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TITLE: Response Assessment to Pembrolizumab with Standard of Care Therapy in Glioblastoma Using Ferumoxytol Steady State Imaging– A Pilot Study

Principal Investigator:
Leslie Muldoon, PhD
Associate Professor
Department of Neurology
Oregon Health & Science University
(503) 494-5626

Co-Investigators:

Guang Fan, 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
Department of Neurology
Oregon Health & Science University
(503) 494-5626
(503) 494-5627 (FAX)

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Version 11	Submitted to IRB 05/22/23; approved 05/30/23

1.0 TRIAL SUMMARY

Abbreviated Title	Assessment Of Response To Pembrolizumab In Primary Brain Tumors Using Ferumoxytol Steady State Imaging– A Pilot Study.
Trial Phase	Pilot study, phase II
Clinical Indication	Metastatic melanoma (closed after enrolling 2 subjects), glioblastoma
Trial Type	Single institution, multi-arm, phase II pilot study for safety and efficacy in conjunction with experimental imaging for disease response
Type of control	None
Route of administration	Intravenous pembrolizumab and intravenous ferumoxytol
Trial Blinding	None
Treatment Groups	Two arms; Arm 1: melanoma (closed after enrolling 2 subjects); Arm 2: glioblastoma
Number of trial subjects	Total 45 (Glioblastoma: 43; Melanoma 2 (closed))
Estimated enrollment period	3 years
Estimated Duration of Trial	5 years
Duration of Participation	5 years

2.0 TRIAL DESIGN

This study will evaluate incidence, magnitude and timing of true vascular tumor progression, pseudoprogression and immune response to pembrolizumab in patients with primary brain tumors using ferumoxytol steady state MRI imaging.

Originally, the study had two arms:

ARM 1: Metastatic brain tumors - Metastatic melanoma
ARM 2: Primary brain tumors - Glioblastoma (GBM)

In December 2019, we decided to stop the enrollment to the melanoma arm and allocate the remaining 18 slots to the Glioblastoma arm – a total of 43 subjects will now be enrolled in the GBM arm.

Treatment

Subjects scheduled to receive standard of care stereotactic radiosurgery (SRS) for brain metastases in Arm 1 or standard of care chemoradiation (CRT) with temozolomide for GBM in Arm 2, are eligible. All subjects will receive pembrolizumab (200mg intravenous every 3 weeks) in addition to standard of care SRS or CRT in the respective arms, until toxicity, or persistent radiographic progression is confirmed, whichever comes first.

Imaging

All subjects will be scanned with clinical MRI at baseline prior to radiation, 4 weeks after the last day of radiation and every 9 weeks thereafter until completion or until taken off-study for

progression or toxicity. Ferumoxytol MRI (FeMRI) will be performed in the same day and sitting as the clinical MRI at baseline and 4 weeks after the last day of radiation timepoints. FeMRI may be performed independently (on a different day or different MRI setting) of the clinical MRI if there is suspected radiographic progression or to confirm radiographic progression. If there is suspected progression, subjects undergo a ferumoxytol steady state MRI and continue on pembrolizumab for one more cycle. The decision regarding continuation of pembrolizumab will be made by the principle investigator based on clinical findings that include (1) stable ECOG performance status with no more than 8mg of dexamethasone (or equivalent) a day; (2) absence of new or worsening symptoms not managed with steroids; and (3) absence of progressive tumor at critical anatomical sites (e.g., spinal cord compression) requiring urgent alternative medical intervention. Follow up clinical standard of care and ferumoxytol MRIs will be repeated to confirm persistent radiographic progression in 4 weeks. During this 4 week follow up period, subjects will be followed with a clinical exam weekly to monitor for clinical stability. If the 4 week follow up scan confirms progression (as defined in section 8.1), then pembrolizumab is discontinued and subject is evaluated for clinical indication of standard of care surgery or biopsy. Surgery or biopsy is performed only if 1) the lesion is deemed easily accessible 2) surgery is not expected to worsen neurological outcomes and 3) it is in the best clinical interest of the subject. The decision regarding surgery will be consensus based and when possible, will be made after each individual case has been presented at the OHSU multi-disciplinary brain tumor board. 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. Subjects with evidence of new lesions distant from the primary lesion/s (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.

An optional, additional third imaging session may be performed with no additional contrast 24 hours after ferumoxytol administration. This optional day of imaging will be used to evaluate delayed ferumoxytol enhancement and will be performed in only those patients who have consented.

