor immune response by blocking the interaction of PD-1 with tumor cells. Pembrolizumab received FDA approval for advanced metastatic melanoma based on Phase I and II clinical studies in subjects who had previously progressed on ipilimumab [8]. These studies indicate an overall response rate of

around 26%, a majority of which were durable responses, some over 6 months. Similar studies comparing efficacy in advanced NSCLC are currently underway. The FDA recently granted pembrolizumab breakthrough therapy designation for advanced NSCLC based on the Phase 1b KEYNOTE -001 study [9]. There are two other ongoing Phase 2 and 3 studies in advanced lung cancer (KEYNOTE-010 and KEYNOTE-024). In contrast to similar drugs that work on the PD-1 ligand inhibitors present on tumor cells, pembrolizumab acts on the peripheral T-cells and is hence expected to activate antitumor immunity even if it does not cross the blood brain and blood tumor barrier (neuro-vascular bundle), which is a major hurdle for any drug targeting the CNS.

Early studies with PD-1 inhibitors evaluating systemic tumors excluded patients with brain metastases. Although promising early data is emerging in brain metastases from various tumor types, very limited mature prospective data is available regarding their efficacy in brain tumors, specifically in brain metastases from NSCLC [10-15]. There are several ongoing clinical trials but the results are not available yet. Recently reported data from an open-label phase II trial evaluating the safety and activity of pembrolizumab monotherapy in brain metastases in 36 untreated brain metastases(n=36, 18 melanoma, 18 NSCLC) showed a brain response rate of 33% and was tolerated [16]. Similarly, initial reports on the safety of combining pembrolizumab with radiation suggest that it is well tolerated with no reported grade 3 or greater acute toxicities [17, 18]. Our study will thus be vital in gathering additional vital data in a homogeneous group of patients with NSCLC brain metastases that will be helpful in designing future studies.

4.3 Challenges in Trial Design for Brain Metastases

A major challenge in managing and designing trials for brain metastases is correctly determining tumor response to therapy, especially differentiating true progression from pseudoprogression or treatment-related changes. After radiation, a large percentage of brain tumors can paradoxically increase in size due to an inflammatory response called ‘pseudoprogression’. The term ‘pseudoprogression’ describes the phenomenon of subacute radiochemotherapy treatment-related sequelae in the CNS in subjects with brain metastases that presents as increasing or new contrast enhancement on MRI [19, 20]. These subjects stabilize or recover spontaneously, usually without any change in treatment. Although the exact pathophysiology of pseudoprogression is yet to be understood, it is widely believed to be mediated by an inflammatory response. The incidence of pseudoprogression is increasing and a becoming major challenge in clinical trials with immune modulatory agents [21].

Radiation alone can cause a spectrum of injury to the CNS and range from reversible edema to radiation necrosis. Pseudoprogression is believed to fall in this spectrum and is better characterized in high grade glioma [22]. Our data suggests that the true incidence of pseudoprogression and radiation injury may have been significantly underestimated [23, 24]. The risk for radiation necrosis increases with high dose fractions, hyperfractionation or stereotactic radiosurgery (SRS), all of which are frequently used in the treatment of brain metastases [25, 26]. We expect a higher incidence of pseudoprogression in this study due to the combination of radiation with immunotherapy.

4.4 Response Assessment in Brain Metastases

Radiological assessment guidelines such as RECIST 1.1 and WHO criteria for response assessment in systemic solid tumors have consistently proven inadequate in the CNS due to the unique anatomy of brain vasculature including the blood-brain barrier (BBB) and the neurovascular unit (NVU), as well as the inherent heterogeneity of brain metastases [2, 27-29]. It is now well recognized that enhancement seen on MRI represents just the area with disrupted BBB and does not show micrometastatic or infiltrative tumor, or lesions with a relatively normal BBB. The lack of reliable imaging biomarkers for response assessment is a major limiting factor in the development of new and effective therapies for brain tumors [29]. Currently the consensus-based updated Response Assessment in Neuro-Oncology Working Group (RANO) criteria is used for evaluation of brain metastases [30]. Among other drawbacks, all radiographic progression after the 12 week window following radiation is attributed to active tumor growth and considered progressive disease by RANO criteria. Another major problem with response assessment is the use of vascular endothelial growth factor (VEGF) inhibitors like bevacizumab or corticosteroids. These agents can alter the NVU around the tumor and decrease contrast enhancement (pseudo-response) when measured by conventional response criteria. Bevacizumab has failed to show significant survival benefits but is frequently used as an anti-edema measure [31-33].

Inaccurate attribution of progression, pseudoprogression, or radiation necrosis at enrollment or at the time of response assessment may significantly bias clinical trial outcomes. In addition, incorrect response assessment may lead to premature changes in therapy, potentially negating the benefits of a particular therapy. Radiographic false-positive changes are expected to increase after immune-modulatory therapy like PD-L1 inhibitors such as pembrolizumab. A subject on a potentially beneficial immune-modulatory therapy could be taken off a clinical trial because the subject may falsely fulfill RANO or RECIST criteria for progression even though in reality, the radiographic progression could be due to pseudoprogression or response to immunotherapy. This is especially true in trials with immunotherapies where response may be slow or delayed. A novel guideline for evaluation of immune therapy activity called Immune-Related Response Criteria (irRC) [34] in systemic solid tumors proposed to assess immune responses in systemic malignancies. The apparent increase in tumor burden sometimes precedes eventual response and this is believed to be mediated by a transient immune response and local immune cell infiltration with or without edema. Similar results are expected in brain tumor therapies with immune modulatory agents such as pembrolizumab. However, irRC is also not expected to be suitable for response assessment in brain tumors due to its inability to differentiate true progression from pseudoprogression and effects of radiation.

4.5 Assessing Pseudoprogression with Ferumoxytol

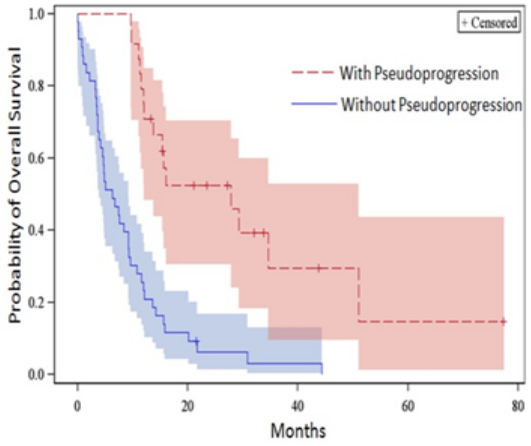
We have developed a novel functional imaging technique that may help us differentiate radiation necrosis, immune response and pseudoprogression from true disease progression in brain tumors. Ferumoxytol(Feraheme)[35] iron oxide nanoparticles, an FDA-approved iron supplement, is a blood-pool contrast agent at early time points after infusion (minutes to hours). Dynamic susceptibility contrast (DSC) ferumoxytol perfusion MRI (feMRI) provides consistent measurement of rCBV, which has been used as a marker of actively growing tumor in glioblastoma multiforme (GBM). At later time points (over hours to days) ferumoxytol is

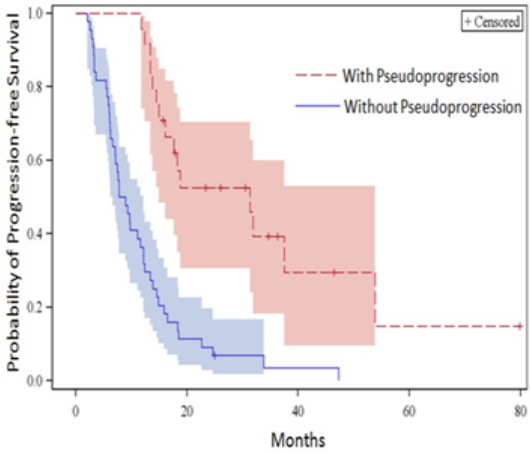
phagocytosed by macrophages and activated glial cells in and around the CNS lesion, providing a marker of neuroinflammation. We hypothesize that early feMRI measurement of rCBV and delayed ferumoxytol uptake can help us differentiate brain tumor response and progression. Ferumoxytol is being used in this study off-label and under a physician's IND.

Preliminary results: Imaging response and inflammation in CNS malignancies.
We have used DSC feMRI to evaluate outcomes in high grade glioma [36]. Prior studies have demonstrated elevated rCBV (rCBV>1.75) in actively growing tumor, while pseudoprogression shows low rCBV in primary brain tumors. [19, 20, 36-41]. We have also validated the relevance of the 1.75 rCBV cut-off for survival in high grade gliomas [42]. Our retrospective neuroimaging study in 68 subjects with high grade gliomas treated with chemoradiation (CRT) suggests that pseudoprogression significantly increases overall survival (OS) from around 13 months to over 34 months [36, 43] (Figure 1). Around 30% of all pseudoprogression occurred beyond the 12-week RANO cut-off, including 2 cases occurring over 6 months after CRT [41]. Figure 2 shows a representative case in high grade glioma, where the subject fulfilled criteria for progressive disease (PD) by RANO criteria over 3 months after radiation, but was shown to have pseudoprogression by the demonstration of low blood volume in the area of interest on feMRI. The rate of pseudoprogression in high grade gliomas diagnosed at any time after CRT range from 9.3 -31% in large studies [19, 44]. Our studies using DSC feMRI as well as similar rCBV measurements using small molecular weight gadolinium (Gd) agents with mathematical leakage correction suggest that pseudoprogression can occur at extended time frames [41].

A representative case showing pseudoprogression in a GBM on immune-modulatory therapy with EGFRv3 based vaccine, is shown in Figure 3. Figure 4 shows an example of the use of feMRI to evaluate treatment efficacy in brain metastasis.

Figure 1. Pseudoprogression and tumor progression in GBM. Kaplan-Meier estimates of overall survival (top) and progression-free survival (bottom) by the presence of pseudoprogression





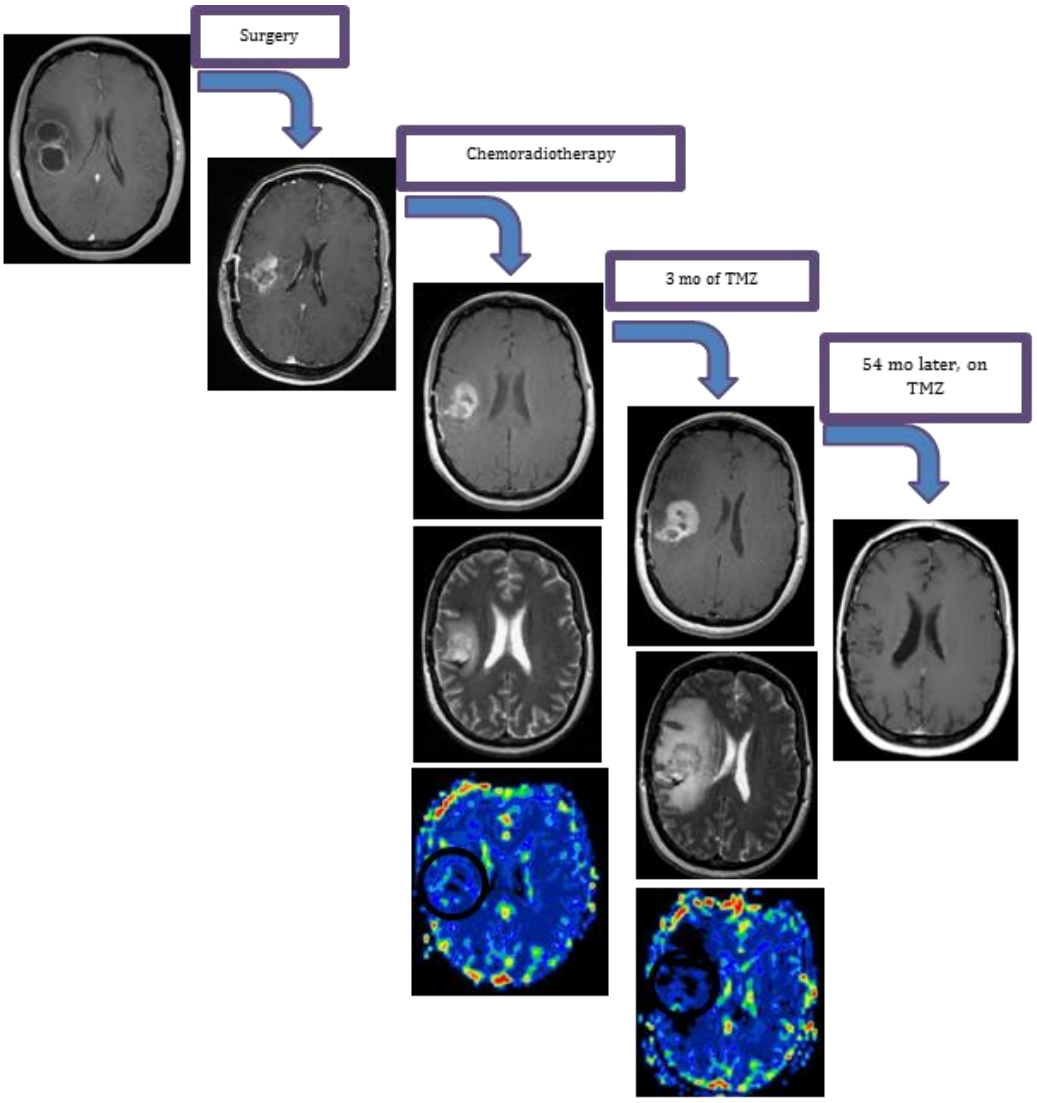


Figure 2. Imaging pseudoprogression. 48 y/o female with glioblastoma. **Column 1**: preoperative T1+Gd MRI. **Column 2**: T1+Gd MRI after maximal safe resection. **Column 3**: T1+Gd, T2 and ferumoxytol perfusion MRI after completion of standard CRT. Note increased enhancement and low rCBV in the lesion (circle). **Column 4**: T1+Gd, T2 and Ferumoxytol perfusion MRI after completion of 3 months of adjuvant TMZ. Note further increase in enhancement with continued low rCBV (circle). **Column 5**: T1+Gd MRI after 54 months of adjuvant TMZ and resolution of pseudoprogression showing sustained complete response.

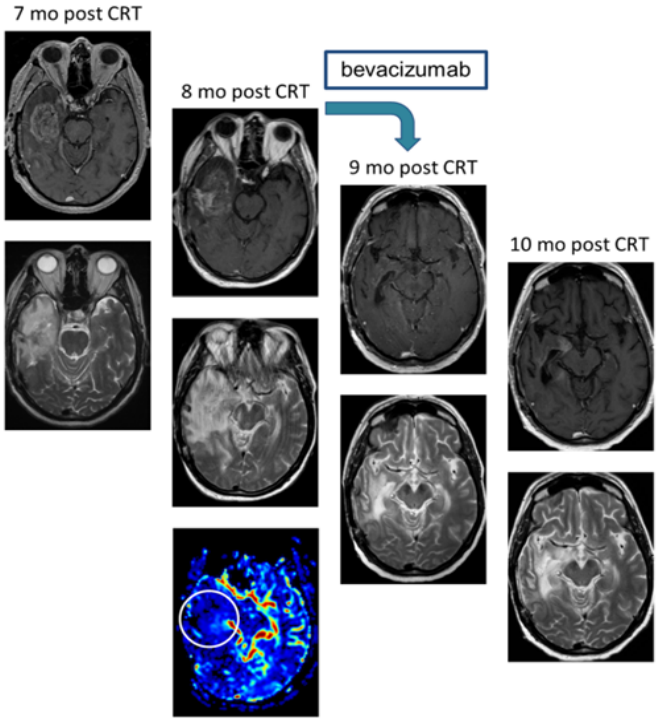


Figure 3: Pseudoprogression after immunotherapy. This Patient received Celldex vaccine (an investigational immunotherapy that targets the tumor specific oncogene EGFRv3) at 5 and 6 months after CRT and 4 monthly cycles of adjunct temozolomide for glioblastoma. A dramatic increased mass effect was noted 7-8 months post CRT and 1-2 months post Celldex vaccine. T1+Gd MRI Column 1: 7 months after CRT showing residual enhancing lesion in the right temporal lobe. Column 2: 8 months after CRT showing increase size of the lesion and exuberant vasogenic edema and mass effect on the lateral ventricle and midline shift. DSC ferumoxytol perfusion MRI 8 months after CRT showing decreased rCBV in the enhancing area in the right temporal lobe, suggesting pseudoprogression. At this point the patient might be falsely classified as disease progression by updated RANO criteria. Patient was continued on temozolomide based on our blood volume measurements. He was given one dose of bevacizumab as an anti-edema measure in addition to high dose steroids. He had to be taken off the clinical trial because the trial excluded patients on bevacizumab. Column 3: 9 months after CRT and 4 weeks after one dose of bevacizumab MRI showed significant improvement in the enhancement, vasogenic edema and mass effect. Column 4: 10 months after CRT and 8 weeks after bevacizumab showing minimal increase of enhancement and mass effect, still significantly better than before bevacizumab. This case clearly demonstrates the inadequacies of conventional MRI scans and RANO criteria and shows blood volume measurements is better imaging biomarker than enhancement.

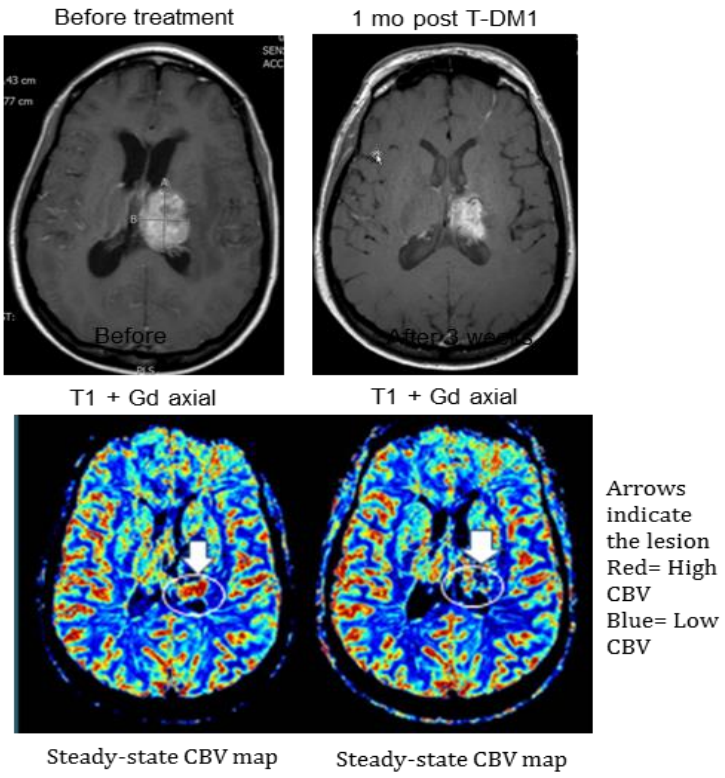


Figure 4. rCBV to evaluate response in a brain metastasis. A 38 year old woman with HER2-overexpressing, node-positive breast cancer was found to have an asymptomatic left frontal brain metastasis during screening central nervous system imaging. The patient received multiple treatments, including stereotactic radiosurgery, systemic therapy with lapatinib and docetaxel, whole brain radiation therapy at 3000cGy, and IV trastuzumab and lapatinib, followed by recurrence each time. Subsequent neuroimaging with ferumoxytol (feMRI) showed increased relative cerebral blood volume (rCBV) along the superior and posterior margin of the thalamic lesion, indicative of active tumor. The patient was then started on trastuzumab-emtansine (T-DM1) an antibody drug conjugate. Three weeks later, there was a marked decrease in rCBV on steady-state feMRI and a partial response with 50% decrease of the enhancing area on MRI.

Recently, Varallyay and colleagues have further improved rCBV measurement by measuring steady-state perfusion using high resolution imaging with ferumoxytol in brain tumors [42]. We can now obtain high-resolution steady state-CBV images that differentiate regions of high vascularity and active tumor growth [36, 42]. Figure 5 shows a comparison of the DSC and

steady-state feMRI techniques. Steady-state MRI with ferumoxytol is particularly helpful in imaging cortical lesions with very high resolution.

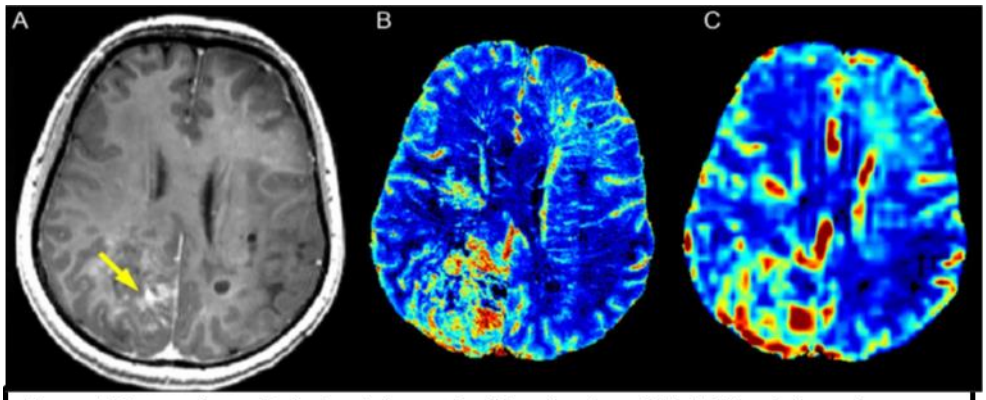


Figure 5. Comparison of steady-state-cerebral blood volume (SS-CBV) and dynamic susceptibility contrast (DSC)-CBV maps in a glioblastoma patient. (A) T1-weighted postgadoteridol scan describes the multifocal signal abnormalities. In corresponding slices, the SS-CBV (B) and DSC-CBV (C) maps show increased areas of CBV referring to highly vascular tumor areas. Note the mismatch between the most enhancing region (arrow) and the highest CBV values. Reprinted from Varallyay et al. (2013).

Ferumoxytol and other iron oxide nanoparticles can be taken up by phagocytic cells of the reticuloendothelial system as well as tumor-associated macrophages [45-47] and brain microglia [48]. We are actively investigating the utility of ferumoxytol for imaging inflammation, and assessing nanoparticle uptake into tumor-associated macrophages/microglia in inflammatory lesions in a rat model (Figure 6). We have demonstrated that ferumoxytol nanoparticles are taken up by phagocytic cells in the brain over hours to days after IV infusion (Figure 6). Similar patterns of ferumoxytol uptake are seen in a rat neuroinflammation model compared to a subject with GBM (Figure 7). Figure 8 shows typical early and delayed imaging in a subject with GBM.

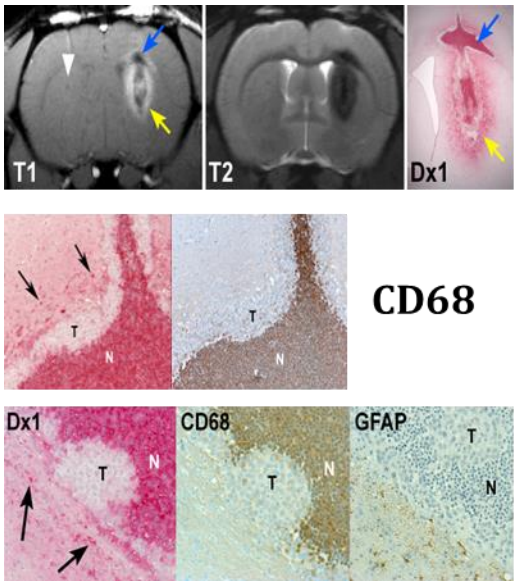


Figure 6. Neuroinflammation model. (TOP) delayed MRI sequences of rat brain after ferumoxytol injection with corresponding histopathology stained for the dextran coating (Dx1) on ferumoxytol. (Bottom) Immunohistochemistry demonstrates ferumoxytol uptake and immune cell infiltration at inflammatory lesions. Twenty-four hours after ferumoxytol administration, 7 µm-thick serial rat brain sections were stained with anti-Dx1 to detect the ferumoxytol coating and with anti-CD68 to detect macrophages. There was no Dx1 staining in live tumor cells (T), but the necrotic tumor cells (N) showed strong Dx1 staining that colocalized with macrophages. Dx1 also stained brain cells outside the lesion (arrows) that colocalized with astroglial cells (GFAP staining).

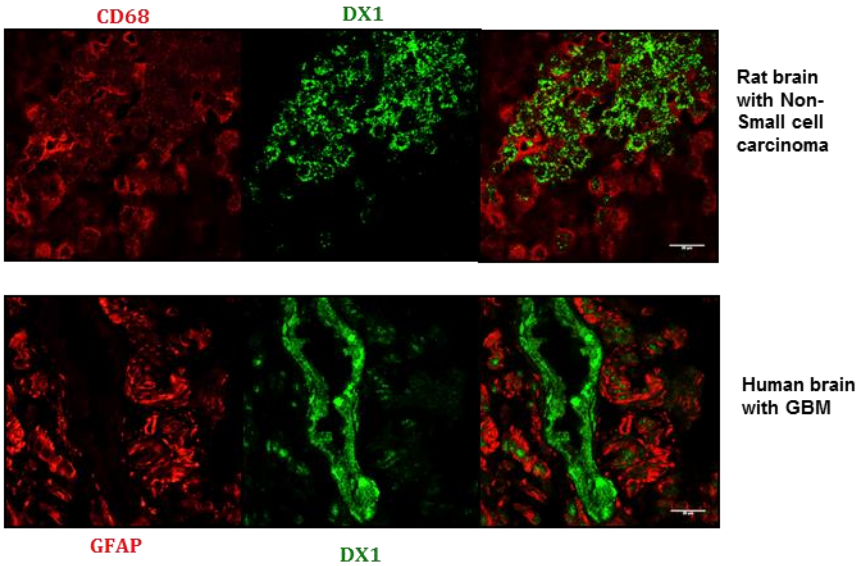


Figure 7. Immunofluorescent histochemistry confirms ferumoxytol uptake by macrophages and astrocytes at and around inflammatory lesions. Twenty-four hours after ferumoxytol administration, 7 µm-thick serial rat and human brain sections were simultaneously incubated with anti-Dx1 and either anti-CD68 or anti-GFAP, respectively. There was intracellular Dx1 staining with nuclear exclusion in CD68-positive cells in rat, as well as within GFAP-positive cells outside of a vessel in human brain. Scale bar = 20 microns

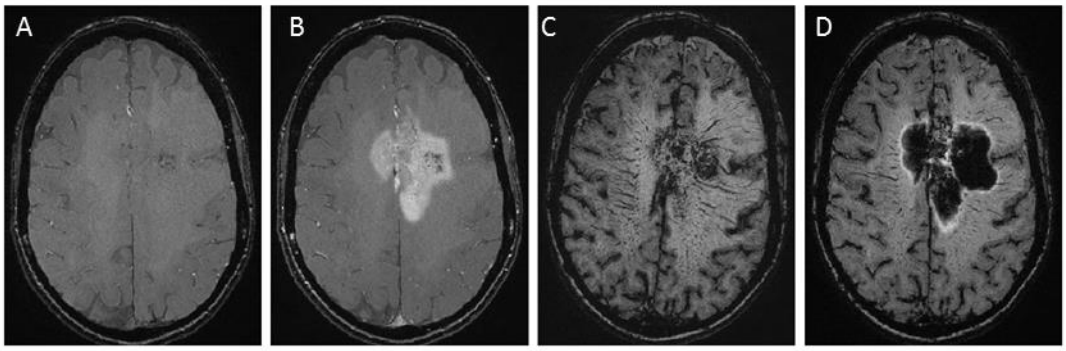
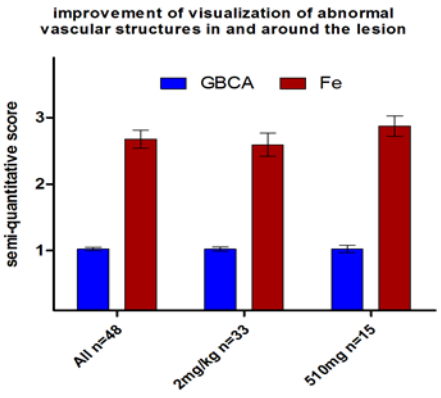


Figure 8: Perfusion imaging and delayed imaging with ferumoxtyol. T2* weighted gradient echo images (A) without contrast (B) Gadolinium based contrast agent (c) early scan(<1 hr) after Ferumoxytol injection (D)delayed (24hr) post ferumoxytol injection. Notice the high resolution of small vessels in the tumors and surrounding normal brain that in (C) is not visualized with Gadolinium based contrast agent (B). Delayed uptake of ferumoxytol (D)is a biomarker of inflammatory response

For the past three decades the OHSU Blood-Brain Barrier Program has developed significant laboratory and clinical expertise in imaging the CNS [49, 50]. We have shown that ferumoxytol is a safe and effective contrast agent that is not inferior to GBCA for brain tumor imaging, and can provide significantly better information regarding the tumor vasculature (Figure 9) [51]. We have developed multiple clinical trials to assess ferumoxytol as a MRI contrast agent for anatomic and dynamic MRI of intracerebral tumors. Based on our studies, ferumoxytol has been granted orphan drug status for the imaging of brain tumors and we are working with the FDA to move towards market approval of ferumoxytol for brain tumor MRI. We have clarified with the FDA that ferumoxytol can be used as diagnostic imaging agent in addition to pembrolizumab which is the therapeutic agent being evaluated, both under separate investigational new drug applications (IND).

Figure 9. Ferumoxytol for MRI contrast. The improved visualization of abnormal vasculature with ferumoxytol at 510 mg was non inferior to gadoteridol and provides additional information to gadoteridol. A multicenter phase 3 clinical trial is being designed to support FDA market approval of ferumoxytol as an MR imaging agent in CNS neoplasms. (Varallyay et al Poster presented at RSNA , December 2014).



Although our work concentrates on CNS metastases, similar imaging modalities may be useful for the assessment of therapeutic efficacy and immune response in systemic metastases. The

phenomenon of immune flare in solid non-CNS tumors may be equivalent to pseudoprogression in CNS malignancies. Steady-state feMRI has been used to assess tumor vasculature in cancers of the breast, pancreas and prostate [52-54]. A subset of subjects enrolled in this study may also have systemic metastases. This imaging technique uses ferumoxytol to assess tumor vascularity, and is analogous to our work in the CNS. This will allow direct comparison of these metastatic sites, to determine if we can delineate immune flare from therapeutic response and the immune component of flare. In both CNS and systemic metastases, we hypothesize that perfusion MRI using steady-state ferumoxytol imaging will provide a measure of rCBV indicative of active tumor growth, while delayed imaging of ferumoxytol may provide an important biomarker of inflammation and immune response. We hypothesize that feMRI techniques will provide a better modality than immune response criteria for evaluating the response to pembrolizumab in NSCLC metastases.

4.6 Trial Description

This study will focus on NSCLC subjects with brain metastases that have failed or refused first line therapy with standard platinum doublet therapy and have 10 or less brain metastases. A significant survival benefit has been shown with radiation in this population, and surgery or SRS is the standard of care for subjects with fewer than 10 brain lesions. The JLGK0901 Study evaluated the role of SRS for brain metastasis patients[55-57]. This prospective observational study, including 1194 brain metastasis patients, clearly showed the non-inferiority of SRS without WBRT as the initial treatment for those with 5–10 lesions versus patients with 2–4 in terms of overall survival (OS). Their results are considered the highest level of evidence which would allow SRS without whole brain radiation to be advocated for patients with up to 10 metastatic brain lesions. This clinical trial will study will evaluate the sensitivity and specificity of ferumoxytol steady state images to detect true tumor progression from pseudo progression in subjects receiving SRS with a PD-1 inhibitor, pembrolizumab[58].

A total of twenty (20) subjects scheduled to receive stereotactic radiosurgery (SRS) for brain metastases will be enrolled in this single institution pilot study. Subjects with up to ten measurable target metastatic brain lesions secondary to non-small cell lung carcinoma (NSCLC), with adequate performance status, baseline hematological and metabolic parameters will receive pembrolizumab (200mg intravenous every 3 weeks) in addition to standard of care SRS, until persistent radiographic progression is confirmed. Subjects may undergo surgery to one or more of the target lesions if clinically indicated prior to enrollment. Subjects will be allowed to continue on pembrolizumab 200mg every 3 weeks until suspected radiographic progression on clinical standard of care MRI. At suspected radiographic progression, subjects will be allowed to continue on Pembrolizumab for 2 more doses until follow up clinical and feMRI within 6 weeks (+/- 5 days) confirms persistent radiographic progression.

Upon confirmation of persistent radiographic progression, subjects will be evaluated for repeat surgery. After surgery, if histopathology confirms true progression, subjects will be taken off study; patients who are not candidates for surgery will also be taken off the study. Subjects whose biopsy suggests pseudoprogression will be allowed to continue on pembrolizumab 200 mg Q weekly until further radiographic progression is confirmed.

If, after initial suspected radiographic progression, persistent radiographic progression is not confirmed at follow up, subjects will continue on pembrolizumab until further persistent radiographic progression is detected and confirmed. Subjects with evidence of new lesions (not present at entry to the study) in the CNS or systemically that fulfil criteria for progression by immune related response criteria (irRC) [1] will be taken off study drug, but will be followed with serial clinical MRI scans until death.

In addition, subjects that are already enrolled in the study and have measurable systemic disease by RECIST 1.1 Criteria [2] at screening may undergo additional MRI sequences of the systemic disease to evaluate the immunological response to pembrolizumab outside the CNS (systemic disease). Patients who are taken off study due to persistent radiographic progression , who are not candidates for biopsy, refuse biopsy or have stable brain disease but have systemic disease progression may be continued on pembrolizumab at the discretion of the treating physician, if there is evidence of clinical benefit from continuation of therapy. They will be followed up with clinical MRI scans and evaluated for survival, but will not be included in the analysis for primary end-points

Blood for biomarker evaluation will be drawn as described in section 8.5. These markers will be correlated with imaging findings and survival.

If available, any archival biopsy, fresh tissue obtained up to 2 months prior to enrollment as well as tissue obtained at persistent radiographic progression will be obtained for clinical pathology review at OHSU. Brain Tissue samples will also be sent to QualTek Molecular Laboratories for PD-1 biomarker assay as described in section 8.5.

The trial population will correspond to those subjects within protocol MK-3475-PN010 that would otherwise be excluded due to brain metastases. We feel this data would be adjunct to what Merck will obtain from protocol 10 and help strengthen the assessment of PD1 activity in both brain metastases and systemic disease. OHSU/Portland VA is a MK-3475-PN010 clinical trial site. A separate but similar study design may be considered for evaluating subjects with advanced malignant melanoma with brain metastases in the future.

OHSU, along with the Veterans Administration Medical center, Portland, Oregon is the largest academic medical center and cancer center in the state with a large referral base from adjacent states. The high volume of subjects with lung cancer ensures attainment of anticipated accrual targets for this trial.

4.7 Pharmaceutical and Therapeutic Background

The importance of intact immune surveillance in controlling outgrowth of neoplastic transformation has been known for decades. Accumulating evidence shows a correlation between tumor-infiltrating lymphocytes (TILs) in cancer tissue and favorable prognosis in various malignancies. In particular, the presence of CD8+ T-cells and the ratio of CD8+ effector T-cells / FoxP3+ regulatory T-cells seems to correlate with improved prognosis and long-term survival in many solid tumors.

The PD-1 receptor-ligand interaction is a major pathway hijacked by tumors to suppress immune control. The normal function of PD-1, expressed on the cell surface of activated T-cells under healthy conditions, is to down-modulate unwanted or excessive immune responses, including autoimmune reactions. PD-1 (encoded by the gene Pdcd1) is an Ig superfamily member related to CD28 and CTLA-4 which has been shown to negatively regulate antigen receptor signaling upon engagement of its ligands (PD-L1 and/or PD-L2). The structure of murine PD-1 has been resolved. PD-1 and family members are type I transmembrane glycoproteins containing an Ig Variable-type (V-type) domain responsible for ligand binding and a cytoplasmic tail which is responsible for the binding of signaling molecules. The cytoplasmic tail of PD-1 contains 2 tyrosine-based signaling motifs, an immunoreceptor tyrosine-based inhibition motif (ITIM) and an immunoreceptor tyrosine-based switch motif (ITSM). Following T-cell stimulation, PD-1 recruits the tyrosine phosphatases SHP-1 and SHP-2 to the ITSM motif within its cytoplasmic tail, leading to the dephosphorylation of effector molecules such as CD3ζ, PKCθ and ZAP70 which are involved in the CD3 T-cell signaling cascade. The mechanism by which PD-1 down modulates T-cell responses is similar to, but distinct from that of CTLA-4 as both molecules regulate an overlapping set of signaling proteins. PD-1 was shown to be expressed on activated lymphocytes including peripheral CD4+ and CD8+ T-cells, B-cells, T regs and Natural Killer cells. Expression has also been shown during thymic development on CD4-CD8- (double negative) T-cells as well as subsets of macrophages and dendritic cells. The ligands for PD-1 (PD-L1 and PD-L2) are constitutively expressed or can be induced in a variety of cell types, including non-hematopoietic tissues as well as in various tumors. Both ligands are type I transmembrane receptors containing both IgV- and IgC-like domains in the extracellular region and contain short cytoplasmic regions with no known signaling motifs. Binding of either PD-1 ligand to PD-1 inhibits T-cell activation triggered through the T-cell receptor.

PD-L1 is expressed at low levels on various non-hematopoietic tissues, most notably on vascular endothelium, whereas PD-L2 protein is only detectably expressed on antigen-presenting cells found in lymphoid tissue or chronic inflammatory environments. PD-L2 is thought to control immune T-cell activation in lymphoid organs, whereas PD-L1 serves to dampen unwarranted T-cell function in peripheral tissues. Although healthy organs express little (if any) PD-L1, a variety of cancers were demonstrated to express abundant levels of this T-cell inhibitor. PD-1 has been suggested to regulate tumor-specific T-cell expansion in subjects with melanoma (MEL). This suggests that the PD-1/PD-L1 pathway plays a critical role in tumor immune evasion and should be considered as an attractive target for therapeutic intervention.

Pembrolizumab is a potent and highly selective humanized monoclonal antibody (mAb) of the IgG4/kappa isotype designed to directly block the interaction between PD-1 and its ligands, PD-L1 and PD-L2. Pembrolizumab has recently been approved in the United Stated for the treatment of subjects with unresectable or metastatic melanoma and disease progression following ipilumumab and, if BRAF V600 mutation positive, a BRAF inhibitor.