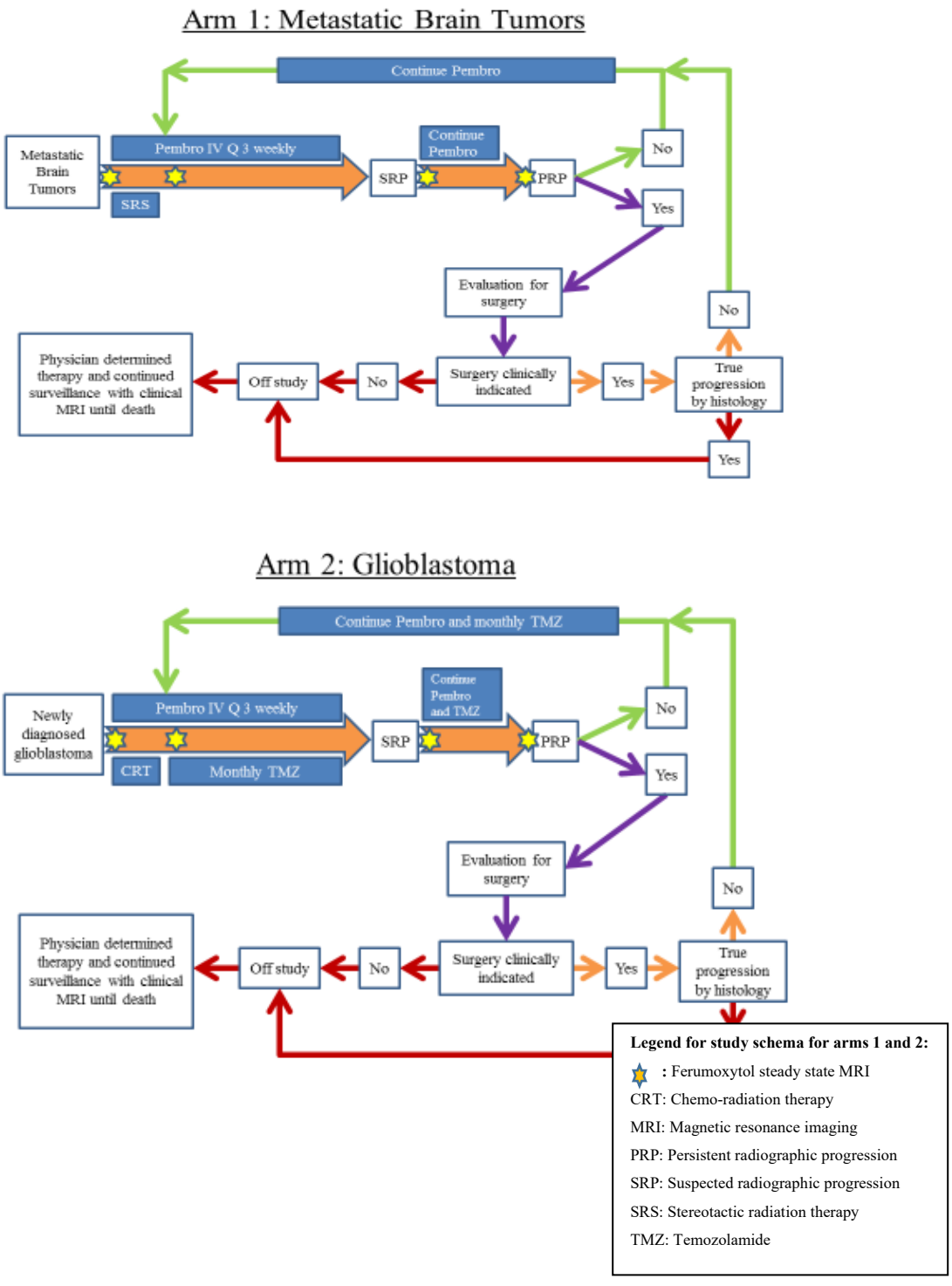
Biomarker

Optional blood for biomarker evaluation may be drawn as described in section 8.3.1. These markers will be correlated with imaging findings and survival. Analysis will be performed by the OHSU clinical flow cytometry laboratory.

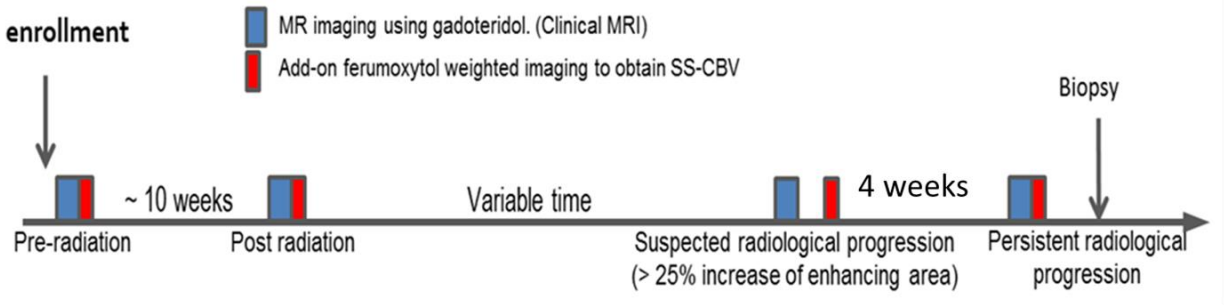
Any archival biopsy, fresh tissue, as well as tissue obtained at persistent radiographic progression will be obtained for clinical pathology review at OHSU. Tissue samples will be sent to QualTek Molecular Laboratories for PD-L1 biomarker assay as described in section 8.3.2.

For Group 1 only: In addition, subjects that are already enrolled in the study and have measurable systemic disease (outside the CNS) by RECIST 1.1 Criteria [2] at screening, may undergo optional additional MRI sequences of the systemic disease to evaluate the immunological response to pembrolizumab outside the CNS (systemic disease).

2.1 Trial schema:



Imaging schema:



Timeline of MRIs: subjects will be scanned at multiple stages of disease. Clinical and ferumoxytol MRIs will be performed on the same day in each time point except for the one when clinical MRI follow up reveals radiological progression. In that case ferumoxytol imaging alone will be performed shortly after the radiological diagnosis. Note that this study will include only short study MRI with ferumoxytol, which may be attached to clinical MRI. Pembrolizumab treatment will start at day 1 of radiation and continue till persistent radiological progression.

3.0 OBJECTIVES

3.1 Primary Objective

Determine the sensitivity and specificity of relative cerebral blood volume (rCBV) measured by steady state MRI with ferumoxytol in identifying true vs pseudoprogression in patients with newly diagnosed GBM receiving pembrolizumab with standard of care chemo-radiation.

3.2 Secondary Objectives:

1. Determine the safety and toxicity of pembrolizumab when used in combination with standard of care chemo radiation.
2. Determine the progression free survival (PFS), overall survival (OS), clinical response and duration of best response.

Rationale: Given the relatively small sample size, we aim to collect preliminary data on safety and toxicity as well as progression free survival, overall survival, best response and duration of best response survival. Data from this pilot study will be used to power a larger study.

3.3 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. This is optional and will be performed only in patients who have provided additional consent.

2. Investigate the serum immunological parameters (serum biomarker) and correlate clinical as well as radiological response with systemic immune response to pembrolizumab as measured by immunological panel.

3. Compare the changes in PDL-1 expression in the biopsy tissue before and after therapy at the time of progression and correlate PD-L1 expression with response rates and survival.

4. In subjects in metastatic arm with any 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 determined by RECIST 1.1 and irRC [1, 2].

3.4 Hypotheses

Since this is a pilot study to determine feasibility of administration of ferumoxytol with pembrolizumab and imaging logistics, no formal hypothesis is being tested. We expect rCBV measurements using ferumoxytol steady state MRI will be able to accurately identify true vs pseudoprogression. Neovascularization is pivotal for tumor viability and high grade tumors are expected to have a higher vascularity and hence a higher blood volume. An immune response or pseudoprogression is expected to have low vascularity and hence a lower rCBV. Prior studies have shown that rCBV value greater than or equal to 1.75 indicated active tumor while rCBV below 1.75 suggest an inflammatory response [3, 4]. Approximately 24 -72 hours after intravenous injection, ferumoxytol is taken up by activated inflammatory cells including M1 macrophages. An increased uptake on delayed scans suggests a robust inflammatory response and hence pseudoprogression and is seen as increased enhancement in T1 weighed images obtained 24 hours after Ferumoxytol injection.

There will be evidence of up regulation of the immune system serum biomarker assay at various time points. Multicolor flow cytometry as described in section 8.3.1 will be used to evaluate various immune cell populations to correlate clinical and radiological response with systemic immune responses.

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. Tissue obtained at initial surgery will be stained for PD-L1. We expect to see a linear correlation between tumor PD-L1 expression, immune infiltrate, and response rates and survival.

4.0 BACKGROUND & RATIONALE

Refer to the Investigator's Brochure (IB)/approved labeling for detailed background information on pembrolizumab and ferumoxytol, and the product insert for temozolomide.

Introduction

Immune checkpoints, particularly inhibitors of programmed cell death protein (PD-1), have emerged as important mechanisms for immunosuppression and immune-evasion in malignancies [5] . High PD-1ligand expression is seen in malignancies including melanoma, non-small cell lung carcinoma, triple-negative (ER, PR, and HER2 negative) breast cancer and glioblastoma multiforme (GBM). There is great enthusiasm in the neuro-oncology community to design trials to test pembrolizumab in primary and metastatic brain tumors [1, 6-11]. Unfortunately, most prior studies looking at systemic malignancies excluded brain metastases and hence there is limited data on its efficacy in this patient population.

Immunotherapy for brain tumors:

Prognosis for primary and metastatic brain tumors continues to be poor [12-14]. Current standard of care therapies includes surgery, radiation and cytotoxic chemotherapy. Over the last decade, targeted molecules and better radiation techniques have failed to deliver the paradigm improvement in progression free or overall survival rates seen with systemic cancers [15].

Challenges unique to brain tumors include drug delivery across the neurovascular unit (NVU), limiting neurotoxicity, and preserving quality of life. In addition, these tumors evade immune surveillance and dampen the anti-tumor immune response by down regulation of HLA molecules, expression of immunosuppressive cytokines, increasing the activation of T-regulatory (T_{reg}) cells, and increasing the T helper cell phenotype [16]. The brain was previously thought be an immune-privileged organ due to the paucity of native antigen presenting cells (APCs), low baseline levels of major histocompatibility complex (MHC) expression, absence of traditional lymphatic system and the notion that the NVU was impermeable to large molecular weight antibodies and lymphocytes and relative lethargy in rejection of foreign tissue engrafted into the CNS [17, 18].

Our understanding of the brain immune micro-environment has undergone a major shift in the recent years. It is now clear that microglia, macrophages, and dendritic cells act as powerful APCs in the CNS [19-21]. Recent demonstration of the perivascular lymphatic pathway in the CNS, now known as the ‘glympatics’, furthers our understanding of the drainage route through which the CNS microenvironment can interact with the better understood systemic immune system [22]. Animal studies from our lab previously demonstrated trafficking of superparamagnetic iron oxide particles from brain to cervical lymph nodes and that this interaction can be visualized using MRI [23].

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 [24-29]. 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 [30]. 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 [31, 32]. 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.

Pembrolizumab in Brain Tumors

Pembrolizumab, is a monoclonal antibody that blocks the programmed death-1 receptor (PD-1, CD279), resulting in dis-inhibition of tumor-specific immune responses and is now FDA approved for the treatment of advanced and metastatic melanoma. PD-1 blockade is thought to prevent PD-L1 expressed on tumor cells, stromal cells, and antigen-presenting cells (APC) from engaging PD-1 expressed on T cells, leading to more robust T cell activity resulting in anti-tumor effects. Pembrolizumab is now FDA approved for the treatment of advanced melanoma, 1st and 2nd line non-small cell lung carcinoma, head and neck cancer, and classical hodgkins lymphoma [6, 33]. Similarly, based on the excellent efficacy noted in the Keynote 01 study, pembrolizumab is now approved for patients with metastatic advanced non-small cell carcinoma lung (NSCLC) across histologies [34]. Promising clinical activity was also noted in patients with recurrent/metastatic triple-negative (ER, PR, and HER2 negative) breast cancer [35]. There are multiple studies currently testing the efficacy of PD-1 inhibitors including pembrolizumab with bevacizumab in patients with recurrent glioblastoma (NCT02017717). The encouraging systemic anti-tumor effects makes pembrolizumab an ideal candidate for testing in primary and metastatic brain tumors that are frequently excluded from such studies. Patients with brain tumors have a very poor prognosis, and pembrolizumab is expected to provide good responses that are sustained in this population.

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 [24-29]. 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 [30]. 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 [31, 32]. 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.