Our preliminary data suggests that use of bevacizumab may alter rCBV measurements in high grade gliomas. For the purpose of this study, a short course of up to 3 doses of bevacizumab

may be allowed, when clinically indicated, as an anti-edema measure and will not be criteria for exclusion. Bevacizumab is preferred over steroids when studying immune-modulatory therapies, since use of steroids can potentially dampen the immune response and outcomes. Study MRI will be performed prior to the start of bevacizumab therapy whenever possible. Imaging data from subjects who require more than 3 doses of bevacizumab will be stratified and analyzed separately. A separate analysis of subjects based on their avastin and steroid dose will be performed as part of the final analysis. Currently, the differences in the pathophysiology or response to bevacizumab in pseudoprogression resulting radiation or PD-1 inhibitors is not clear. This study will provide critical data that can provide insight to the use of bevacizumab as a steroid sparing agent to control the edema and mass effect. There are several ongoing studies in several tumor types (including brain metastases) evaluating the efficacy of the pembrolizumab and bevacizumab combination, however no study has a completed dataset. However, none of the studies are investigating the role of bevacizumab in pseudoprogression.

4.8 Clinical Safety Data with Ferumoxytol

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 in this study will be given in a minimum of two separate, fractionated doses. The rate of administration of the second dose 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		Rapid Injection (2009-31 Mar 2015) Estimated Exposure: 556,177	
	No of Cases	Reporting Rate (Estimated)	No of Cases	Reporting Rate (Estimated)
Anaphylaxis	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 submitted for publication in December of 2016)

	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.

4.8.1 Rationale for the Trial and Selected Subject Population

Subjects with metastatic brain tumors are frequently excluded from Clinical trials of systemic disease. Systemic therapy has consistently failed to show benefit in brain metastases due to the presence of the blood brain barrier, among other things [59]. Pembrolizumab acts on the T-cells in the periphery to augment systemic immune response and thus does not have to cross the blood brain barrier and. A synergetic effect of combined immunotherapy and stereotactic

radiation is expected to promote the release of tumor specific antigens that can then direct antigen- presenting cells to induce a T cell mediated immune response [60-63]. Prior studies have demonstrated the safety and efficacy of pembrolizumab for systemic non–small-cell lung cancer [9]. Since pembrolizumab acts at the level of T–cells, it does not have to cross the blood brain barrier and hence is expected to be efficacious in our cohort with brain metastases as well.

Half of all patients with NSCLC develop brain metastases during the course of their disease. Approximately 10% of US patients with NSCLC have an activating mutation with in the epidermal growth factor receptor (EGFR) while 3-5% will have chromosomal translocation involving the gene coding for the protein anaplastic lymphoma kinase (ALK).[64, 65] Patients with oncogenic driver mutations within the EGFR or ALK are currently treated with individual TKI with specific activity against such mutations. The ability of these TKIs to penetrate the BBB and control CNS metastatic disease vary significantly between different TKIs with 3rd generation EGFR-TKI (e.g. osimertinib) and 2nd and 3rd generation ALK-TKI (alectinib, ceritinib, brigatinib and lorlatinib) achieving therapeutic CSF levels while earlier generation TKIs (e.g. erlotinib, gefitinib, afatinib and crizotinib) failing to reach therapeutic CNS levels. Pharmacologic difference in CNS penetrance among various TKIs accounts for the wide variations in incidence and control rate of CNS metastases among EGFR and ALK-mutated NSCLC patients treated with different TKIs, as well as the intra-patient discrepancy observed between visceral and CNS disease control rate. For enrollment in this study, Subjects with EGFR or ALK driver mutations should have disease progression on FDA-approved therapy for these driver mutations. Subjects who develop brain metastases (while on FDA-approved therapies for EGFR or ALK aberrations) may be included at the discretion of the treating oncologist, depending on whether an EGFR or ALK- specific TKI with proven CNS activity is available or not and whether visceral disease outside the CNS is controlled or progressing on the current treatment.

Patients for whom an FDA-approved TKI with adequate CNS penetration is available will be excluded. Our accrual goal is a total of 20 patients over 3 years and excluding these patients will not affect the study accrual.

Significance:
With immunotherapy, a transient increase in size of lesion on imaging called flair response is frequently seen before a response is noted [34]. A similar increase in size of the lesion is noted after radiation therapy in primary brain tumors [19, 43, 66]. Currently, subjects who have imaging changes within the first 12 weeks after chemo-radiation are followed up with serial scans to confirm true vs pseudoprogression and may sometimes be excluded from clinical trials due to the lack of validated noninvasive methods to distinguish true progression from pseudoprogression [30, 67].

Our group has demonstrated that rCBV measured by ferumoxytol steady state imaging is very helpful in distinguishing pseudoprogression from true disease progression in brain tumors. [43]. Accurate diagnosis of pseudoprogression will prevent subjects being taken off a potentially beneficial clinical trial when there is an anatomical increase in tumor burden after radiation or immunotherapy. This study will validate and determine the sensitivity and specificity of ferumoxytol steady state imaging in distinguishing the two entities. This study

will also provide information that will be invaluable in designing future trials for metastatic as well as primary brain tumors and help improve the practice of neuro-oncology.

4.8.2 Rationale for Dose Selection/Regimen/Modification

An open-label Phase I trial (Protocol 001) is being conducted to evaluate the safety and clinical activity of single agent MK-3475. The dose escalation portion of this trial evaluated three dose levels, 1 mg/kg, 3 mg/kg, and 10 mg/kg, administered every 2 weeks (Q2W) in subjects with advanced solid tumors. All three dose levels were well tolerated and no dose-limiting toxicities were observed. This first in human study of MK-3475 showed evidence of target engagement and objective evidence of tumor size reduction at all dose levels (1 mg/kg, 3 mg/kg and 10 mg/kg Q2W). No MTD has been identified to date. 10.0 mg/kg Q2W, the highest dose tested in PN001, will be the dose and schedule utilized in Cohorts A, B, C and D of this protocol to test for initial tumor activity. Recent data from other clinical studies within the MK-3475 program has shown that a lower dose of MK-3475 and a less frequent schedule may be sufficient for target engagement and clinical activity.

PK data analysis of MK-3475 administered Q2W and Q3W showed slow systemic clearance, limited volume of distribution, and a long half-life (refer to IB). Pharmacodynamic data (IL-2 release assay) suggested that peripheral target engagement is durable (>21 days). This early PK and pharmacodynamic data provides scientific rationale for testing a Q2W and Q3W dosing schedule.

A population pharmacokinetic analysis has been performed using serum concentration time data from 476 subjects. Within the resulting population PK model, clearance and volume parameters of MK-3475 were found to be dependent on body weight. The relationship between clearance and body weight, with an allometric exponent of 0.59, is within the range observed for other antibodies and would support both body weight normalized dosing or a fixed dose across all body weights. MK-3475 has been found to have a wide therapeutic range based on the melanoma indication. The differences in exposure for a 200 mg fixed dose regimen relative to a 2 mg/kg Q3W body weight based regimen are anticipated to remain well within the established exposure margins of 0.5 – 5.0 for MK-3475 in the melanoma indication. The exposure margins are based on the notion of similar efficacy and safety in melanoma at 10 mg/kg Q3W vs. the proposed dose regimen of 2 mg/kg Q3W (i.e. 5-fold higher dose and exposure). The population PK evaluation revealed that there was no significant impact of tumor burden on exposure. In addition, exposure was similar between the NSCLC and melanoma indications. Therefore, there are no anticipated changes in exposure between different indication settings.

The choice of the 200 mg every 3 weeks is an appropriate dose based on simulations performed using the population PK model of pembrolizumab showing that the fixed dose of 200 mg every 3 weeks will provide exposures that 1) are optimally consistent with those obtained with the 2 mg/kg dose every 3 weeks, 2) will maintain individual subject exposures in the exposure range established in melanoma as associated with maximal efficacy response and 3) will maintain individual subjects exposure in the exposure range established in melanoma that are well tolerated and safe.

This fixed FDA approved dose regimen (200 mg, intravenously every 3 weeks) will simplify the dosing regimen to be more convenient for physicians and to reduce potential for dosing errors. A fixed dosing scheme will also reduce complexity in the logistical chain at treatment facilities and reduce wastage.

4.8.3 Rationale for Endpoints

4.8.3.1 Efficacy Endpoints

This pilot study is designed to determine the sensitivity and specificity of ferumoxytol steady state imaging in distinguishing true progression in subjects who will receive SRS with Immunotherapy (pembrolizumab) for brain metastases. This information will be used to validate this novel imaging biomarker and help design future brain tumor studies that incorporate immunotherapy. We will use radiographic progression to determine a subject's suitability to continue on treatment. Furthermore, since the benefits of immunotherapy can take months to become apparent, we will not make treatment decisions within the first 12 weeks and at the time of suspected progression, no change in therapy will be made until persistent radiographic progression is noted on a follow up scan at least 6 weeks later.

The secondary endpoints will provide preliminary data on safety, toxicity, and efficacy (progression free survival, overall survival, best response and duration of best response survival) in brain metastasis from NSCLC receiving pembrolizumab in addition to standard of care treatments. Data from this pilot study will be used to power a larger study.

4.8.4 Biomarker Research End Points

Blood Biomarker

The number and type of T cell can suggest response to immunotherapy in various cancers [68]. This exploratory study will look at serum immune cell subtypes at various time points and correlate them with radiographic and clinical outcomes. This is further elaborated in section 8.5.

Tissue Biomarker

The type and number of tumor infiltrating immune cells including lymphocytes and macrophages correlate with prognosis [69]. Data in melanoma and breast cancer suggest a correlation between the immunophenotype to treatment response [70, 71]. This study will explore the changes in the PDL-1 expression (before and after therapy with pembrolizumab) and types of tumor infiltrating immune cells with radiographic and clinical outcomes. This is further elaborated on in section 8.5.

5.0 METHODOLOGY

5.1 Diagnosis/Condition for Entry into the Trial

Subjects with up to ten measurable target metastatic brain lesions secondary to non-small cell lung carcinoma (NSCLC) to include adenocarcinoma and squamous cell carcinoma by histology

5.1 Subject Inclusion Criteria

In order to be eligible for participation in this trial, the subject must:

1. Be willing and able to provide written informed consent/assent for the trial.
2. Be ≥ 18 years of age on day of signing informed consent.
3. Have a histologically confirmed diagnosis of NSCLC
4. Have up to ten measurable (by RANO) brain metastasis planned for stereotactic radiosurgery.
5. Have PD-L1 expression of greater than 1%.
6. Subjects may already be receiving PD-1/L1 inhibitors (including pembrolizumab or other PD-(L)1 inhibitors such as nivolumab, atezolizumab, avelumab, durvalumab) for the treatment of systemic disease. A washout period of at least 3 weeks is required from the last dose of PD-(L)1 inhibitor.
7. Subjects with EGFR or ALK genomic tumor aberrations should have documented disease progression on FDA-approved therapy for these aberrations. Subjects with EGFR or ALK genomic tumor aberrations who develop new brain metastases may be included at the discretion of the treating physician.
8. Have a performance status of 0-2 on the ECOG Performance Scale.
9. Demonstrate adequate organ function as defined in Table 3.
10. Female subject of childbearing potential should have a negative urine or serum pregnancy within 72 hours prior to receiving the first dose of study medication. If the urine test is positive or cannot be performed or confirmed as negative, a serum pregnancy test will be required.
11. Female subjects of childbearing potential should be willing to use 2 methods of birth control or be surgically sterile, or abstain from heterosexual activity for the course of the study through 120 days after the last dose of study medication. Subjects of childbearing potential are those who have not been surgically sterilized or have not been free from menses for > 1 year. Male subjects should agree to use an adequate method of contraception starting with the first dose of study therapy through 120 days after the last dose of study therapy. (Section 7.16).

Table 3 Adequate Organ Function Laboratory Values

System	Laboratory Value
Hematological	
Absolute neutrophil count (ANC)	≥1,500 /mcL
Platelets	≥100,000 / mcL
Hemoglobin	≥9 g/dL or ≥5.6 mmol/L without transfusion or EPO dependency (within 7 days of assessment)
Renal	
Serum creatinine **OR** Measured or calculated[a] creatinine clearance (GFR can also be used in place of creatinine or CrCl)	≤1.5 X upper limit of normal (ULN) **OR** ≥60 mL/min for subject with creatinine levels > 1.5 X institutional ULN
Hepatic	
Serum total bilirubin	≤ 1.5 X ULN **OR**
	Direct bilirubin ≤ ULN for subjects with total bilirubin levels > 1.5 ULN
AST (SGOT) and ALT (SGPT)	≤ 2.5 X ULN **OR** ≤ 5 X ULN for subjects with liver metastases

Albumin	≥2.5 mg/dL
Coagulation	
International Normalized Ratio (INR) or Prothrombin Time (PT) Activated Partial Thromboplastin Time (aPTT)	≤1.5 X ULN unless subject is receiving anticoagulant therapy as long as PT or PTT is within therapeutic range of intended use of anticoagulants ≤1.5 X ULN unless subject is receiving anticoagulant therapy as long as PT or PTT is within therapeutic range of intended use of anticoagulants
[a]Creatinine clearance should be calculated per institutional standard.	

5.2 Subject Exclusion Criteria

The subject must be excluded from participating in the trial if the subject:

1. Has evidence of leptomeningeal disease on MRI or in CSF.
2. If tumor demonstrates EGFR or ALK genomic tumor aberrations, subject should have documented disease progression on FDA-approved therapy for these aberrations.
3. Has a diagnosis of immunodeficiency and is not on continuous daily immunosuppressive therapy within 7 days prior to the first dose of trial treatment. (Subjects may receive steroids before or after SRS to prevent or manage cerebral edema; inhalational steroids are permitted)
4. Has previously progressed on a PD-1 or PD-L1 checkpoint inhibitor for systemic disease.
5. Has a known history of active TB (Bacillus Tuberculosis)
6. Has hypersensitivity to pembrolizumab, or ferumoxytol or any of their excipients.
7. Has had a prior anti-cancer monoclonal antibody (mAb) within 4 weeks prior to study Day 1 or who has not recovered (i.e., ≤ Grade 1 or at baseline) from adverse events due to agents administered more than 4 weeks earlier.
    a. Note: The use of Denosumab is an exception to this criterion.
8. Subject who has not recovered (i.e., ≤ Grade 1 or at baseline) from adverse events due to a previously administered agent.
    a. Note: Subjects with ≤ Grade 2 neuropathy are an exception to this criterion and may qualify for the study.
    b. Note: Subjects with ≤ Grade 2 hematologic toxicities are an exception to this criterion and may qualify for the study.
    c. Note: Subjects with ≤ Grade 2 fatigue are an exception to this criterion and may qualify for the study.
9. Has a known additional malignancy that is progressing or requires active treatment. Exceptions include basal cell carcinoma of the skin or squamous cell carcinoma of the skin that has undergone potentially curative therapy or in situ cervical cancer.
10. Has active autoimmune disease that has required systemic treatment in the past 2 years (i.e. with use of disease modifying agents, corticosteroids or immunosuppressive drugs). Replacement therapy (eg., thyroxine, insulin, or physiologic corticosteroid replacement therapy for adrenal or pituitary insufficiency, etc.) is not considered a form of systemic treatment.
11. Has known history of, or any evidence of active, non-infectious pneumonitis.
12. Has an active infection requiring systemic therapy.
13. Has a history or current evidence of any condition, therapy, or laboratory abnormality that might confound the results of the trial, interfere with the subject's participation

for the full duration of the trial, or is not in the best interest of the subject to participate, in the opinion of the treating investigator.

14. Has known psychiatric or substance abuse disorders that would interfere with cooperation with the requirements of the trial.
15. Is pregnant or breastfeeding, or expecting to conceive or father children within the projected duration of the trial, starting with the pre-screening or screening visit through 120 days after the last dose of trial treatment.
16. Has a known history of Human Immunodeficiency Virus (HIV) (HIV 1/2 antibodies).
17. Has known active Hepatitis B (e.g., HBsAg reactive) or Hepatitis C (e.g., HCV RNA [qualitative] is detected).
18. Has received a live vaccine within 30 days of planned start of study therapy.
    a. *Note: Seasonal influenza vaccines for injection are generally inactivated flu vaccines and are allowed; however intranasal influenza vaccines (e.g., Flu-Mist®) are live attenuated vaccines, and are not allowed.*
19. 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, are not eligible.
20. Subjects with known allergic or hypersensitivity reactions to parenteral iron, parenteral dextran, parenteral iron-dextran, or parenteral iron-polysaccharide preparations (Ferumoxytol Investigator's Drug Brochure, 2009). *Subjects with significant drug or other allergies or autoimmune diseases may be enrolled at the investigator's discretion.*
21. Subjects who have a contraindication for 3T MRI: metal in their bodies (a cardiac pacemaker or other incompatible device), are severely agitated, or have an allergy to gadolinium containing contrast material.
22. Subjects with known iron overload (genetic hemochromatosis). In subjects with a family history of hemochromatosis, hemochromatosis must be ruled out prior to study entry with normal values of the following blood tests: Transferrin saturation (TS) test and Serum ferritin (SF) test. All associated costs will be paid by the study.
23. Subject who have received ferumoxytol within 3 weeks of study entry.
24. Subjects with three or more drug allergies from separate drug classes.

5.3 Subject Withdrawal/Discontinuation Criteria

Subjects may withdraw consent at any time for any reason or be dropped from the trial at the discretion of the investigator should any untoward effect occur. In addition, a subject may be withdrawn by the investigator or the Sponsor if enrollment into the trial is inappropriate, the trial plan is violated, or for administrative and/or other safety reasons.

A subject must be discontinued from the trial for any of the following reasons:

- The subject or legal representative (such as a parent or legal guardian) withdraws consent.
- Persistent radiographic progression confirmed by tissue biopsy.
- Persistent radiographic progression if subject is not a candidate for repeat surgery or subject refuses a repeat biopsy to pathological confirmed of disease progression

Note: A subject may be granted an exception to continue on treatment with confirmed radiographic progression if clinically stable or clinically improved, at the discretion of the treating physician. Please see Section 7.4.9

- Unacceptable adverse experiences as described in Section 9.0
- Miss two consecutive treatments for any reason other than surgery
- Intercurrent illness that prevents further administration of treatment
- Investigator's decision to withdraw the subject
- The subject has a confirmed positive serum or urine pregnancy test. If the urine test is positive, cannot be performed or cannot be confirmed as negative, a serum pregnancy test will be required. Noncompliance with trial treatment or procedure requirements
- The subject is lost to follow-up
- Completed 24 months of uninterrupted treatment with pembrolizumab or 35 administrations of study medication, whichever is later.
  Note: 24 months of study medication is calculated from the date of first dose. Subjects who stop pembrolizumab after 24 months may be eligible for up to one year of additional study treatment if they progress after stopping study treatment provided they meet the requirements detailed in Section 5.4.1
- Administrative reasons

The End of Treatment and Follow-up visit procedures are listed in the Schedule of Events (Section 6). After the end of treatment, each subject will be followed for 30 days for adverse event monitoring (serious adverse events will be collected for 90 days after the end of treatment as described in Section 9). Subjects who discontinue for reasons other than progressive disease will have post-treatment follow-up for disease status until disease progression, initiating a non-study cancer treatment, withdrawing consent or becoming lost to follow-up. After documented disease progression each subject will be followed by telephone for overall survival until death, withdrawal of consent, or the end of the study, whichever occurs first.