Immune Surveillance in the CNS And Blood Tumor Barrier:

Despite significant improvement in our understanding the molecular pathogenesis of these brain tumors,drug deliver across the neurovascular unit and blood tumor barrier continues to be a major challenge [14]. Pembrolizumab is a large molecular weight monoclonal antibody. There is justifiable concern of large molecules being delivered into the CNS. However, the mechanism of action of PD-1 inhibitors such as pembrolizumab is at the systemic T-cell side of the immune synapse and not at the level of the PD-L1 ligand on the tumor cells that is inside the blood brain barrier.[36, 37] Immune cells that are part of the CNS immunosurveillance mechanism are known to be able to migrate in and out of the CNS more so under inflammatory

conditions and into the tumor microenvironment.[38, 39] One of the mechanisms by which tumors including triple negative breast cancer, melanoma and glioblastoma evade the immune surveillance is due to over expression of PD-L1 and its interactions with PD-1 receptors on T cells. Pembrolizumab blocks these interactions and hence is expected to enhance the antitumor immune response [6, 34, 40-44]. The concurrent use of standard of care including radiation in addition to pembrolizumab is justified by the synergistic action of radiation and checkpoint blockade immunotherapy [45].

Challenges in brain tumor trial design: pseudoprogression

A major challenge in managing and designing trials for brain tumors is determining tumor response to therapy, and then differentiating response from recurrence or treatment-related changes. After radiation, a large majority of these tumors can paradoxically increase in size due to an inflammatory response called 'pseudoprogression'. The term pseudoprogression describes the phenomenon of subacute radio-chemotherapy treatment-related sequelae in patients with brain tumors that presents as increasing or new contrast enhancement on MRI [46, 47]. These patients stabilize or recover spontaneously, usually without any change in treatment. As cancer immunotherapeutics are being tested in clinical trials, pseudoprogression is increasingly becoming a growing challenge that needs to be addressed [48]. It is estimated that over 10 -12% of patients develop pseudoprogression before eventually showing a response that can be captured by conventional response assessment criteria after immunotherapy. Similarly, over 30% develop imaging changes that are consistent with pseudoprogression after radiation [49]. This number is significantly higher when immunotherapy is combined with standard of care radiation. Current standard of care imaging or response assessment criteria do not have the ability to differentiate this immune response from true progression at any given time point.

Currently used response assessment guidelines used such as RECIST 1.1 and WHO (for response assessment in systemic solid tumors) have consistently proven inadequate due to the unique anatomy of the brain, presence of the neuro vascular unit (also referred to as the blood brain barrier (BBB)), blood tumor barrier and heterogeneity of tumors like glioblastoma [2, 50-52] [39-42]. It is now well understood that enhancement seen in MRI represents just the area with disrupted BBB and does not represent the whole tumor. 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 [51]. Further, agents like steroids and bevacizumab that are frequently used for symptom management in brain tumors can significantly alter imaging and alter response assessment. This lead to the development of the consensus based Response Assessment in Neuro-Oncology Working Group (RANO) criteria [53]. Based on RANO, all radiographic progression after 12 weeks post radiation is considered disease progression. The rate of pseudoprogression diagnosed at any time after radiation ranges from 9.3-31% [46]. Furthermore, around 30% of all pseudoprogression occurred beyond the 12-week RANO cut off including 2 cases occurring over 6 months after chemoradiation [3].

Figure 1 shows a case where the patient fulfilled criteria for progressive disease by RANO at 3 months after radiation, but was shown to have pseudoprogression by low blood volume on ferumoxytol (an iron oxide nanoparticle blood pool contrast agent) imaging.

Ferumoxytol Imaging: Differentiating Pseudoprogression from True Progression

Preliminary retrospective data from our group suggests that a novel MRI sequence called steady state (SS) imaging with ferumoxytol provides excellent spatial and temporal resolution and will fulfil this need. SS imaging cannot be performed with conventional gadolinium based MR contrast agents [54]. Ferumoxytol (Feraheme), is an iron oxide nanoparticle that is FDA-approved as an intravenous iron supplement [55]. Over the last 20 years, we have demonstrated that ferumoxytol is a safe and effective contrast agent for brain tumor imaging, especially evaluating tumor vascularity and blood volumes [56-60]. Measurement of brain tumor relative cerebral blood volume (rCBV) with ferumoxytol may be helpful to clearly differentiate true tumor progression from pseudoprogression after chemo-radiotherapy [3, 54, 61, 62]. Based on our studies, ferumoxytol has been granted orphan drug status for the imaging of brain tumors. Dr. Edward Neuwelt holds the investigational new drug (IND) for ferumoxytol for the imaging indication, and the FDA has approved the use of ferumoxytol in conjunction with other therapeutic investigational drugs, in the setting of a clinical trial.

This proposed pilot study will provide preliminary prospective data regarding the sensitivity and specificity of ferumoxytol steady state technology as a noninvasive biomarker in differentiating true progression from pseudoprogression after immunotherapy with the check point inhibitor pembrolizumab. Additionally, this study will generate pilot data regarding clinical activity of adding pembrolizumab to standard of care for primary and brain metastases.

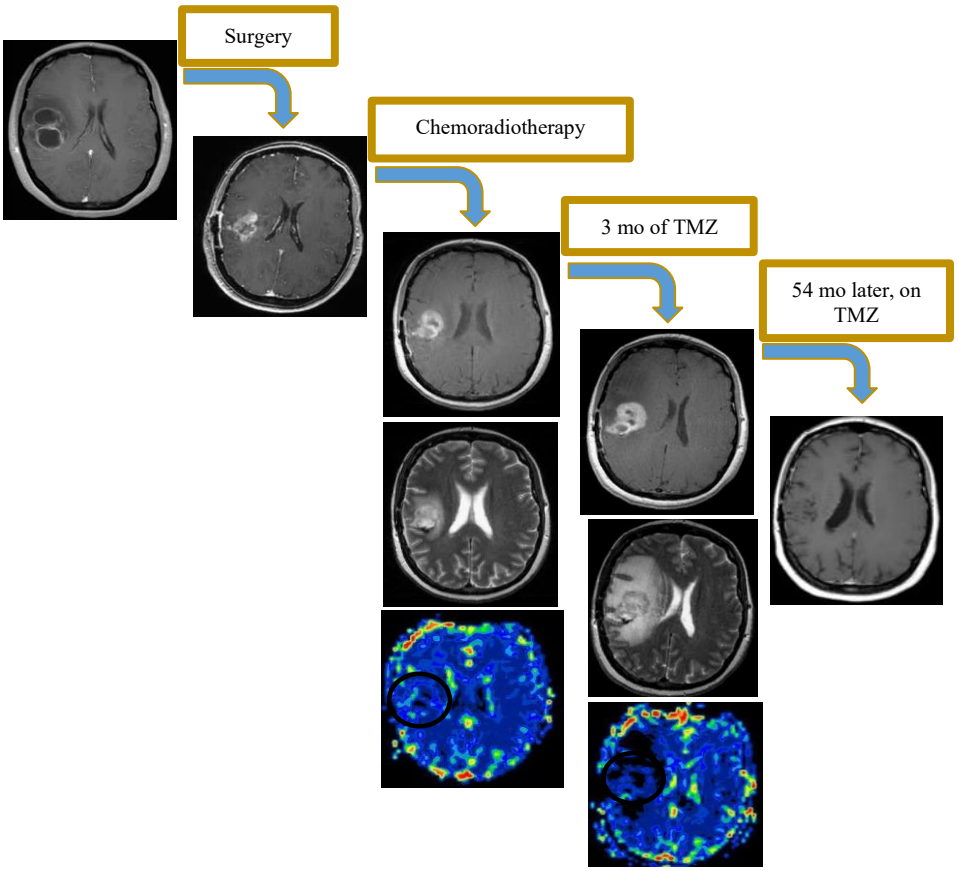
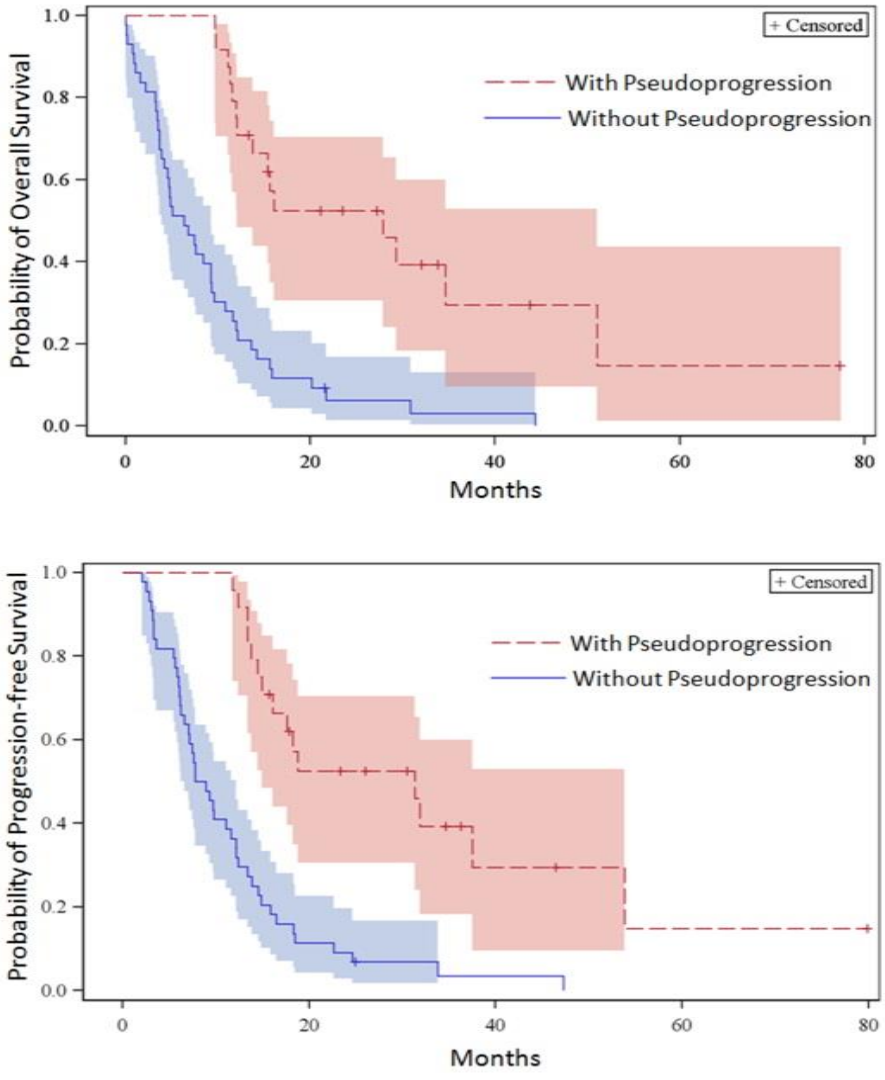


Figure 1. 48 y/o F with glioblastoma.**Column 1**: preoperative T1+Gd MRI. **Column 2**: T1+Gd (gadolinium) MRI after maximal safe resection. **Column 3**: T1+Gd, T2 and Ferumoxytol perfusion MRI after completion of standard CRT. Note increased enhancement and low rCBV in the lesion (circle). **Column 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

Our retrospective data from 68 patients with high grade gliomas suggest that pseudoprogression significantly increases overall survival from around 13 months to over 34 months (Figure 2).

Figure 2: Kaplan-Meier estimates of overall survival and progression-free survival by the presence of pseudoprogression



This difference was more pronounced in patients with MGMT methylation) [63]. It is now well recognized that inaccurate attribution of progression or pseudoprogression at enrollment or at the time of response assessment can significantly bias clinical trial outcomes based on progression free survival. In addition, it leads to premature therapy switching, potentially negating the benefits of a particular therapy. These radiographic changes are expected to increases after immune-modulatory therapy like PD-1 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 fulfills 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. Figures 1 and 3 show pseudoprogression in glioblastoma. The patient described in figure 3 was on immune-modulatory therapy (EGFRv3 based vaccine study) and was taken of study due to erroneous diagnosis of true progression when the patient in fact had pseudoprogression and subsequently showed clinical and radiographic improvement.