5.3.1 Discontinuation of Study Therapy after CR

Discontinuation of treatment may be considered for subjects who have attained a confirmed CR in the brain and have been treated for at least 24 months with pembrolizumab.

At the discretion of the investigator, subjects who have had CR in the brain but develop progressive systemic disease may be eligible for up to one year of additional treatment with pembrolizumab via the Second Course Phase, if no additional cancer treatment was administered since the last dose of pembrolizumab, the subject meets the safety parameters listed in the Inclusion/Exclusion criteria, and the trial is open. Subjects will resume therapy at the same dose and schedule at the time of initial discontinuation. Additional details are provided in Section 7.4.9.

5.3.2 Subject Replacement Strategy

Subjects that are unevaluable will be replaced. Subjects that are unevaluable are those who do not reach the first follow-up imaging time point (12 weeks after first pembroluzimab infusion).

5.3.3 Clinical Criteria for Early Trial Termination

Early trial termination will be the result of the criteria specified below:

1. Quality or quantity of data recording is inaccurate or incomplete
2. Poor adherence to protocol and regulatory requirements
3. Incidence or severity of adverse drug reaction in this or other studies indicates a potential health hazard to subjects
4. Plans to modify or discontinue the development of the study drug

In the event of Merck decision to no longer supply study drug, ample notification will be provided so that appropriate adjustments to subject treatment can be made.

6.0 SCHEDULE OF EVENTS

	Screening visit: within 30 days of first treatment	Treatment: every 3 weeks (+15 days) for up to 2 years or 32 cycles	At suspected radiographic progression (±15 days)	At persistent radiographic progression (±15 days)	At discontinuation of treatment (±15 days) (unless testing has been done within 1 month)	Safety follow-up: 30 days (±15 days) post discontinuation of pembro	Follow-up: for subjects who complete 2 years of treatment or 32 cycles	Survival: follow-up once off study[8]
Informed Consent	X							
Inclusion/Exclusion Criteria	X							
Archival Tissue Collection (if available)	X							
Biopsy or Resection of Metastatic Brain Lesion and biomarkers[1]	X[1]			X[1]				
Demographics and Medical History	X							
Medication, herbal or dietary supplement review	X	X			X	X	X	
Trial Treatment Administration (Pembrolizumab)		X[11]						
Review Adverse Events, include Events of Clinical Interest[10]		X				X		
Physical Examination[2]	X	X			X	X	X	
Vital Signs and Weight	X	X			X	X	X	
ECOG Performance Status	X	X			X	X	X	
CBC with Differential[3]	X	X			X	X		
Comprehensive Serum Chemistry Panel[3]	X	X			X	X		
Urinalysis	X							
FT4 and TSH[4]	X	X				X		
PT/INR, aPTT	X					X		
Pregnancy Test (urine or serum, females only)	X							
Clinical Tumor Imaging	X	X[5]					X[7]	
Ferumoxytol imaging		X[6]						
Biomarker Collection (serum) - optional		X[6]						
PET scan abd, chest, pelvis	X[9]							
Post-study anticancer therapy status							X	
Survival Status							X	X
Telephone contact								X[8]

[1]Brain surgery is not necessary before enrollment. However, a brain surgery may be done prior to enrollment or at the time of persistent radiographic progression if clinically indicated. Pembrolizumab needs to be stopped for 4 weeks prior to and will not be restarted for at least 4 weeks after surgery (section 7.2). All tissue will be sent for biomarkers.

[2]Physical exam only required with every pembrolizumab infusion.

[3]CBC and CMP to be done with every cycle while on pembrolizumab – labs must be done within 30 days before first pembrolizumab infusion, and within 72 hours before subsequent infusions.

[4] FT4 and TSH with every cycle while on pembrolizumab

[5]Clinical standard of care MRIs (gadolinium with perfusion) are done at baseline, 12 weeks post radiation, then every 9 weeks, and 6 weeks after suspected radiographic progression (see special instructions below for evaluating persistent radiographic progression). All timepoints are ±15 days. Clinical scans may be done more frequently, at physician's discretion. Each MRI scan will be evaluated for progression.

[6]Ferumoxytol steady state MRIs (within 10 days of clinical standard of care MRIs) are done baseline, 12 weeks post radiation, anytime there is suspected radiographic progression and 6 weeks after suspected radiographic progression. All timepoints are ±15 days. Serum biomarkers will be obtained ±15 days of ferumoxytol MRI scan.

[7] Follow-up after completing 2 years of treatment or 32 cycles (whichever comes later): clinical standard of care MRI and clinical visit every 12 weeks (±30 days) for up to 1 year, and every 6 months (±30 days) until death.

[8] Survival follow-up once off study: Telephone contact every 12 weeks (±30 days) to assess for survival status until death, withdrawal of consent, the end of the study, or loss to follow-up, whichever occurs first.

[9]Evaluation of systemic disease burden**:** A standard of care PET /CT or CT abdomen, chest and pelvis with contrast will be obtained within 3 months of enrollment and every 6 months while on study thereafter.

[10]Adverse event review will be recorded in Epic continuously while on study. A separate Epic note will be done with each course. All AEs are assessed for attribution to pembrolizumab, ferumoxytol, and standard of care therapy.

[11] First dose of pembrolizumab will be given on the first day of radiation (±15 days), and every 3 weeks thereafter (+15 days).

Imaging instructions for evaluating persistent radiographic progression: if there is suspected progression, subjects undergo a ferumoxytol steady state MRI and continue on pembrolizumab for two more cycles (total of 6 weeks). At the 6 week point, clinical standard of care and ferumoxytol MRIs will be repeated to confirm persistent radiographic progression. If the 6 week scan confirms progression, then pembrolizumab is discontinued and subject is evaluated for surgery. If histopathology confirms pseudoprogression or is inconclusive, the subject will continue on pembrolizumab every 3 weeks until further radiographic progression is

suspected. If histopathology confirms progression, the subject will be removed from the study. If the subject is not a surgical candidate, they will be taken off study and move into the survival follow up stage.

7.0 TREATMENT PLAN AND PROCEDURES

The Schedule of Events - Section 6.0 summarizes the trial procedures to be performed at each visit. Individual trial procedures are described in detail below. It may be necessary to perform these procedures at unscheduled time points if deemed clinically necessary by the investigator.

Furthermore, additional evaluations/testing may be deemed necessary by the Sponsor and/or Merck for reasons related to subject safety. In some cases, such evaluation/testing may be potentially sensitive in nature (e.g., HIV, Hepatitis C, etc.), and thus local regulations may require that additional informed consent be obtained from the subject. In these cases, such evaluations/testing will be performed in accordance with those regulations.

7.1 Trial Treatments

The treatment to be used in this trial is outlined below in Table 4

Table 4 Trial Treatment

Drug	Dose/Potency	Dose Frequency	Route of Administration	Regimen/Treatment Period	Use
Therapeutic Agent					
Pembrolizumab	200 mg	Every 3 weeks (+15 days)	IV infusion	Day 1 of each 3 week cycle	Experimental
Imaging Agent					
Ferumoxytol		Baseline, 12 weeks post radiation, anytime there is suspected radiographic progression and 6 weeks after suspected radiographic progression (±15 days).	IV infusion	Baseline, 12 weeks post radiation, anytime there is suspected radiographic progression and 6 weeks after suspected radiographic progression (±15days).	

Trial treatment should begin on the day of randomization or as close as possible to the date on which treatment is allocated/assigned.

7.1.1 Timing of Dose Administration

Pembrolizumab: Trial drug treatment should be administered on Day 1(+15 days) of each cycle after all procedures/assessments have been completed as detailed on the Trial Flow Chart (Section 6.0). Pembrolizumab will not be administered less than 14 days after any major surgeries including brain biopsy.

All trial treatments will be administered on an outpatient basis.

Pembrolizumab 200mg will be administered as IV infusion every 3 weeks as per institutional guidelines. The expiration of the reconstituted pembrolizumab is 6 hours at room temperature and 20 hours refrigerated. The total cumulative storage time at room temperature and refrigeration should not exceed 24 hours. The Pharmacy Manual contains specific instructions for the preparation of the Pembrolizumab infusion fluid and administration of infusion solution.

Ferumoxytol (only for study MRIs): The total dose of ferumoxytol will be 7mg/kg (not exceeding 510mg total). The ferumoxytol will be mixed with normal saline 1:1 solution to dilute to 15mg/ml. 1 mg/Kg IV will be injected at the start of the 7th dynamic cycle during the DSC acquisition at a flow rate of 3ml/s followed by 20ml saline flush. The rest of the ferumoxytol will be given as IV injections at a low flow rate (.1ml/s) followed by 20 mL saline flush. The flow rates may be adjusted at the physician's discretion.

Vitals monitoring for ferumoxytol MRIs: Blood pressure and pulse will be taken at the start of MRI (± 5 minutes), after each ferumoxytol injection (± 5 minutes), and again 30 minutes (± 5 minutes) after the first injection of ferumoxytol.

7.2 Study procedures narrative

Enrolled subjects will receive pembrolizumab (200 mg I.V. every 3 weeks) starting on the first day of radiation (±15 days) until persistent radiographic progression is confirmed. Subjects may receive standard of care cytotoxic chemotherapy (carboplatin and pemetrexed) while on trial, at the treating physician's discretion. [72, 73] MRI scans will be performed to assess response to therapy. Clinical standard of care MRIs are done at baseline, 12 weeks post radiation and then every 9 weeks thereafter until suspected radiographic progression. An early follow up clinical standard of care MRI will be performed 6 weeks (±15 days) from the date of MRI showing suspected radiographic progression to confirm persistent radiographic progression. Clinical scans may be done more frequently, based on physician's discretion. Each MRI scan will be evaluated for progression.

Additional ferumoxytol steady state MRIs will be done at baseline, 12 weeks after radiation, at suspected radiographic progression and within 6 weeks (±15 days) from suspected radiographic progression to confirm persistent radiographic progression.

When persistent radiographic progression is confirmed, subjects will be evaluated for a craniotomy with intent to complete resection and /or biopsy of the enhancing lesion, if this is thought to be the clinical standard of care.

Biopsy tissue obtained from surgery at persistent radiographic progression will be evaluated by the pathologists at OHSU. The pathologist will determine if the tissue is consistent with progression or pseudoprogression, where progression is defined as presence of viable metastatic carcinoma in the biopsy tissue and absence of tumor cell is assumed to reflect pseudoprogression. If histopathology confirms pseudoprogression the subject will continue on pembrolizumab every 3 weeks until further radiographic progression is suspected. If histopathology confirms progression, the subject will be removed from the study, followed clinically with serial clinical stand of care MRIs every 3 months for up to a year and then every 6 months until death or lost to follow-up.

The decision regarding surgery will be consensus based and will be made after each individual case has been presented at the OHSU interdisciplinary brain tumor board. If a subject is not a surgical candidate, or is unwilling to undergo surgery, they will be taken off study. They will, however, be eligible for continuation of pembrolizumab, at the physician's discretion, at the same dose and frequency, if clinical benefit is noted. They will be followed clinically with serial clinical standard of care MRI scans every 3 months for up to a year and then every 6 months until death or until lost to follow up. These subjects will not be included in the analysis the primary end-points but will be included in the survival analysis.

In subjects where the lesion remains unchanged or regresses in volume at 6 weeks) after the date of suspected radiographic progression, pembrolizumab will be continued until further persistent radiographic progression is confirmed.

All subjects will be followed with clinical examination and clinical standard of care MRI scans until death or lost to follow up. Subjects will be treated for up to 2 years, toxicity, or progression whichever comes first. Dose reductions/discontinuations for pembrolizumab will be as per Merck guidelines.

Pembrolizumab may be stopped for up to 3 months in the context of a craniotomy to confirm persistent radiographic progression or unrelated major surgery. Dosing delays of up to 7 days are permitted at physician discretion for medical / surgical events or logistical reasons. Dosing interruptions (missing a dose) are permitted in the case of medical / surgical events or logistical reasons not related to study therapy (e.g., elective surgery, medical events, subject vacation, and/or holidays), not to exceed two consecutive doses or a maximum of 9 weeks. The reason for interruption will be documented in the subject's study record. Pembrolizumab will not be administered less than 14 days after any major surgeries including brain biopsy.

7.3 Informed Consent

The Investigator must obtain documented consent from each potential subject prior to participating in a clinical trial.

Consent must be documented by the subject's dated signature or by the subject's legally acceptable representative's dated signature on a consent form along with the dated signature of the person conducting the consent discussion.

A copy of the signed and dated consent form should be given to the subject before participation in the trial.

The initial informed consent form, any subsequent revised written informed consent form and any written information provided to the subject must receive the IRB/ERC's approval/favorable opinion in advance of use. The subject or his/her legally acceptable representative should be informed in a timely manner if new information becomes available that may be relevant to the subject's willingness to continue participation in the trial. The communication of this information will be provided and documented via a revised consent form or addendum to the original consent form that captures the subject's dated signature or by the subject's legally acceptable representative's dated signature.

Specifics about a trial and the trial population will be added to the consent form template at the protocol level.

The informed consent will adhere to IRB/ERC requirements, applicable laws and regulations and Sponsor requirements.

7.4 Trial Procedures

See the Schedule of Events for when tests should be completed.

7.4.1 Clinical procedures:

- Demographics
- Medical history
- Prior medication review
    - The investigator or qualified designee will review prior medication and herbal and dietary supplement use, including any protocol-specified washout requirement, and record prior medication taken by the subject within 28 days before starting the trial. Treatment for the disease for which the subject has enrolled in this study will be recorded separately and not listed as a prior medication.
    - The investigator or qualified designee will record medication, if any, taken by the subject during the trial. All medications related to reportable SAEs and ECIs should be recorded as defined in Section 7.2.
    - Subjects will be asked to stop all nonprescription herbal supplements and over the counter medication.
- Full physical examination
- Vital signs
    - temperature, pulse, respiratory rate, height, weight and blood pressure. Height will be measured at screening only.
- ECOG performance status (Appendix)
- Archival tissue collection:
    - If available, any tumor biopsy tissue (lung, mediastinal, or brain) that was obtained prior to screening for the study will be requested and sent to the Merck-designated Contract Research Organization (CRO) QualTek Molecular Laboratories for PD-1 biomarker assay.
- Biopsy or resection of metastatic brain lesion
    - When clinically indicated, a biopsy or resection will be done of the brain lesion(s) 1) prior to enrollment and SRS, and 2) at the time of persistent radiographic progression. Any tumor biopsy tissue will be sent to the Merck-designated Contract Research Organization (CRO) QualTek Molecular Laboratories for PD-1 biomarker assay.
- Radiation therapy:
    - All patients will receive standard of care stereotactic radiosurgery in 1-5 fractions, at the discretion of the treating radiation oncologist. If all lesions cannot be treated on the same day, all lesions MUST be treated ≤14 days of treatment of the first lesion. Gamma knife or X-rays with nominal energy of 4 megavoltage (MV) or greater for accelerator-based treatments, including

isocentric conical collimators, mini-multi-leaf (5 mm or less) technology or linear accelerators mounted on robotic arms will be used to deliver the target dose.

- Target Volume: The volumes shall be defined by a planning MRI brain scan, with the patient in the treatment position. The target volume should include the enhancement seen on planning MRI with 0-1 mm margin. The maximal cross-sectional diameter must be < 3.0 cm for a lesion treated with a single fraction.
- Target Dose: The dose should be prescribed to the highest isodose line encompassing the 90-95% target volume. General dose guidelines are as follows
  - Lesion diameter < 1cm – 20-22 Gy in 1 fraction
  - Lesion diameter 1-2 cm – 18-20 Gy in 1 fraction
  - Lesion diameter 2-3 cm – 18 Gy in 1 fraction, or 27 Gy in 3 fraction or 30-35 Gy in 5 fractions
  - Lesion diameter 3-5cm – 30 – 35 Gy in 5 fractions
- Up to 8 of dexamethasone can be administered on the day of single fraction SRS, at the discretion of the treating physician.
- Radiation therapy to an additional symptomatic solitary lesion or to the brain may be allowed at the investigator's discretion at any time during the study if :
  - they fulfill other criteria to remain in the study and
  - this lesion has remained stable or shows radiographic response compared to baseline.
  - this additional lesion was not in the initial radiation field.

- Subjects may receive standard of care cytotoxic chemotherapy (carboplatin and pemetrexed) while on trial, at the treating physician's discretion. Chemotherapy to be administered per package insert.
- Subject may also provide consent/assent for future correlative research (optional). Subjects may participate in the main trial without participating in future correlative research.

7.4.2 Lab tests: see Table 5

- CBC with differential
- Comprehensive serum chemistry panel
- Urinalysis
- FT4, TSH
- PT/INR
- aPTT
- Pregnancy test (urine or serum) (women). If the urine test is positive, cannot be performed or cannot be confirmed as negative, a serum pregnancy test will be required.
- Serum biomarker assay (see Section 8.5.1)

Table 5 Laboratory Tests

Hematology	**Chemistry**	**Urinalysis**	**Other**
Hematocrit	Albumin	Blood	Serum β-human chorionic gonadotropin†
Hemoglobin	Alkaline phosphatase	Glucose	(β-hCG)†
Platelet count	Alanine aminotransferase (ALT)	Protein	PT (INR)
WBC (total and differential)	Aspartate aminotransferase (AST)	Specific gravity	aPTT
Red Blood Cell Count	Carbon Dioxide ‡	Microscopic exam *(If abnormal)*	Free tyroxine (T4)
Absolute Neutrophil Count	*(CO_2 or biocarbonate)*	results are noted	Thyroid stimulating hormone (TSH)
Absolute Lymphocyte Count	Calcium	Urine pregnancy test†	
	Chloride		Blood for correlative studies
	Glucose		
	Potassium		
	Sodium		
	Total Bilirubin		
	Direct Bilirubin *(If total bilirubin is elevated above the upper limit of normal)*		
	Total protein		

Laboratory tests for screening or entry into the Second Course Phase should be performed within 10 days prior to the first dose of treatment. After Cycle 1, pre-dose laboratory procedures can be conducted up to 72 hours prior to dosing. Results must be reviewed by the investigator or qualified designee and found to be acceptable prior to each dose of trial treatment.

7.4.3 Imaging

Standard of care clinical brain MRI: Clinical MRIs are done at baseline, 12 weeks post radiation and then every 9 weeks (see special instructions below for evaluating persistent radiographic progression). Gadolinium is the preferred contrast agent, when subject qualifies (per institutional policy) for gadolinium infusion, but it is not required. Clinical scans may be done more frequently, at physician's discretion. Each MRI scan will be evaluated for progression.

Ferumoxtyol MR: ferumoxytol steady state MRIs of the brain are done baseline, 12 weeks post radiation, anytime there is suspected radiographic progression and 6 weeks after suspected radiographic progression. Additional MR imaging of systemic lesions may be performed in the same setting and time points as the brain imaging. Details are explained in section 8.4.2. Blood pressure and pulse will be taken at the start of MRI ($\pm$ 5 minutes), after each ferumoxytol injection ($\pm$ 5 minutes), and again 30 minutes ($\pm$ 5 minutes) after the first injection of ferumoxytol.

Evaluation of systemic disease burden: A standard of care PET/CT or CT abdomen, chest and pelvis with contrast will be obtained within 3 months of enrollment and at least every 6 months thereafter. This is considered standard of care for patients with NSCLC. Stable systemic disease burden should be demonstrated at enrollment and for continuation of study drug.

7.4.3.1 Imaging instructions for evaluating persistent radiographic progression:

If there is suspected progression, subjects undergo a ferumoxytol steady state MRI and continue on pembrolizumab for two more cycles (total of 6 weeks). At the 6 week point, clinical standard of care and ferumoxytol MRIs will be repeated to confirm persistent radiographic progression. If the 6 week scan confirms progression, then pembrolizumab is discontinued and subject is evaluated for surgery. If histopathology confirms pseudoprogression, the subject will continue on pembrolizumab every 3 weeks until further radiographic progression is suspected. If histopathology confirms progression, the subject will be removed from the study.

7.4.4 Adverse Event Monitoring

The investigator or qualified designee will assess each subject to evaluate for potential new or worsening AEs as specified in the Schedule of Events and more frequently if clinically indicated. Adverse experiences will be graded and recorded throughout the study and during the follow-up period according to NCI CTCAE Version 4.0. Toxicities will be characterized in terms regarding seriousness, causality, toxicity grading, and action taken with regard to trial treatment. All AEs will be attributed to pembrolizumab or ferumoxytol separately.

For subjects receiving treatment with pembrolizumab all AEs of unknown etiology associated with pembrolizumab exposure should be evaluated to determine if it is possibly an event of clinical interest (ECI) of a potentially immunologic etiology (termed immune-related adverse events, or irAEs)

Please refer to Section 9 for detailed information regarding the assessment and recording of AEs.

7.4.5 Post-study anticancer therapy status

The investigator or qualified designee will review all new anti-neoplastic therapy initiated after the last dose of trial treatment. If a subject initiates a new anti-cancer therapy within 30 days after the last dose of trial treatment, the 30 day Safety Follow-up visit must occur before the first dose of the new therapy. Once new anti-cancer therapy has been initiated the subject will move into survival follow-up.

7.4.6 Safety Follow-Up Visit: 30 days post discontinuation

The mandatory Safety Follow-Up Visit should be conducted approximately 30 days after the last dose of trial treatment or before the initiation of a new anti-cancer treatment, whichever comes first. All AEs that occur prior to the Safety Follow-Up Visit should be recorded. Subjects with an AE of Grade > 1 will be followed until the resolution of the AE to Grade 0-1 or until the beginning of a new anti-neoplastic therapy, whichever occurs first. SAEs that occur within 90 days of the end of treatment or before initiation of a new anti-cancer treatment should also be followed and recorded. Subjects who are eligible for retreatment with pembrolizumab (as described in Section 7.4.9) may have up to two safety follow-up visits, one after the Treatment Period and one after the Second Course Phase.

7.4.7 Follow-up

Subjects who complete 2 years of treatment or 32 cycles (whichever comes later) will move into the Follow-Up Phase and should be assessed every 12 weeks (±30 days) by clinical standard of care MRI scans and clinical assessment to monitor disease status for up to 1 year or until they progress or death. After 1 year, the imaging and clinical assessment time point will occur every 6 months (± 30 days) until death. Follow-up may be done more frequently, at physician's discretion.

7.4.8 Survival Follow-up when off study

Once a subject is taken of study for any reason, the subject moves into the survival follow-up phase. Survival data will be collected from the medical record when available. When possible, the patient will be contacted by telephone approximately every 12 weeks to assess for survival status until death, withdrawal of consent, or the end of the study, whichever occurs first.

7.4.9 Second Course Phase (Retreatment Period)

Subjects who stop pembrolizumab with CR in the brain may be eligible for up to one year of additional pembrolizumab therapy if they progress systemically. This retreatment is termed the Second Course Phase of this study and is only available if the study remains open and the subject meets the following conditions:

Either

- Stopped initial treatment with pembrolizumab after attaining an investigator-determined confirmed CR, and
  - Was treated for at least 24 weeks with pembrolizumab before discontinuing therapy
  - Received at least two treatments with pembrolizumab beyond the date when the initial CR was declared

OR

- Had CR in the brain and stopped pembrolizumab treatment after 24 months of study therapy for reasons other than disease progression or intolerability

AND

- Experienced an investigator-determined confirmed radiographic disease progression after stopping their initial treatment with pembrolizumab
- Did not receive any anti-cancer treatment since the last dose of pembrolizumab
- Has a performance status of 0 or 1 on the ECOG Performance Scale
- Demonstrates adequate organ function as detailed in Section 5.2

Female subject of childbearing potential should have a negative serum or urine pregnancy test within 72 hours prior to receiving retreatment with study medication. If the urine test is positive, cannot be performed or cannot be confirmed as negative, a serum pregnancy test will be required.

- Female subject of childbearing potential should be willing to use 2 methods of birth control or be surgically sterile, or abstain from heterosexual activity for the course of the study through 120 days after the last dose of study medication. Subjects of child bearing potential are those who have not been surgically sterilized or have been free from menses for > 1 year.
- Male subject should agree to use an adequate method of contraception starting with the first dose of study therapy through 120 days after the last dose of study therapy.
- Does not have a history or current evidence of any condition, therapy, or laboratory abnormality that might interfere with the subject's participation for the full duration of the trial or is not in the best interest of the subject to participate, in the opinion of the treating investigator.

Subjects who restart treatment will be retreated at the same dose and dose interval as when they last received pembrolizumab. Treatment will be administered for up to one additional year.

Visit requirements are outlined in Section 6.0 – Schedule of Events.

7.5 Dose Modification

Adverse events (both non-serious and serious) associated with pembrolizumab exposure may represent an immunologic etiology. These adverse events may occur shortly after the first dose or several months after the last dose of treatment. Pembrolizumab must be withheld for drug-related toxicities and severe or life-threatening AEs as per Table 6 below.

Table 6 Dose Modification and Toxicity Management Guidelines for Immune-related AEs Associated with Pembrolizumab

General instructions:

Corticosteroid taper should be initiated upon AE improving to Grade 1 or less and continue to taper over at least 4 weeks.
For situations where pembrolizumab has been withheld, pembrolizumab can be resumed after AE has been reduced to Grade 1 or 0 and corticosteroid has been tapered. Pembrolizumab should be permanently discontinued if AE does not resolve within 12 weeks of last dose or corticosteroids cannot be reduced to ≤10 mg prednisone or equivalent per day within 12 weeks.
For severe and life-threatening irAEs, IV corticosteroid should be initiated first followed by oral steroid. Other immunosuppressive treatment should be initiated if irAEs cannot be controlled by corticosteroids.

Immune-related AEs	Toxicity grade or conditions (CTCAEv4.0)	Action taken to pembrolizumab	irAE management with corticosteroid and/or other therapies	Monitor and follow-up
Pneumonitis	Grade 2	Withhold	• Administer corticosteroids (initial dose of 1-2 mg/kg prednisone or equivalent) followed by taper	• Monitor participants for signs and symptoms of pneumonitis • Evaluate participants with suspected pneumonitis with radiographic imaging and initiate corticosteroid treatment • Add prophylactic antibiotics for opportunistic infections
	Grade 3 or 4, or recurrent Grade 2	Permanently discontinue		
Diarrhea / Colitis	Grade 2 or 3	Withhold	• Administer corticosteroids (initial dose of 1-2 mg/kg prednisone or equivalent) followed by taper	• Monitor participants for signs and symptoms of enterocolitis (ie, diarrhea, abdominal pain, blood or mucus in stool with or without fever) and of bowel perforation (ie, peritoneal signs and ileus). • Participants with ≥ Grade 2 diarrhea suspecting colitis should consider GI consultation and performing endoscopy to rule out colitis. • Participants with diarrhea/colitis should be advised to drink liberal quantities of clear fluids. If sufficient oral fluid intake is not feasible, fluid and electrolytes should be substituted via IV infusion.
	Grade 4	Permanently discontinue		
AST / ALT elevation or Increased bilirubin	Grade 2	Withhold	• Administer corticosteroids (initial dose of 0.5- 1 mg/kg prednisone or equivalent) followed by taper	• Monitor with liver function tests (consider weekly or more frequently until liver enzyme value returned to baseline or is stable
	Grade 3 or 4	Permanently discontinue	• Administer corticosteroids (initial dose of 1-2 mg/kg prednisone or equivalent) followed by taper	

Type 1 diabetes mellitus (T1DM) or Hyperglycemia	Newly onset T1DM or Grade 3 or 4 hyperglycemia associated with evidence of β-cell failure	Withhold	• Initiate insulin replacement therapy for participants with T1DM • Administer anti-hyperglycemic in participants with hyperglycemia	• Monitor participants for hyperglycemia or other signs and symptoms of diabetes.
Hypophysitis	Grade 2	Withhold	• Administer corticosteroids and initiate hormonal replacements as clinically indicated.	• Monitor for signs and symptoms of hypophysitis (including hypopituitarism and adrenal insufficiency)
	Grade 3 or 4	Withhold or permanently discontinue[1]		
Hyperthyroidism	Grade 2	Continue	• Treat with non-selective beta-blockers (eg, propranolol) or thionamides as appropriate	• Monitor for signs and symptoms of thyroid disorders.
	Grade 3 or 4	Withhold or permanently discontinue[1]		
Hypothyroidism	Grade 2-4	Continue	• Initiate thyroid replacement hormones (eg, levothyroxine or liothyroinine) per standard of care	• Monitor for signs and symptoms of thyroid disorders.
Nephritis and Renal dysfunction	Grade 2	Withhold	• Administer corticosteroids (prednisone 1-2 mg/kg or equivalent) followed by taper.	• Monitor changes of renal function
	Grade 3 or 4	Permanently discontinue		
Myocarditis	Grade 1 or 2	Withhold	• Based on severity of AE administer corticosteroids	• Ensure adequate evaluation to confirm etiology and/or exclude other causes
	Grade 3 or 4	Permanently discontinue		
All other immune-related AEs	Intolerable/ persistent Grade 2	Withhold	• Based on type and severity of AE administer corticosteroids	• Ensure adequate evaluation to confirm etiology and/or exclude other causes
	Grade 3	Withhold or discontinue based on the type of event. Events that require discontinuation include and not limited to: Gullain-Barre Syndrome, encephalitis		
	Grade 4 or recurrent Grade 3	Permanently discontinue		

1. Withhold or permanently discontinue pembrolizumab is at the discretion of the investigator or treating physician.

NOTE:

For participants with Grade 3 or 4 immune-related endocrinopathy where withhold of pembrolizumab is required, pembrolizumab may be resumed when AE resolves to ≤ Grade 2 and is controlled with hormonal replacement therapy or achieved metabolic control (in case of T1DM).

Dosing interruptions are permitted in the case of medical/surgical events or logistical reasons not related to study therapy (e.g., elective surgery, unrelated medical events, subject vacation,

and/or holidays), not to exceed two cycles. Subjects should be placed back on study therapy within 3 weeks of the scheduled interruption in all cases other than when pembrolizumab is stopped for craniotomy to confirm progression or unless otherwise discussed with the Sponsor. The reason for interruption should be documented in the subject's study record.

There is limited data regarding optimal therapy for refractory and/or persistent side effects from pembrolizumab (after discontinuation of pembrolizumab and initiation of steroids). These subjects will be managed according to best available practice and guidelines/treatment algorithms provided by the drug manufacturer Merck & Co. This may include but not limited to the use of drugs such as infliximab (5 mg/kg). If symptoms persist after the first infliximab dose, a second dose of infliximab (5 mg/kg) may be repeated two weeks after the initial dose.

7.6 Trial Blinding/Masking

This is an open-label pilot trial; therefore, the Sponsor, investigator and subject will know the treatment administered.

7.7 Randomization or Treatment Allocation

N/A

7.8 Stratification

N/A

7.9 Concomitant Medications/Vaccinations (allowed & prohibited)

Medications or vaccinations specifically prohibited in the exclusion criteria are not allowed during the ongoing trial. If there is a clinical indication for one of these or other medications or vaccinations specifically prohibited during the trial, discontinuation from trial therapy or vaccination may be required. The investigator should discuss any questions regarding this with the Merck Clinical team. The final decision on any supportive therapy or vaccination rests with the investigator and/or the subject's primary physician.

7.9.1 Acceptable Concomitant Medications

All treatments that the investigator considers necessary for a subject's welfare may be administered at the discretion of the investigator in keeping with the community standards of medical care. All concomitant medication will be recorded in Epic including all prescription, over-the-counter (OTC), herbal supplements, and IV medications and fluids.

All concomitant medications received within 28 days before the first dose of trial treatment and 30 days after the last dose of trial treatment should be recorded. Concomitant medications administered after 30 days after the last dose of trial treatment should be recorded for SAEs and ECIs as defined in Section 7.2.

7.9.2 Prohibited Concomitant Medications and Therapies

Subjects are prohibited from receiving the following therapies during the Screening and Treatment Phase (including retreatment for post-complete response relapse) of this trial:

- Antineoplastic systemic chemotherapy or biological therapy not specified in this protocol

- Immunotherapy not specified in this protocol
- Chemotherapy not specified in this protocol
- Live vaccines within 30 days prior to the first dose of trial treatment and while participating in the trial. Examples of live vaccines include, but are not limited to, the following: measles, mumps, rubella, varicella/zoster, yellow fever, rabies, BCG, and typhoid vaccine.

- All herbal and natural supplement that are being used will be recorded at all clinic visits. Natural therapies and herbal supplements may stimulant or suppress subjects immune system. Immune suppressants may reduce the efficacy of pembrolizumab and their concomitant use may potentially increase the risk of infections. Concomitant use of immune stimulants may potentially increase the risk of adverse auto-immune responses. Concomitant use of the natural therapies and herbal supplements in Table 7 below are prohibited

- Emerging data suggests that marijuana can affect response rates. The use of marijuana and marijuana derived produces are allowed in this study. Subjects will be made aware (in the consent form) of the potential risks of using marijuana while on the study.

Table 7 Prohibited Concomitant Natural Therapies and Herbal Supplements

Name of herbal supplement	
Immune stimulating agents	
1. Coriolus MushroomDHEA 2. 7-keto-DHEA 3. Echinacea* 4. European mandrake 5. European mistletoe 6. Fo-Ti Root 7. Ginseng, panaxand siberian 8. Glossy Privet (ligustrum) 9. Greater Celandine 10. Jiaogulan 11. Larch Arabinogalactan 12. Lycium 13. Melatonin 14. MGN-3 15. Podophyllum 16. Pokeweed 17. Pycnogenol 18. Coriolus Mushroom 19. DHEA 20. 7-keto-DHEA 21. Echinacea* 22. European mandrake	23. European mistletoe 24. Fo-Ti Root 25. Ginseng, panaxand siberian 26. Glossy Privet (ligustrum) 27. Greater Celandine 28. Jiaogulan 29. Larch Arabinogalactan 30. Lycium 31. Melatonin 32. MGN-3 33. Podophyllum 34. Pokeweed 35. Pycnogenol 36. Quillaia 37. Reishi Mushroom(gandoderma) 38. Shitake Mushroom 39. Sweet Annie 40. Terminalia chebula 41. Thunder god vine 42. Wild indigo 43. Zinc *Note:*Can be immune suppressing also*
Immune suppressing agents	
1. Bitter Melon 2. Caramel Color	8. Fish Oils 9. Licorice

3. Carrageenan(intravenous only) 4. Echinacea** 5. Indole-3-carbinol 6. Ipriflavone 7. Hydrazine	10. Periwinkle(vinca) 11. Poria Mushroom *Note : ** Can be immunestimulating also*

Subjects who, in the assessment by the investigator, require the use of any of the aforementioned treatments for clinical management should be removed from the trial. Subjects may receive other medications that the investigator deems to be medically necessary.

The Exclusion Criteria describes other medications which are prohibited in this trial.
There are no prohibited therapies during the Post-Treatment Follow-up Phase.

7.10 Pharmacokinetic/Pharmacodynamic Evaluations
NA

7.11 Blood Collection for Anti-Pembrolizumab Antibodies
NA

7.12 Withdrawal/Discontinuation
When a subject discontinues/withdraws prior to trial completion, all applicable activities scheduled for the final trial visit should be performed at the time of discontinuation. Any adverse events which are present at the time of discontinuation/withdrawal should be followed in accordance with the safety requirements outlined in Section 9 - Assessing and Recording Adverse Events. Subjects who a) attain a CR or b) complete 24 months of treatment with pembrolizumab may discontinue treatment with the option of restarting treatment if they meet the criteria specified in Section 7.4.9. After discontinuing treatment following assessment of CR, these subjects should return to the site for a Safety Follow-up Visit (described in Section 7.4.6) and then proceed to the Follow-Up Period of the study (described in Section 7.4.7).

7.13 Blinding/Unblinding
N/A

7.14 Diet/Activity/Other Considerations
Subjects should maintain a normal diet unless modifications are required to manage an AE such as diarrhea, nausea or vomiting.

7.15 Contraception
Pembrolizumab may have adverse effects on a fetus in utero. Furthermore, it is not known if pembrolizumab has transient adverse effects on the composition of sperm. Non-pregnant, non-breast-feeding women may be enrolled if they are willing to use 2 methods of birth control or are considered highly unlikely to conceive. Highly unlikely to conceive is defined as 1) surgically sterilized, or 2) postmenopausal (a woman who is ≥45 years of age and has not had menses for greater than 1 year will be considered postmenopausal), or 3) not heterosexually active for the duration of the study. The two birth control methods can be either two barrier methods or a barrier method plus a hormonal method to prevent pregnancy. Subjects should

start using birth control from study Visit 1 throughout the study period up to 120 days after the last dose of study therapy.

The following are considered adequate barrier methods of contraception: diaphragm, condom (by the partner), copper intrauterine device, sponge, or spermicide. Appropriate hormonal contraceptives will include any registered and marketed contraceptive agent that contains an estrogen and/or a progestational agent (including oral, subcutaneous, intrauterine, or intramuscular agents).

Subjects should be informed that taking the study medication may involve unknown risks to the fetus (unborn baby) if pregnancy were to occur during the study. In order to participate in the study they must adhere to the contraception requirement (described above) for the duration of the study and during the follow-up period. If there is any question that a subject will not reliably comply with the requirements for contraception, that subject should not be entered into the study.

7.16 Use in Pregnancy

If a subject inadvertently becomes pregnant while on treatment with pembrolizumab, the subject will immediately be removed from the study. The site will contact the subject at least monthly and document the subject's status until the pregnancy has been completed or terminated. The outcome of the pregnancy will be reported to the Sponsor and to Merck without delay and within 24 hours to the Sponsor and within 2 working days to Merck if the outcome is a serious adverse experience (e.g., death, abortion, congenital anomaly, or other disabling or life-threatening complication to the mother or newborn).

The study investigator will make every effort to obtain permission to follow the outcome of the pregnancy and report the condition of the fetus or newborn to the Sponsor. If a male subject impregnates his female partner, the study personnel at the site must be informed immediately and the pregnancy reported to the Sponsor and to Merck.