Early ferumoxytol scans: A biomarker for tumor vascularity

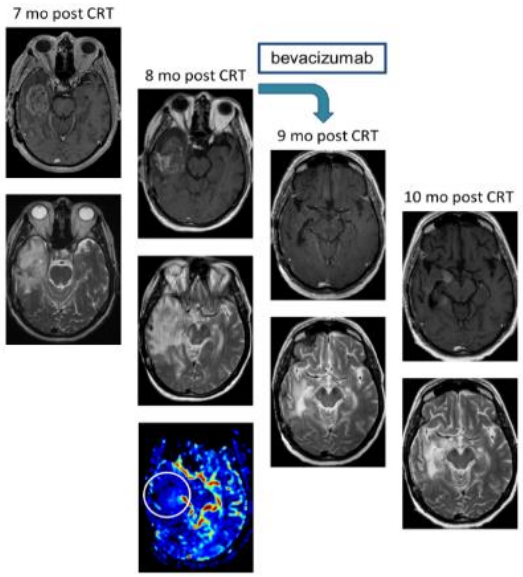


Figure 3: This Patient received Celldex vaccine (an investigational immunotherapy that targets the tumor specific oncogene EGFRv3) at 5 and 6 months after CRT and 4 monthly cycles of adjunct Temozolamide. A Dramatic increased mass effect was noted 7-8 months post CRT and 1-2 months post Celldex vaccine. 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 perfusion MRI 8 months after CRT showing decreased CBV in the enhancing area in the right temporal lobe, suggesting pseudoprogression. At this point Patient might be falsely classified as disease progression by updated RANO criteria. Patient was continued on Temozolamide based on our blood volume measurements. He was given one dose of Avastin as an anti-edema measure in addition to high dose of steroids. He had to be eventually taken off the clinical because the trial excluded patients on Avastin .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.

Prior studies have demonstrated elevated relative cerebral blood volume (rCBV≥1.75) in actively growing tumor while pseudoprogression shows low rCBV in primary brain tumors [3, 4, 46, 47, 61, 64-66]. Dynamic susceptibility contrast (DSC) perfusion, which provides an estimate of rCBV in tumor and brain, has shown value in distinguishing primary from metastatic cerebral tumors and assessing response to therapy. It adds little to no extra cost to a clinical MRI scan, and is rapidly becoming the most widely used advanced MRI technique in brain tumor imaging. rCBV analysis is especially helpful in differentiating progression from response to therapy (pseudoprogression). Using a lesion rCBV threshold of >2.0 yields 56% sensitivity and 100% specificity in the differentiation of tumor progression from treatment injury [54]. The use of ferumoxytol iron oxide nanoparticles which have no early leakage across the blood brain barrier has further improved rCBV analysis. Use of these improved MR perfusion techniques has led to very high accuracy in this important diagnostic dilemma, and may be predictive of outcome and long-term survival in high grade gliomas [61] (Figure 4).Varallyay and colleagues from our group have described the use of ferumoxytol MRI to obtain a high-resolution steady state-CBV image that differentiates regions of high vascularity and active tumor growth (Figure 5, 6) [54, 61]. Steady-state MRI with ferumoxytol further improves rCBV measurement by providing high spatial resolution (Figure 7) [54]. This is particularly helpful in imaging cortical lesions and lesions near blood vessels with very high resolution. High resolution rCBV measurement is very helpful in differentiating pseudoprogression from true progression not only in glioblastoma after CRT but also after SRS in brain metastases as well (Figure 8).

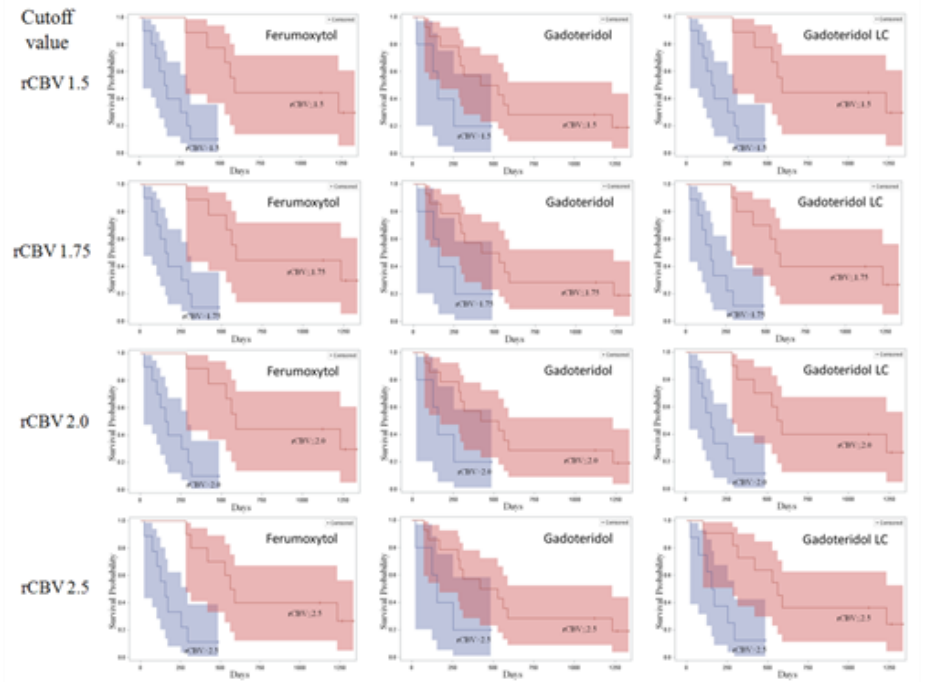


Figure 4: Charts show survival of GBM patients according to rCBV by using four cutoff values. Tumor rCBV was measured by using ferumoxytol versus gadoteridol and gadoteridol with leakage correction (Gadoteridol LC). Kaplan-Meier survival curves show best survival prediction by using rCBV values obtained with ferumoxytol (P , .001) when cutoff range is between 1.5 and 2.0, and the same result was achieved with gadoteridol LC with cutoff of 1.5. By using gadoteridol, survival prediction is similar but not statistically signifi cant (P = .079) for all cutoff values. Colored areas indicate 95% CIs.

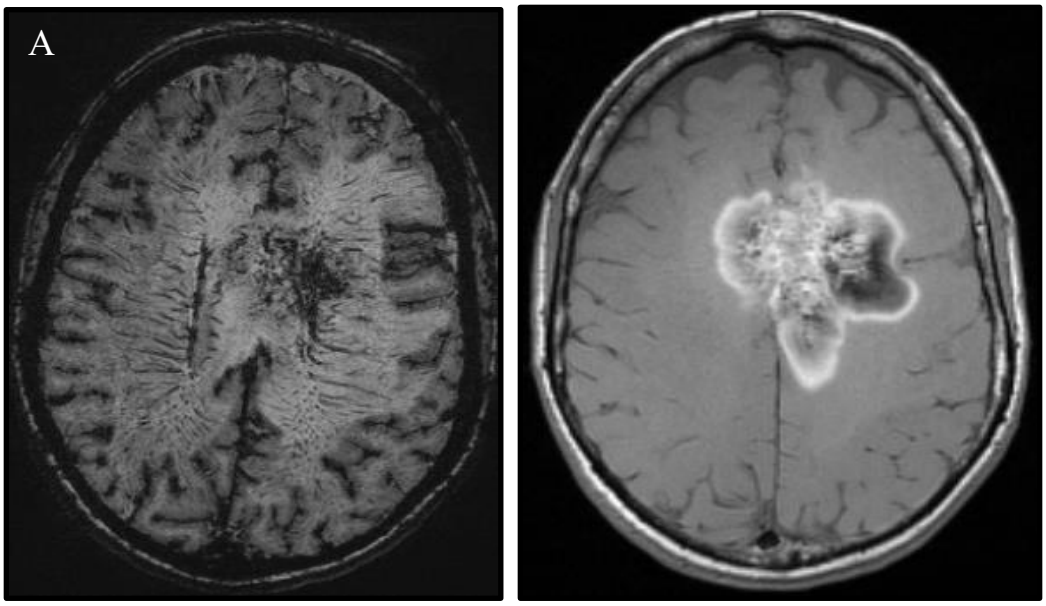


Figure 5: Brain imaging using ferumoxytol: (A) High resolution images showing intravascular iron. (B) Delayed enhancement24 hours after ferumoxytol administration.

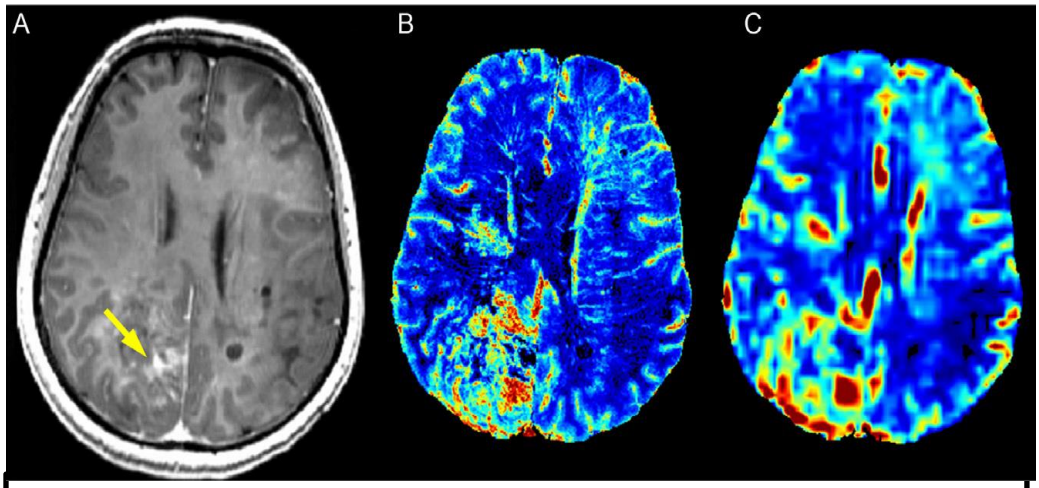


Figure 6. Comparison of steady-state-cerebral blood volume (SS-CBV) and dynamic susceptibility contrast (DSC)-CBV maps in a glioblastoma patient. (A) T1-weighted postgadoteridol scan describes the multifocal signal abnormalities. In corresponding slices, the SS-CBV (B) and DSC-CBV (C) maps show increased 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).