7.17 Use in Nursing Women

It is unknown whether pembrolizumab is excreted in human milk. Since many drugs are excreted in human milk, and because of the potential for serious adverse reactions in the nursing infant, subjects who are breast-feeding are not eligible for enrollment.

8.0 TUMOR IMAGING, ASSESSMENT OF DISEASE AND BIOMARKERS

8.1 Imaging Schedule

- Clinical standard of care MRIs are done at baseline, 12 weeks post radiation and then every 9 weeks (see special instructions below for evaluating persistent radiographic progression).
- Ferumoxytol steady state MRIs and serum for biomarkers are done baseline, 12 weeks post radiation, anytime there is suspected radiographic progression and 6 weeks after suspected radiographic progression.

Imaging instructions for evaluating persistent radiographic progression: if there is suspected progression, subjects undergo a ferumoxytol steady state MRI and continue on pembrolizumab for two more cycles (total of 6 weeks). At the 6 week point, clinical standard of care and ferumoxytol MRIs will be repeated to confirm persistent radiographic progression. If the 6 week scan confirms progression, then pembrolizumab is discontinued and subject is evaluated for surgery. If histopathology confirms pseudoprogression or is inconclusive, the subject will continue on pembrolizumab every 3 weeks until further radiographic progression is suspected. If histopathology confirms progression, the subject will be removed from the study.

8.2 Response assessment

8.2.1 Brain Lesions:

Complete response (CR): Complete disappearance of all enhancing abnormalities on clinical enhanced MRI scan (in the absence of gadolinium, ferumoxytol enhancement will be used). Some subjects will have a small but persistent enhancing abnormality on MRI related to biopsy or focal hemorrhage. It is often difficult to ascertain whether this represents a residual nidus of tumor or scar tissue. Adjunctive radiologic studies such as SPECT or PET as clinically indicated may be helpful, but often the nature of these abnormalities may only be determined by following the subject with serial scans. If this type of abnormality does not change or slowly involutes over time off therapy and corticosteroids, it is reasonable to categorize it as a CR.

Partial Response (PR): At least 50% decrease in the contrast enhancing lesion seen on MRI as compared to baseline imaging. In the event of multiple lesions, the determination of partial response will be based on the sum of the bi-dimensional measurements (greatest diameter x greatest perpendicular) across lesions AND no new sites of disease

Stable disease (SD): less than a PR but is not progressive disease.

Progressive disease (PD):

- **Suspected Radiographic Progression:** progression will be defined by a 20% increase in volume of enhancement compared to the best response on clinical standard of care MRI. At any time point if progression is suspected on the clinical scan, additional steady state ferumoxytol MRI will be performed within 7 days of the clinical scan. Progression will then be confirmed with a follow up scan in 4 weeks +/-5 days with a clinical standard of care MRI scan and additional ferumoxytol scan.

- **Persistent Radiographic Progression:** sustained and evident progression greater than increase in volume of enhancement compared to best response on a follow up scan at least 4 weeks after the scan where progression was suspected as per RANO BM form arm 1 or RANO criteria for subjects in arm 2. In cases with persistent radiographic progression the date of initial scan when progression was suspected will be considered to be the date of progression.

- Appearance of any new lesion or site of disease in the brain during or at the end of therapy.

- **Confirmation of true progression:** For the purpose of this study, Histopathologic evidence of viable tumor cells in patients with persistent radiographic progression will be define to have confirmed true progression. True progression is confirmed by histopathology, Pembrolizumab will be stopped and patient swill be taken off-study.. Patients who refuse biopsy or biopsy is not deemed clinically necessary will be taken off study at persistent radiographic progression. However, they will be continued to be monitored with clinical MRI scan and until death.

- **Pseudoprogression (PsP):** for the purpose of this pilot study, PsP may be confirmed by imaging or by pathology.
    - PsP Imaging: stable or decrease in volume of enhancement at 4 weeks (±5 days) after suspected radiographic progression.
    - PsP pathology: No viable tumor tissue is seen by histopathology evaluation on tissue obtained after confirmed radiographic progression by imaging.

- **Clinical progression:** Subjects may be removed from study if they experience symptomatic deterioration without radiographic evidence of disease progression.

Not evaluable (NE): Only relevant if any of the target lesions had additional interventions for clinical managements, patient is unable to get follow up scans or images are of poor quality.

Duration of Best Response: The duration of best response is measured from the time measurement criteria are met for SD, CR or PR (whichever is first recorded) until the first date that recurrent or PD is objectively documented (taking as reference for PD the smallest measurements recorded since the treatment started).

Progression free survival and overall survival will be calculated from the date of diagnosis to the date of confirmed progression or death, respectively. The date of diagnosis was used because the time between the date of diagnosis and the start of treatment or date of enrollment may vary due to subject variables or logistical issues based on radiation planning.

8.2.2 Systemic Lesions:

Measurements based on RECIST version 1.1 will be used to make a diagnosis of suspected systemic progression; it will be confirmed using irRC at follow-up.

8.3 Contrast agents dosing and administration

Clinical Standard of Care MRI:

Gadolinium is the preferred contrast agent, when subject qualifies (per institutional policy, see section 8.4.2) for gadolinium infusion, but is not required. In subjects that are already enrolled and have underwent at least one gadolinium enhanced MRI, but do not qualify to get Gadolinium at subsequent imaging time points, non-contrast enhanced MRI can be used as clinical MRI.

Dosing of Gadoteridol: After acquiring precontrast images, gadoteridol (Prohance®, Bracco Pharm) will be injected using a power injector. An IV bolus of 0.1 mmol/kg at a flow rate of

up to 3 mL/s will be administered, followed by a saline flush during the first DSC sequence. The appropriate volume of gadoteridol is based on the subject's weight (0.2 ml/kg gadoteridol = 0.1 mmol/Kg) followed by a 20ml saline flush.

Ferumoxytol Imaging

Ferumoxytol imaging consists of two consecutive days of imaging (early and delayed) and will be performed only at time points described in the Schedule of Events (Section 6).

1. Early intravascular phase scan ferumoxytol imaging
   Dosing of ferumoxytol: The total dose of ferumoxytol (AMAG Pharmaceuticals, Inc., Cambridge MA) will be 7mg/kg (not exceeding 510mg total). The ferumoxytol will be mixed with normal saline 1:1 solution to dilute to 15mg/ml. 1 mg/Kg IV will be injected at the start of the 7th dynamic cycle during the DSC acquisition at a flow rate of 3ml/s followed by 20ml saline flush. The rest of the ferumoxytol will be given as IV injections at a low flow rate (.1ml/s) followed by 20mg saline flush. The flow rates may be adjusted at the physician's discretion.

2. Delayed (24h) parenchymal phase ferumoxytol scan (optional)
   No contrast agent will be given during this MRI.

8.4 Image processing

Image processing will be done at OHSU.

8.4.1 For CNS lesions:

SS-CBV maps will be created using the high resolution T2*w scans before and after contrast agent and ΔR2* maps will be created using the formula $\Delta R2^* = \ln(SI_{pre}/SI_{post})/TE$, in which SI_{pre} and SI_{post} indicate the signal intensities pre- and post ferumoxytol, and TE is the echo time. DSC-CBV maps from gadoteridol DSC will be created using a mathematical leakage correction method in NordicICE (Nordic Neurolab, Bergen, Norway) an FDA approved perfusion MRI software (if gadoteridol could not be given per institutional protocol in section 8.4.2, only ferumoxytol maps will be used). The coregistration and overlay of CBV maps on T1-w MPRAGE will be done using NordicICE as well [36, 42]. The clinical MR sequences, such as T1 weighted scans with and without gadoteridol, and T2 weighted pre contrast scans will also be read. Images will be read by 2 radiologists. The radiologists will be blinded to the type of CBV maps (SS and DSC) and subject information.

8.4.2 Oregon Health & Science University policy on assessment of lab results prior to gadolinium based contrast media

For patients with the following risk factors, a serum creatinine level will be obtained with calculation of eGFR according to the following guidelines:

1) Creatinine/eGFR NOT required:
   - Dialysis patients – No eGFR required
   - Dialysis patients are excluded from the requirements in numbers 2 and 3 below.

2) Creatinine/eGFR required within 24 hours of MRI scan:
   - All inpatients (includes Daypatients and ER patients)
   - Outpatients whose most recent eGFR<60

3) Creatinine/eGFR required within 30 days of MRI scan:
   - Patients >60 years of age
   - Outpatients with a history of hypertension requiring medication
   - Outpatients with diabetes mellitus
   - Outpatients with kidney disease: kidney transplant, single kidney, kidney cancer, kidney surgery
   - Outpatient children < 1 year of age

The technologist or RN will notify the radiologist for the following:

1) Patient has a history of NSF (Nephrogenic Systemic Fibrosis
2) Patient is on dialysis
3) Patients with eGFR <30 mL/min/1.73m2
4) Patient received gadolinium based contrast media within the past 24 hours
5) For questions related to kidney function or patient history

Informed Consent: The radiologist will obtain informed consent and document risk/benefit of procedure in the patient's medical record for any of the following patients who will be receiving IV gadolinium-based contrast:

1) Patients with eGFR<30
2)) Patients on dialysis

8.4.3 For systemic lesions

With immunotherapy treatment, an increase in total tumor burden of previously stable systemic lesions (tumor flare reaction) has been described [1]. Based on the location of the systemic lesions, and the feasibility of MR imaging, subjects will be offered optional MR imaging of systemic lesions, to evaluate response as part of the exploratory endpoints. Only subjects with measureable, but stable, systemic involvement at the time of enrollment, will be considered for this imaging. Subjects must provide additional consent for this optional portion of the study.

Fractional Blood Volume (fBV), Vessel Size Index (VSI), and Vessel Density Index (VDI) values will be obtained by defining a region of interest over the entire tumor area. This process will be repeated for 3 central slices of the tumor for every lesion, and the mean value within the region of interest (ROI) will be calculated. T2 and T2* values will be obtained, using GRE and SE data respectively, by plotting mean ROI value at each echo, and calculating the best-fit exponential decay function. R2 and R2* will be defined as the inverse of T2 or T2* values; DR_2 , DR_2^* will be calculated as the ratio of values before and after iron contrast injection.

Fractional Blood Volume of the tumor was derived from the relationship of ΔR_2^*, fractional blood volume (fBV) and magnetic field constants (γ, B_0) to changes in magnetic susceptibility, $\Delta\chi$.

$$\Delta\chi = \frac{3}{4\pi}\frac{\Delta R^*_{2,muscle}}{fBV_{muscle}\gamma B_0}$$

$$= \frac{3}{4\pi}\frac{\Delta R^*_{2,tumor}}{fBV_{tumor}\gamma B_0}$$

$$\frac{3}{4\pi}\frac{\Delta R^*_{2,muscle}}{fBV_{muscle}\gamma B_0} = \frac{3}{4\pi}\frac{\Delta R^*_{2,tumor}}{fBV_{tumor}\gamma B_0}$$

$$fBV_{tumor} = fBV_{muscle}\frac{\Delta R^*_{2,tumor}}{\Delta R^*_{2,muscle}}$$

where fBV_{muscle} is assumed to be a constant of 3%.

Vessel Size Index will be computed using the following equation:

$$VSI = 0.424\left(\frac{ADC}{\gamma\Delta\chi B_0}\right)^{1/2}\frac{\Delta R_2^*}{\Delta R_2}$$

For normalized value calculations, the first two terms will be considered to be constant for all images and will be therefore disregarded.

Vessel Density Index was computed as follows:

$$VDI = 329\frac{\Delta R_2}{\Delta R_2^*}$$

As for the VSI calculations, the first term in the VDI equation will be disregarded during calculation of normalized values.

Paramagnetic maps will also generated by calculating VSI,VDI and fBV values on a voxel-by-voxel basis. Histograms will be obtained by plotting vessel indices within a tumor region-of-interest against the frequency of occurrence.

Data will be reported as fBV, VSI and VDI +/- standard error of the mean.

8.5 Tumor Tissue Collection and Correlative Blood Biomarker Studies

8.5.1 Blood Biomarkers (optional)

- Correlate clinical and radiological response with systemic immune responses using multicolor flow cytometry, measuring total T-cells (CD3+) with CD4/CD8 ratio,
- T-Reg (CD4+/CD127-/bright CD25+) , DNT (CD3+/CD4-/CD8-), Naïve T (CD3+/CD45RA+), memory T (CD3+/CD45RO+), T-cell activation (CD3+/variable CD69+/variable CD25+/variable HLA-DR), NK and NK-activation (CD3-/CD56+/varibleCD16+), total B-cells (CD19+), naïve B-cells (CD19+/IgD+/CD27-), memory B-cells (CD19+/IgD-/CD27+).
- Blood will be collected just prior to first dose of pembrolizumab, at 12 weeks after radiosurgery, at suspicion of radiographic progression, and 6 weeks after suspected radiographic progression.
- Blood samples will be collected at OHSU and processed at the OHSU research laboratory.

8.5.2 Tissue Biomarkers

PD-L1 testing will be performed by the CRO QualTek Molecular Laboratories. Any archived tissue samples or tissue obtained from brain biopsy prior to enrollment (archival tissue) and those obtained at suspected radiographic progression will be sent to the CRO for PD-L1 expression assay. We will correlate outcomes and imaging findings with PD-L1 expression in archival systemic tumor tissue, brain biopsy prior to enrollment, and tissue obtained at persistent radiographic progression. Frozen tissue and fixed tissue blocks from initial lung biopsies will be used. In a subset frozen sections and fixed tissue blocks from subjects who undergo resection of brain metastases for clinical indications will also be utilized.

9.0 ADVERSE EVENTS

9.1 Adverse Event (AE) Monitoring

The investigator or qualified designee will assess each subject to evaluate for potential new or worsening AEs as specified in the Schedule of Events and more frequently if clinically indicated. Adverse experiences will be graded and recorded throughout the study and during the follow-up period according to NCI CTCAE Version 4.0. Toxicities will be attributed and graded, and action taken with regard to trial treatment will be recorded.

AEs will be attributed as unrelated, related or possibly related to:

1) pembrolizumab
2) ferumoxytol
3) standard of care stereotactic radiosurgery

For subjects receiving treatment with pembrolizumab, all AEs of unknown etiology associated with Pembrolizumab exposure should be evaluated to determine if it is possibly an event of clinical interest (ECI) of a potentially immunologic etiology (termed immune-related adverse events, or irAEs).

9.2 Sponsor Responsibility for Reporting Adverse Events

All Adverse Events will be reported to regulatory authorities, IRB/IECs and investigators in accordance with all applicable global laws and regulations.

9.3 OHSU IRB Reporting of Unanticipated Problems and Adverse Events

Unanticipated Problems (UP) and Adverse Events (AE) will be reported to OHSU 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 events must be reported to OHSU IRB within 5 working days after the PI learns of the event. If any of these require a change (as determined by the PI or the IRB) to the protocol or consent form, the PI will make those changes promptly and submit the revised documents to the OHSU IRB.

All other UP reports will be submitted to OHSU IRB no later than 15 calendar days of notification of the event. If the event requires changes as determined by the PI or the IRB) to the protocol or consent form, the PI will make the changes promptly and submit the revised

documents to the IRB. UP and AE reports are submitted through OHSU e-IRB and will be reviewed by OHSU IRB.

9.4 Assessing and Recording Adverse Events

An adverse event is defined as any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have to have a causal relationship with this treatment. An adverse event can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a medicinal product or protocol-specified procedure, whether or not considered related to the medicinal product or protocol-specified procedure. Any worsening (i.e., any clinically significant adverse change in frequency and/or intensity) of a preexisting condition that is temporally associated with the use of the Merck's product, is also an adverse event.

Changes resulting from normal growth and development that do not vary significantly in frequency or severity from expected levels are not to be considered adverse events. Examples of this may include, but are not limited to, teething, typical crying in infants and children and onset of menses or menopause occurring at a physiologically appropriate time.
Merck product includes any pharmaceutical product, biological product, device, diagnostic agent or protocol-specified procedure, whether investigational (including placebo or active comparator medication) or marketed, manufactured by, licensed by, provided by or distributed by Merck for human use.

Adverse events may occur during the course of the use of Merck product in clinical trials or within the follow-up period specified by the protocol, or prescribed in clinical practice, from overdose (whether accidental or intentional), from abuse and from withdrawal.
Adverse events may also occur in screened subjects during any pre-allocation baseline period as a result of a protocol-specified intervention, including washout or discontinuation of usual therapy, diet, placebo treatment or a procedure.

Progression of the cancer under study is not considered an adverse event unless it is considered to be drug related by the investigator.

All adverse events will be recorded from the time the consent form is signed through 30 days following cessation of treatment and at each examination on the Adverse Event case report forms/worksheets. The reporting timeframe for adverse events meeting any serious criteria is described in Section 9.6.3.1.

9.5 Rescue Medications & Supportive Care

9.5.1 Supportive Care Guidelines

Subjects should receive appropriate supportive care measures as deemed necessary by the treating investigator. Suggested supportive care measures for the management of adverse events with potential immunologic etiology are outlined below. Where appropriate, these guidelines include the use of oral or intravenous treatment with corticosteroids as well as additional anti-inflammatory agents if symptoms do not improve with administration of corticosteroids. Note that several courses of steroid tapering may be necessary as symptoms

may worsen when the steroid dose is decreased. For each disorder, attempts should be made to rule out other causes such as metastatic disease or bacterial or viral infection, which might require additional supportive care. The treatment guidelines are intended to be applied when the investigator determines the events to be related to pembrolizumab.

Note: if after the evaluation the event is determined not to be related, the investigator is instructed to follow the ECI reporting guidance but does not need to follow the treatment guidance.. Refer to Section 7.5 for dose modification.

It may be necessary to perform conditional procedures such as bronchoscopy, endoscopy, or skin photography as part of evaluation of the event.

9.5.2 Expected AEs due to Pembrolizumab and Guidelines for Management

- **Pneumonitis**:
    - For **Grade 2 events**, treat with systemic corticosteroids. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks.
    - For **Grade 3-4 events**, immediately treat with intravenous steroids. Administer additional anti-inflammatory measures, as needed.
    - Add prophylactic antibiotics for opportunistic infections in the case of prolonged steroid administration.

- **Diarrhea/Colitis**:
  Subjects should be carefully monitored for signs and symptoms of enterocolitis (such as diarrhea, abdominal pain, blood or mucus in stool, with or without fever) and of bowel perforation (such as peritoneal signs and ileus).
    - All subjects who experience diarrhea/colitis should be advised to drink liberal quantities of clear fluids. If sufficient oral fluid intake is not feasible, fluid and electrolytes should be substituted via IV infusion. For Grade 2 or higher diarrhea, consider GI consultation and endoscopy to confirm or rule out colitis.
    - For **Grade 2 diarrhea/colitis** that persists greater than 3 days, administer oral corticosteroids.
    - For **Grade 3 or 4 diarrhea/colitis** that persists > 1 week, treat with intravenous steroids followed by high dose oral steroids.
    - When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks.

- **Type 1 diabetes mellitus (if new onset, including diabetic ketoacidosis [DKA]) or ≥ Grade 3 Hyperglycemia, if associated with ketosis (ketonuria) or metabolic acidosis (DKA)**

    - For **T1DM** or **Grade 3-4** Hyperglycemia
        - Insulin replacement therapy is recommended for Type I diabetes mellitus and for Grade 3-4 hyperglycemia associated with metabolic acidosis or ketonuria.

        - Evaluate subjects with serum glucose and a metabolic panel, urine ketones, glycosylated hemoglobin, and C-peptide.

- **Hypophysitis**:
    - For **Grade 2** events, treat with corticosteroids. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks. Replacement of appropriate hormones may be required as the steroid dose is tapered.
    - For **Grade 3-4** events, treat with an initial dose of IV corticosteroids followed by oral corticosteroids. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks. Replacement of appropriate hormones may be required as the steroid dose is tapered.

- **Hyperthyroidism or Hypothyroidism**:
  Thyroid disorders can occur at any time during treatment. Monitor subjects for changes in thyroid function (labs every 3 months) and for clinical signs and symptoms of thyroid disorders.
    - **Grade 2** hyperthyroidism events (and **Grade 2-4** hypothyroidism):
        - In hyperthyroidism, non-selective beta-blockers (e.g. propranolol) are suggested as initial therapy.
        - In hypothyroidism, thyroid hormone replacement therapy, with levothyroxine or liothyroinine, is indicated per standard of care.
    - **Grade 3-4** hyperthyroidism
        - Treat with an initial dose of IV corticosteroid followed by oral corticosteroids. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks. Replacement of appropriate hormones may be required as the steroid dose is tapered.

- **Hepatic**:
    - For **Grade 2** events, monitor liver function tests more frequently until returned to baseline values (consider weekly).
        - Treat with IV or oral corticosteroids
    - For **Grade 3-4** events, treat with intravenous corticosteroids for 24 to 48 hours.
    - When symptoms improve to Grade 1 or less, a steroid taper should be started and continued over no less than 4 weeks.

- **Renal Failure or Nephritis**:
    - For **Grade 2** events, treat with corticosteroids.
    - For **Grade 3-4** events, treat with systemic corticosteroids.
    - When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks.

- **Stevens-Johnson Syndrome (SJS) and Toxic Epidermal Necrolysis (TEN):**
    - One fatal case of SJS in a clinical trial and one fatal case of TEN in the post-marketing setting have been reported in patients treated with pembrolizumab.

Including these cases, there have been 8 cases of SJS (6 in clinical trials, and 2 post-marketing) and 2 cases of TEN (both post-marketing) all of which were serious.

- The risk of SJS and TEN is reported at approximately 0.4 - 7 cases per million patient years in the general adult population.
- Independent risk factors include certain medications such as anticonvulsants, sulfonamides, aminopenicillins, allopurinol, and NSAIDs. Non-medication triggers include infection, contrast media, and vaccinations.
- Malignancy is associated with an increased mortality rate in patients with SJS and TEN.

For signs or symptoms of SJS or TEN, withhold Pembrolizumab and refer the patient for specialized care for assessment and treatment. If SJS or TEN is confirmed, permanently discontinue Pembrolizumab.

- **Immune-mediated myocarditis**
  A total of 6 cases of myocarditis have been reported in patients treated with pembrolizumab in clinical trials or in an expanded access program. There were was 1 fatal case reported in a clinical trial.

  A search of the literature identified neither background incidence rates nor prevalence of myocarditis specifically among cancer patients. Immune-mediated myocarditis should be suspected if other causes of myocarditis, such as infection or prior radiation therapy, have been excluded. Risk factors include certain medications and treatment modalities such as radiation, anthracycline, alkylating agents and most recently checkpoint inhibitors. For suspected immune-mediated myocarditis, ensure adequate evaluation to exclude other etiologies, and administer corticosteroids.

- **Management of Infusion Reactions**: Signs and symptoms usually develop during or shortly after drug infusion and generally resolve completely within 24 hours of completion of infusion.

Table 8 below shows treatment guidelines for subjects who experience an infusion reaction associated with administration of pembrolizumab (MK-3475).

Table 8 Infusion Reaction Treatment Guidelines

NCI CTCAE Grade	Treatment	Premedication at subsequent dosing
Grade 1 Mild reaction; infusion interruption not indicated; intervention not indicated	Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator.	None
Grade 2 Requires infusion interruption but responds promptly to symptomatic treatment (e.g., antihistamines, NSAIDS, narcotics, IV fluids);	**Stop Infusion and monitor symptoms.** Additional appropriate medical therapy may include but is not limited to: IV fluids Antihistamines NSAIDS	Subject may be premedicated 1.5h (± 30 minutes) prior to infusion of Pembrolizumab (MK-3475) with: Diphenhydramine 50 mg po (or equivalent dose of antihistamine).

NCI CTCAE Grade	Treatment	Premedication at subsequent dosing
prophylactic medications indicated for < =24 hrs	Acetaminophen Narcotics Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator. If symptoms resolve within one hour of stopping drug infusion, the infusion may be restarted at 50% of the original infusion rate (e.g., from 100 mL/hr to 50 mL/hr). Otherwise dosing will be held until symptoms resolve and the subject should be premedicated for the next scheduled dose. **Subjects who develop Grade 2 toxicity despite adequate premedication should be permanently discontinued from further trial treatment administration.**	Acetaminophen 500-1000 mg po (or equivalent dose of antipyretic).
Grades 3 or 4 Grade 3: Prolonged (i.e., not rapidly responsive to symptomatic medication and/or brief interruption of infusion); recurrence of symptoms following initial improvement; hospitalization indicated for other clinical sequelae (e.g., renal impairment, pulmonary infiltrates) Grade 4: Life-threatening; pressor or ventilatory support indicated	**Stop Infusion.** Additional appropriate medical therapy may include but is not limited to: IV fluids Antihistamines NSAIDS Acetaminophen Narcotics Oxygen Pressors Corticosteroids Epinephrine Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator. Hospitalization may be indicated. **Subject is permanently discontinued from further trial treatment administration.**	No subsequent dosing
Appropriate resuscitation equipment should be available in the room and a physician readily available during the period of drug administration.		

9.5.3 Expected AEs from Ferumoxytol and Guidelines for Management

Possible risks and side effects of the study agent ferumoxytol from the Ferumoxytol Investigator's brochure 2012 are below.

In Phase III clinical studies in subjects with iron deficiency anemia and chronic kidney disease, the most commonly reported side effects (≥2% of subjects) following treatment with ferumoxytol were diarrhea, nausea, dizziness, hypotension, constipation, and peripheral edema.

Pharmacological class effects commonly associated with marketed IV iron products include hypotension and hypersensitivity reactions. In clinical studies of ferumoxytol, hypotension was reported in 1.9% (33/1726) of subjects. Three of these subjects (0.2%) had related serious hypotensive events, one of which was characterized as an anaphylactoid reaction. All of the events resolved on the same day of occurrence without sequelae. Serious hypersensitivity reactions have been observed in 0.2% (3/1726) of subjects following administration of ferumoxytol. Other adverse reactions potentially associated with hypersensitivity, including pruritus, rash, urticaria, or wheezing were reported in 3.7% (63/1726) of subjects; each of these reactions occurred in <1% of subjects exposured to ferumoxytol.

The overall incidence of AEs in a post-marketing study irrespective of relationship to study medication was lower in subjects administered ferumoxytol (48%) than in those treated with iron sucrose (65%). The incidence of SAEs was higher in ferumoxytol-treated subjects than in iron sucrose-treated subjects (9% vs 7%). The incidence of related SAEs was similar between the two treatment groups (1% in both groups). The most common AEs among ferumoxytol-treated subjects were nausea (7.5%) and muscle spasms (5.0%).

In the Phase II imaging study where ferumoxytol was used as an imaging agent (doses 1.0, 2.5 and 4.0 mg/kg) in subjects with peripheral arterial disease (PAD), there was a possible dose dependent increase in overall TEAEs reported in this study. Only 3.5% of the subject population experienced TEAEs that were related to ferumoxytol. The majority of the treatment-related AEs were mild in severity and were resolved. 6.1% of the subject population experienced SAEs that were eventually resolved; none were treatment related. There were 3 AESIs reported in 3 subjects (2.6%); 1 subject in each dose group experienced 1 AESI. In the 1.0 mg/kg dose group 1 subject experienced acute hypotension. In the 2.5 mg/kg dose group 1 subject experienced a hypersensitivity reaction of rash, and in the 4.0 mg/kg dose group 1 subject experienced a drug hypersensitivity reaction (acute hypersensitivity reaction to ferumoxytol).

Given the potential for hypersensitivity and other adverse reactions observed with other IV anemia therapies, subjects should be monitored for signs and symptoms of hypotension and hypersensitivity following administration of ferumoxytol as outlined in study protocols. Ferumoxytol should only be administered when personnel and therapies are immediately available for the treatment of anaphylaxis and other hypersensitivity reactions. In the event of a ferumoxytol-related adverse reaction, the infusion will be stopped and additional medical treatment will be considered depending on the severity of the reaction.

With certain IV iron preparations, mild to severe low blood phosphorus levels (hypophosphatemia) have also frequently been reported following administration, particularly in women with heavy AUB. Mild to moderate, asymptomatic reductions in blood phosphorus levels have been infrequently observed following ferumoxytol administration.

Elemental iron is essential for normal cell and tissue development, as well as for tumor cell growth. There are no clinical data to suggest that therapeutic IV iron supplementation, given to correct anemia, stimulates tumor development, despite the theoretical risk. While free iron has been implicated in increased oxidative stress, which is potentially associated with cellular damage and carcinogenesis, there are no clinical studies to support that transient increases in oxidative stress resulting from iron supplementation result in increased tumor growth.

Other potential risks to subjects receiving ferumoxytol include the temporary discomfort at the site of injection, as well as the potential for bruising, local infection, and pigmentation following IV administration.

There are no studies of ferumoxytol in pregnant women, and the risk to a pregnant mother and unborn baby is unknown. In animal studies, ferumoxytol caused fetal malformations and decreased fetal weights at maternally toxic doses of 6 times the human daily dose. Thus

ferumoxytol should only be used during pregnancy if the potential benefit justifies the potential risk to the fetus.

It is also unknown whether ferumoxytol is present in human milk. Animal studies showed that when administered daily to pregnant and lactating females, a small amount of ferumoxytol was excreted into breast milk. Because many drugs are excreted in human milk and because of the potential for adverse reactions in nursing infants, a decision should be made whether to discontinue nursing or to avoid ferumoxytol, taking into account the importance of ferumoxytol to the mother and the known benefits of nursing.

No data from clinical trials are available regarding overdose of ferumoxytol in humans. During the post-marketing phase, several subjects received an overdose of ferumoxytol ranging from 1 g in 1 day to 2.5 g over 14 days. Only one case of minor rash was observed.

Although this section provides general guidance regarding the risks and side effects associated with administration of ferumoxytol, investigators should refer to the protocol for study-specific follow-up and the monitoring procedures.

9.6 Additional IND Reporting Requirements for Ferumoxytol and Pembrolizumab

Sponsor-investigator of this IND is responsible for compliance with the FDCA (21 U.S.C. §§ 301 et. seq.) as well as the implementing regulations [Title 21 of the Code of Federal Regulations (CFR)]. Responsibilities include:

- Reporting any unexpected fatal or life-threatening suspected adverse reactions to this Division no later than 7 calendar days after initial receipt of the information [21 CFR 312.32(c)(2)]. If IND is in eCTD format, submit 7-day reports electronically in eCTD format. If IND is not in eCTD format, submit 7-day reports by telephone or fax;
- Reporting any (1) serious, unexpected suspected adverse reactions, (2) findings from other clinical, animal, or in-vitro studies that suggest significant human risk, and (3) a clinically important increase in the rate of a serious suspected adverse reaction to this Division and to all investigators no later than 15 calendar days after determining that the information qualifies for reporting [21 CFR 312.32(c)(1)]. If IND is in eCTD format, submit 15- day reports to FDA electronically in eCTD format. If IND is not in eCTD format, submit 15-day reports in paper format; and
- Submitting annual progress reports within 60 days of the anniversary of the date that the IND went into effect (the date clinical studies were permitted to begin) [21 CFR 312.33].

Table 9 Evaluating Adverse Events

An investigator who is a qualified physician, will evaluate all adverse events as to:

V4.0 CTCAE Grading	**Grade 1**	**Mild; asymptomatic or mid symptoms; clinical or diagnostic observations only; intervention not indicated.**
	Grade 2	**Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental ADL.**
	Grade 3	**Severe or medically significant but not immediately life-threatening; hospitalization or prolongation or hospitalization indicated; disabling; limiting self-care ADL.**
	Grade 4	**Life threatening consequences; urgent intervention indicated.**
	Grade 5	**Death related to AE**
Seriousness	A serious adverse event is any adverse event occurring at any dose or during any use of Merck product that:	
	†**Results in death**; or	
	†**Is life threatening; or** places the subject, in the view of the investigator, at immediate risk of death from the event as it occurred (Note: This does not include an adverse event that, had it occurred in a more severe form, might have caused death.); or	
	†**Results in a persistent or significant disability/incapacity** (substantial disruption of one's ability to conduct normal life functions); or	
	†**Results in or prolongs an existing inpatient hospitalization** (hospitalization is defined as an inpatient admission, regardless of length of stay, even if the hospitalization is a precautionary measure for continued observation. (Note: Hospitalization [including hospitalization for an elective procedure] for a preexisting condition which has not worsened does not constitute a serious adverse event.); or	
	†**Is a congenital anomaly/birth defect** (in offspring of subject taking the product regardless of time to diagnosis);or	
	Is a new **cancer;** (that is not a condition of the study) **or**	
	Is an overdose (whether accidental or intentional). Any adverse event associated with an overdose is considered a serious adverse event. An overdose that is not associated with an adverse event is considered a non-serious event of clinical interest and must be reported within 24 hours.	
	Other important medical events that may not result in death, not be life threatening, or not require hospitalization may be considered a serious adverse event when, based upon appropriate medical judgment, the event may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed previously (designated above by a †).	
Duration	Record the start and stop dates of the adverse event. If less than 1 day, indicate the appropriate length of time and units	
Action taken	Did the adverse event cause the Merck product to be discontinued?	
Relationship to test drug	Did the Merck product cause the adverse event? The determination of the likelihood that the Merck product caused the adverse event will be provided by an investigator who is a qualified physician. The investigator's signed/dated initials on the source document or worksheet that supports the causality noted on the AE form, ensures that a medically qualified assessment of causality was done. This initialed document must be retained for the required regulatory time frame. The criteria below are intended as reference guidelines to assist the investigator in assessing the likelihood of a relationship between the test drug and the adverse event based upon the available information. **The following components are to be used to assess the relationship between the Merck product and the AE**; the greater the correlation with the components and their respective elements (in number and/or intensity), the more likely the Merck product caused the adverse event (AE):	
	Exposure	Is there evidence that the subject was actually exposed to the Merck product such as: reliable history, acceptable compliance assessment (pill count, diary, etc.), expected pharmacologic effect, or measurement of drug/metabolite in bodily specimen?
	Time Course	Did the AE follow in a reasonable temporal sequence from administration of the Merck product? Is the time of onset of the AE compatible with a drug-induced effect (applies to trials with investigational medicinal product)?
	Likely Cause	Is the AE not reasonably explained by another etiology such as underlying disease, other drug(s)/vaccine(s), or other host or environmental factors

Relationship to Merck product (continued)	**The following components are to be used to assess the relationship between the test drug and the AE: (continued)**	
	Dechallenge	Was the Merck product discontinued or dose/exposure/frequency reduced? If yes, did the AE resolve or improve? If yes, this is a positive dechallenge. If no, this is a negative dechallenge. (Note: This criterion is not applicable if: (1) the AE resulted in death or permanent disability; (2) the AE resolved/improved despite continuation of the Merck product; or (3) the trial is a single-dose drug trial); or (4) Merck product(s) is/are only used one time.)
	Rechallenge	Was the subject re-exposed to the Merck product in this study? If yes, did the AE recur or worsen? If yes, this is a positive rechallenge. If no, this is a negative rechallenge. (Note: This criterion is not applicable if: (1) the initial AE resulted in death or permanent disability, or (2) the trial is a single-dose drug trial); or (3) Merck product(s) is/are used only one time). NOTE: IF A RECHALLENGE IS PLANNED FOR AN ADVERSE EVENT WHICH WAS SERIOUS AND WHICH MAY HAVE BEEN CAUSED BY THE MERCK PRODUCT, OR IF REEXPOSURE TO THE MERCK PRODUCT POSES ADDITIONAL POTENTIAL SIGNIFICANT RISK TO THE SUBJECT, THEN THE RECHALLENGE MUST BE APPROVED IN ADVANCE BY THE U.S. CLINICAL MONITOR AS PER DOSE MODIFICATION GUIDELINES IN THE PROTOCOL.
	Consistency with Trial Treatment Profile	Is the clinical/pathological presentation of the AE consistent with previous knowledge regarding the Merck product or drug class pharmacology or toxicology?
The assessment of relationship will be reported on the case report forms /worksheets by an investigator who is a qualified physician according to his/her best clinical judgment, including consideration of the above elements.		
Record one of the following	**Use the following scale of criteria as guidance (not all criteria must be present to be indicative of a Merck product relationship).**	
Yes, there is a reasonable possibility of Merck product relationship.	There is evidence of exposure to the Merck product. The temporal sequence of the AE onset relative to the administration of the Merck product is reasonable. The AE is more likely explained by the Merck product than by another cause.	
No, there is not a reasonable possibility Merck product relationship	Subject did not receive the Merck product OR temporal sequence of the AE onset relative to administration of the Merck product is not reasonable OR there is another obvious cause of the AE. (Also entered for a subject with overdose without an associated AE.)	

9.7 Definition of an Overdose for This Protocol and Reporting of Overdose to the Sponsor and to Merck

For purposes of this trial, an overdose of pembrolizumab will be defined as any dose of 1,000 mg or greater (≥5 times the indicated dose). No specific information is available on the treatment of overdose of pembrolizumab. Appropriate supportive treatment should be provided if clinically indicated. In the event of overdose, the subject should be observed closely for signs of toxicity. Appropriate supportive treatment should be provided if clinically indicated.

If an adverse event(s) is associated with ("results from") the overdose of a Merck product, the adverse event(s) is reported as a serious adverse event, even if no other seriousness criteria are met.

If a dose of Merck's product meeting the protocol definition of overdose is taken without any associated clinical symptoms or abnormal laboratory results, the overdose is reported as a non-serious Event of Clinical Interest (ECI), using the terminology "accidental or intentional overdose without adverse effect."

All reports of overdose with and without an adverse event must be reported within 24 hours to the Sponsor and within 2 working days hours to Merck Global Safety (Attn: Worldwide Product Safety; FAX 215 993-1220).

9.8 Reporting of Pregnancy and Lactation to the Sponsor and to Merck

Although pregnancy and lactation are not considered adverse events, it is the responsibility of investigators or their designees to report any pregnancy or lactation in a subject (spontaneously reported to them), including the pregnancy of a male subject's female partner that occurs during the trial or within 120 days of completing the trial completing the trial, or 30 days following cessation of treatment if the subject initiates new anticancer therapy, whichever is earlier. All subjects and female partners of male subjects who become pregnant must be followed to the completion/termination of the pregnancy. Pregnancy outcomes of spontaneous abortion, missed abortion, benign hydatidiform mole, blighted ovum, fetal death, intrauterine death, miscarriage and stillbirth must be reported as serious events (Important Medical Events). If the pregnancy continues to term, the outcome (health of infant) must also be reported.

Such events must be reported within 24 hours to the Sponsor and within 2 working days to Merck Global Safety (Attn: Worldwide Product Safety; FAX 215 993-1220).

9.9 Immediate Reporting of Adverse Events to the Sponsor and to Merck

9.9.1 Serious Adverse Events

A serious adverse event is any adverse event occurring at any dose or during any use of Merck's product that:

- Results in death;
- Is life threatening;
- Results in persistent or significant disability/incapacity;

- Results in or prolongs an existing inpatient hospitalization;
- Is a congenital anomaly/birth defect;
- Is a new cancer (that is not a condition of the study);
- Is associated with an overdose;
- Is another important medical event

Refer to Table 8 for additional details regarding each of the above criteria.

Any serious adverse event, or follow up to a serious adverse event, including death due to any cause other than progression of the cancer under study that occurs to any subject from the time the consent is signed through 90 days following cessation of treatment, or the initiation of new anti-cancer therapy, whichever is earlier, whether or not related to Merck product, must be reported within 24 hours to the Sponsor and within 2 working days to Merck Global Safety.

Non-serious Events of Clinical Interest will be forwarded to Merck Global Safety and will be handled in the same manner as SAEs.

Additionally, any serious adverse event, considered by an investigator who is a qualified physician to be related to Merck product that is brought to the attention of the investigator at any time outside of the time period specified in the previous paragraph also must be reported immediately to the Sponsor and to Merck.

SAE reports and any other relevant safety information are to be forwarded to the Merck Global Safety facsimile number: +1-215-993-1220

A copy of all 15 Day Reports and Annual Progress Reports is submitted as required by FDA, European Union (EU), Pharmaceutical and Medical Devices agency (PMDA) or other local regulators. Investigators will cross reference this submission according to local regulations to the Merck Investigational Compound Number (IND, CSA, etc.) at the time of submission. Additionally investigators will submit a copy of these reports to Merck & Co., Inc. (Attn: Worldwide Product Safety; FAX 215 993-1220) at the time of submission to FDA.

All subjects with serious adverse events must be followed up for outcome.

9.9.1.1 Events of Clinical Interest

Selected non-serious and serious adverse events are also known as Events of Clinical Interest (ECI) and must be reported within 24 hours to the Sponsor and within 2 working days to Merck Global Safety. (Attn: Worldwide Product Safety; FAX 215 993-1220).

For the time period beginning when the consent form is signed until treatment allocation/randomization, any ECI, or follow up to an ECI, that occurs to any subject must be reported within 24 hours to the Sponsor and within 2 working days to Merck Global Safety if it causes the subject to be excluded from the trial, or is the result of a protocol-specified intervention, including but not limited to washout or discontinuation of usual therapy, diet, placebo treatment or a procedure.

For the time period beginning at treatment allocation/randomization through 90 days following cessation of treatment, or 30 days following cessation of treatment if the subject initiates new anticancer therapy, whichever is earlier, any ECI, or follow up to an ECI, whether or not related to Merck product, must be reported within 24 hours to the Sponsor and within 24 hours to Merck Global Safety.

Events of clinical interest for this trial include:

1. an overdose of Merck product, as defined in Section 7.2.1 - Definition of an Overdose for This Protocol and Reporting of Overdose to the Sponsor, that is not associated with clinical symptoms or abnormal laboratory results.

2. an elevated AST or ALT lab value that is greater than or equal to 3X the upper limit of normal and an elevated total bilirubin lab value that is greater than or equal to 2X the upper limit of normal and, at the same time, an alkaline phosphatase lab value that is less than 2X the upper limit of normal, as determined by way of protocol-specified laboratory testing or unscheduled laboratory testing.*

*Note: These criteria are based upon available regulatory guidance documents. The purpose of the criteria is to specify a threshold of abnormal hepatic tests that may require an additional evaluation for an underlying etiology.

9.9.2 Evaluating Adverse Events

An investigator who is a qualified physician will evaluate all adverse events according to the NCI Common Terminology for Adverse Events (CTCAE), version 4.0. Any adverse event which changes CTCAE grade over the course of a given episode will have each change of grade recorded on the adverse event case report forms/worksheets.

All adverse events regardless of CTCAE grade must also be evaluated for seriousness.

10.0 STATISTICAL ANALYSIS PLAN

Given the exploratory nature of this study, the statistical analyses will be descriptive in nature. For the primary and secondary objectives, sensitivity, specificity, rate of adverse event and distribution of best responses will be analyzed using proportions and exact 95% confidence intervals. Progression free survival and overall survival will be assessed using the Kaplan-Meier product limit estimates. Outcomes in the exploratory objectives will be summarized using descriptive statistics as appropriate and associations between immunological parameters, clinical responses, radiological responses and other variables will be explored using two sample tests or correlation measures, as appropriate.

10.1 Power and sample size calculation

This pilot study will enroll 20 subjects. Here we roughly estimate the precision of sensitivity and specificity in the primary objective based on 20 subjects.

Assuming that on average, we have n = 2 lesions/subject. Further, we assume that these lesions are totally independent based on their biologically and physiologically features (i.e., two

lesions grown in the same subject are not more similar or different compared to two lesions grown in two subjects). Further, we assume that all subjects will eventually have disease progression and due to dropout and inoperable cases, a total of n = 15 subjects will undergo surgery.

For sensitivity, assume that we will have data from 12 subjects with true progression who also undergo surgery. (Typically subjects will only be operated on one lesion; some subjects may not undergo surgery due to increased volume in more than one lesion; subjects may die due to systematic disease etc.). Then based on n = 12, if we observe 10 subjects (lesions) for whom the true progression is correctly identified by Ferumoxytol MRI, then the sensitivity will be 83% with a 95% CI between 52% and 98% (Table 10).

Table 10: Estimated sensitivity and 95% CI based on 12 subjects (lesions).

Number of subjects (lesions) with true progression	Number of subjects (lesions) whose true progression was detected by ferumoxytol MRI	Sensitivity	Exact 95% CI of sensitivity
12	12	100%	74% to 100%
12	11	92%	62% to 99.8%
12	10	83%	52% to 98%
12	9	75%	43% to 95%
12	8	67%	35% to 90%

For specificity, we assume that we will observe 8 subjects (about ~40%) who have pseudo-progression based on followup scan or surgery. Further, assume that among the 8 patients, 50% will have pseudo-progression in two lesions and 50% will have pseudo-progression in one lesion. Then we will have 12 lesions with pseudo-progression. If we observe 10 lesions for whom the pseudo-progression status is correctly identified by Ferumoxytol MRI, then the specificity will be 83% with a 95% CI between 52% and 98% (Table 11).

Table 11: Estimated specificity and 95% CI based on 12 lesions.

Number of lesions with pseudo-progression	Number of lesions with pseudo-progression detected by ferumoxytol MRI	Specificity	Exact 95% CI of specificity
12	12	100%	74% to 100%
12	11	92%	62% to 99.8%
12	10	83%	52% to 98%
12	9	75%	43% to 95%
12	8	67%	35% to 90%

10.2 Analysis plan

For sensitivity and specificity, the unit of the analysis is lesion since lesions within the same patients are biologically and physiologically independent based on the opinions of clinical experts. For sensitivity, since each subject only contributes one lesion so the number of subjects equal to the number of lesions. Given this is a pilot study for a rare disease, we aim to maximize the use of available data and plan such analysis *a-priori.*

For the primary and secondary endpoints, sensitivity, specificity, rate of adverse events for tolerability and safety, including any potential toxicities involving SOC chemotherapy, and the distribution of best responses will be analyzed using proportions and exact 95% confidence intervals. In the primary analysis of sensitivity and specificity, the cutoff for rCBV will be specified as 1.75, sensitivity analysis will be based on subjects (lesions) with surgical results, and specificity analysis will be based on lesions with pseudo-progression determined based on surgical results or follow-up scan, as detailed in the power and sample size calculation section. In secondary analysis, we will try other cutoff points for rCBV, and include all subjects that will be confirmed with true progression through surgery or follow up imaging. Progression free survival and overall survival will be assessed using the Kaplan-Meier product limit estimates for all patients, taking censoring into account.

For exploratory objectives, continuous variables will be summarized using mean and standard deviation, or median and range based on the distribution of data. The categorical variables will be summarized using frequencies and proportions, with exact 95% CI as appropriate.

Immune responses will be determined by the volume, pattern and intensity of delayed (24hr) ferumoxytol uptake and compared between subjects who develop true vs pseudoprogression and provide data using Wilcoxon Rank Sum test (volume and intensity) or Fisher's exact test (pattern). ECD8, CD19, CD25, CD27, CD45, CD56, CD45R0, CD45RA, titers of IgD, $T\gamma\delta$ and $T\alpha\beta$) and correlated with clinical responses (complete response, disease progression, pseudoprogression etc.) using Wilcoxon Rank Sum test, and with radiological response (size of tumor) using Pearson's correlation coefficient or Spearman's correlation coefficient.

Among the 20 subjects, 5 to 10 subjects are expected to have data on PD-1 receptor expression (Yes vs. No) and immune cells (T cells, CD4+, CD8+, macrophages, M1, M2 etc) before initiation of Pembrolizumab and at the time of progression. If data allow, we will compare the PD-1 receptor expression and immune cells data before initiation of pembrolizumab and at the time of progression using exact McNemar's test or Wilcoxon signed test to accommodate the paired nature of the data.

Lastly, in subjects with measurable systemic lesions, vascular volume fraction (VVF), vessel size index (VSI) and vessel density index (VDI) will be measured and compared between subjects with true vs. pseudoprogression, if data allow.

In summary, all these proposed analyses will be conducted on an exploratory basis. A two-sample t-test may be used if the data show adequate normality. We will only provide descriptive statistics if the comparison mentioned above will not yield clinical meaningful

results. The purpose of these exploratory analyses is to see whether some preliminary signals in the data could be detected.

11.0 LABELING, PACKAGING, STORAGE AND RETURN OF CLINICAL SUPPLIES

11.1 Investigational Product

The investigator shall take responsibility for and shall take all steps to maintain appropriate records and ensure appropriate supply, storage, handling, distribution and usage of investigational product in accordance with the protocol and any applicable laws and regulations.

Clinical Supplies will be provided by Merck and AMAG Pharmaceuticals as summarized in Table 12.

Table 12 Investigational Product Descriptions

Product Name & Potency	**Dosage Form**
Investigational Drug - Merck	
1. Pembrolizumab 50 mg	Lyophilized Powder for Injection
2. Pembrolizumab 100 mg/4mL	Solution for Injection
Investigational Contrast Agent – AMAG Pharmaceuticals	
1. Ferumoxytol 510mg/17mL	Single Dose Vial

11.2 Packaging and Labeling Information

Clinical supplies will be affixed with a clinical label in accordance with regulatory requirements.

11.3 Clinical Supplies Disclosure

Pembrolizumab

This trial is open-label; therefore, the subject, the trial site personnel, the Sponsor and/or designee are not blinded to treatment. Drug identity (name, strength) is included in the label text; random code/disclosure envelopes or lists are not provided.

Ferumoxytol

Ferumoxytol is a commercially available agent and is supplied to investigators by purchasing through the OHSU research pharmacy. It is FDA approved for iron replacement therapy. It is supplied in single dose vials containing 510 mg per 17 ml. It is being used in this study off-label and under a physicians IND.

11.4 Storage and Handling Requirements

Clinical supplies must be stored in a secure, limited-access location under the storage conditions specified on the label. Receipt and dispensing of both agents will be recorded by the OHSU Research Pharmacy. Clinical supplies may not be used for any purpose other than that stated in the protocol.

11.5 Returns and Reconciliation

The investigator is responsible for keeping accurate records of the clinical supplies received from Merck or designee, the amount dispensed to and returned by the subjects and the amount remaining at the conclusion of the trial.

Upon completion or termination of the study, all unused and/or partially used investigational product will be destroyed at the site per institutional policy. It is the Investigator's responsibility to arrange for disposal of all empty containers, provided that procedures for proper disposal have been established according to applicable federal, state, local and institutional guidelines and procedures, and provided that appropriate records of disposal are kept.

12.0 ADMINISTRATIVE AND REGULATORY DETAILS

12.1 Compliance with Trial Registration and Results Posting Requirements

Under the terms of the Food and Drug Administration Modernization Act (FDAMA) and the Food and Drug Administration Amendments Act (FDAAA), the Sponsor of the trial is solely responsible for determining whether the trial and its results are subject to the requirements for submission to the Clinical Trials Data Bank, http://www.clinicaltrials.gov. Information posted will allow subjects to identify potentially appropriate trials for their disease conditions and pursue participation by calling a central contact number for further information on appropriate trial locations and trial site contact information.

12.2 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.

For studies conducted outside the U.S. under a U.S. IND, the Principal Investigator must comply with the record retention requirements set forth in the U.S. FDA IND regulations and the relevant national and local health authorities, whichever is longer.

12.3 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.

Data will not be released to any entity other than Merck, the IRB and the Knight Cancer Institute, 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.

Data Storage and disposition: the study team will maintain a password protected database (REDCap) for subject enrollment and all data collection. The database (REDCap) will be maintained on an OHSU secured, password protected network, and only the study team will have access to the spreadsheet, 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, another investigator, or to OHSU Knight Cancer Institute (KCI) Clinical Trials Office. Records must be maintained according to sponsor or FDA requirements.

12.4 OHSU IRB Reporting of Reportable New Information and Adverse Events (only if applicable)

Reportable New Information (RNI) and Adverse Events (AE) will be reported to IRB according to the policies, procedures and guidelines posted on the OHSU IRB web site:

- Fatal and life-threatening RNI will be reported to OHSU IRB within 5 days of notification of the event. All other reports will also be submitted to OHSU IRB no later than 5 days of occurrence or notification of the event. Copies of the report documents will be kept in the study regulatory binder.
- Reportable New Information is submitted through OHSU eIRB and will be reviewed by OHSU Knight Cancer Institute and IRB.

12.5 OHSU Knight Cancer Institute Data and Safety Monitoring Plan

In addition to complete study and pharmacy files, complete records must be maintained on each subject treated on this protocol. OHSU Knight Cancer Institute, through the auditing function of the Knight Clinical Trials Office, is responsible for ensuring that all member investigators and affiliate investigators conduct clinical research studies in compliance with local IRB standards, FDA regulations and NIH policies and in accordance with the Data and Safety Monitoring Plan policies and procedures.

The study will be audited by an OHSU KCI Auditor. Newly approved studies may be audited any time after enrollment has been initiated. Each OHSU Knight approved treatment protocol will be audited on an annual basis in accordance with the Knight Data and Safety Monitoring Plan.

The Knight DSMC will review and monitor study progress, toxicity, safety and other data from this study. Information that raises any questions about participant safety or protocol performance will be addressed by the Investigator, statistician and study team. Should any major concerns arise, the Knight DSMC may recommend corrective action and determine

whether or not to suspend the study.

The Knight DSMC will review each protocol every 6 months, but may occur more often, if required, to review toxicity and accrual data (please refer to Knight DSMP for additional details on audit frequency). The Knight DSMC will review accrual, toxicity, response and reporting information. Information to be provided to the DSMC may include: participant accrual; treatment regimen information; AEs and SAEs reported by category; summary of any deaths on study; audit results; and a summary provided by the study team. Other information (e.g. scans, laboratory values) will be provided upon request.

Clinical Data & Safety Monitoring
Monitoring visits will be performed 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 Assurance & 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.

Quality assurance (QA) auditing activities will occur as detailed in the Knight DSMP. All discrepancies, queries, deviations, observations, and findings will be compiled into a final audit report along with a Corrective and Preventative Action Plan.

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)).

12.5.1 Changes to Protocol

Any modification of this protocol must be documented in the form of a protocol revision or amendment signed by the PI and approved by the KCI and IRB, before the revision or amendment may be implemented. 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 KCI and IRB in writing within 10 working days after the implementation. Investigators holding the IND must notify FDA of substantive changes to the protocol.

13.0 INCLUSION OF WOMEN, MINORITIES AND CHILDREN IN CLINICAL TRIALS

The following protocol attachment is required by the OHSU Knight Cancer Institute, Clinical Research Review Committee in order to meet NIH guidelines on the inclusion of women, minorities and children as subjects in clinical research. This policy conforms to section 492B of the Public Health Service Act, NIH Revitalization Act of 1993, and Public Law 103-43.
No OHSU Knight Cancer Institute study will focus on any particular gender, racial or ethnic subset. No subject will be excluded from the study on the basis of gender, racial or ethnic origin. Male, female and minority volunteers will be recruited for this study from the general population and approximately 50% men and 50% women will be studied.

The projected gender, racial, and ethnic composition of the study will represent that of the state of Oregon.

Table 13 Population Demographics - Oregon (%)

Ethnic Category	**Sex/Gender**		
	Females	Males	Total
Hispanic or Latino			11.7
Not Hispanic or Latino			88.3
Ethnic Category: Total of all subjects*			100*

Racial Category			
American Indian or Alaskan Native			1.4
Asian			3.7
Black or African American			1.8
Native Hawaiian or other Pacific Islander			0.3
White			83.6
More than one race			3.8
Unknown/Other			5.3
Racial Category: Total of all subjects*			100*
TOTALS	50.4	49.6	100*

Source: U.S. Census Bureau, 2010 *Totals may not equal 100 due to rounding.

Table 14 Projected Accrual

Ethnic Category	**Sex/Gender**			
	Females	Males	Unknown	Total
Hispanic or Latino	1	1		2
Not Hispanic or Latino	9	9		18
Unknown				
Ethnic Category: Total of all subjects*	10	10		20
Racial Category				
American Indian or Alaskan Native				
Asian	1			1
Black or African American				
Native Hawaiian or other Pacific Islander				
White	9	8		17
More than one race		1		1
Unknown		1		1
Racial Category: Total of all subjects*	10	10		20

Source: Adapted from U.S. Census Bureau, 2010 *Totals may not equal 100 due to rounding.

Inclusion of Children

In accordance with NIH guidelines on the inclusion of children as participants in research involving human subjects, children under the age of 18 years must be included in all human subjects' research, conducted or supported by the NIH, unless there are clear and compelling reasons not to include them. Therefore, proposals for research involving human subjects must include a description of plans for the inclusion of children.

This protocol does not include children for the following reason: No dosing or adverse event data are currently available on the use of this Study Agent in this way in subjects <18 years of age, therefore, children are excluded from this study but will be eligible for future pediatric trials with this Study Agent.

14.0 SUPPLEMENTAL MATERIAL

14.1 ECOG Performance Status

Grade	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (e.g., light housework, office work).
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.
5	Dead.
* As published in Am. J. Clin. Oncol.: *Oken, M.M., Creech, R.H., Tormey, D.C., Horton, J., Davis, T.E., McFadden, E.T., Carbone, P.P.: Toxicity And Response Criteria Of The Eastern Cooperative Oncology Group. Am J Clin Oncol 5:649-655, 1982.* The Eastern Cooperative Oncology Group, Robert Comis M.D., Group Chair.	

14.2 Common Terminology Criteria for Adverse Events V4.0 (CTCAE)

The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for adverse event reporting. (http://ctep.cancer.gov/reporting/ctc.html)

14.3 Ferumoxytol Investigator's Brochure

15.0 APPENDICES

Appendix A: Ferumoxytol Adverse Events Form
Appendix B: Pembrolizumab Adverse Events Form

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