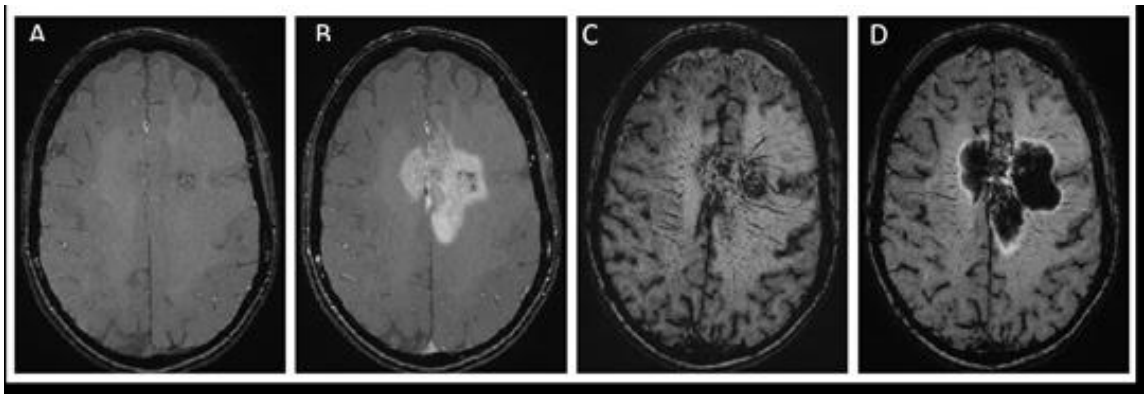


Figure 7: T2* weighted gradient echo images (A) without contrast (B) Gadolinium based contrast agent (c) early scan(<1 hr) after Ferumoxytol injection (D)delayed (24hr) post ferumoxytol injection. Notice the high resolution of small vessels in the tumors and surrounding normal brain that in (C) is not visualized with Gadolinium based contrast agent (B). Delayed uptake of ferumoxytol (D)is a biomarker of inflammatory response

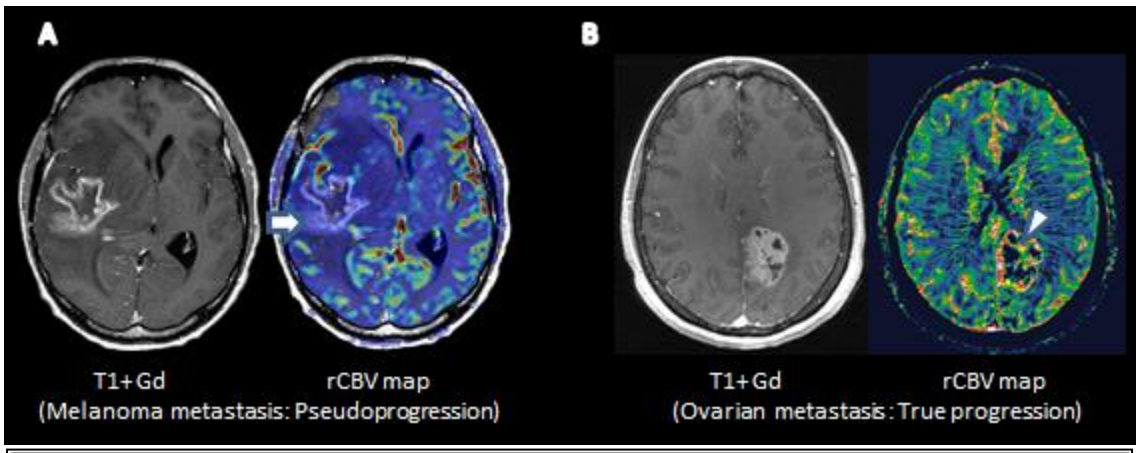


Figure 8: (A) Demonstrates low rCBV (White arrow) in the area of interest in a case with pseudoprogression (metastatic melanoma). (B) High relative blood volume (arrow head) is noted in a case with true progression (Ovarian metastases). Both cases would be characterized as 'progression' by conventional RANO criteria

Another major problem with response assessment is the use of VEGF inhibitors like bevacizumab or corticosteroids. These agents can alter the neurovascular unit 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 [67-69].

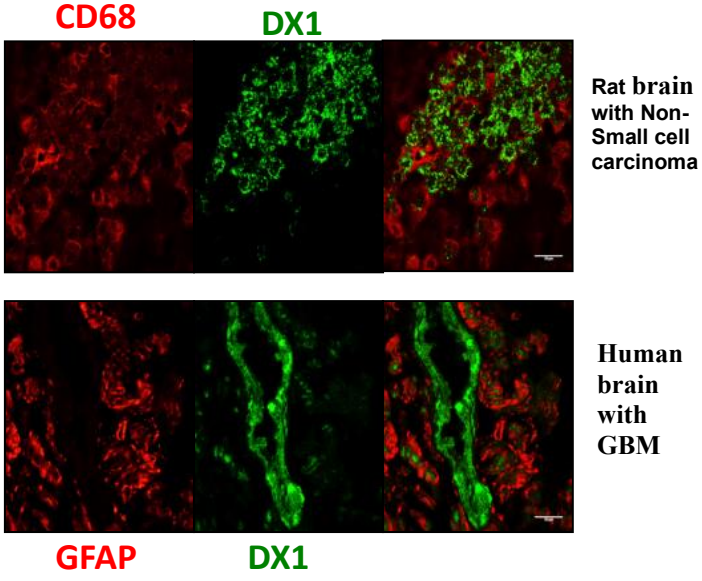
Our preliminary data suggests that use of bevacizumab may alter rCBV measurements in high grade gliomas. For the purpose of this study, a short course of up to 3 doses of bevacizumab may be allowed, when clinically indicated, as an anti-edema measure and will not be criteria for exclusion. Bevacizumab 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 bevacizumab and steroid dose will be performed as part of the final analysis. Since this is not a randomized trial and the need for bevacizumab cannot be predicted prior to enrollment, stratification prior to enrollment is not possible and analysis will be performed post enrollment. 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 evaluating the efficacy of the combination in brain tumors.

Delayed Ferumoxytol imaging: A biomarker for inflammation

In addition to uptake by the reticuloendothelial system, ferumoxytol and other iron oxide nanoparticles are taken up by tumor-associated macrophages [70-72] and brain microglia [73]. Delayed imaging of ferumoxytol in the CNS (figure 7) may therefore provide an important biomarker of inflammation and immune response in brain tumors [70, 74-76]. We have demonstrated that ferumoxytol iron oxide nanoparticles are taken up by phagocytic cells over hours to days after IV infusion (Figure 9). We are actively investigating the utility of ferumoxytol for imaging inflammation, assessing nanoparticle uptake into tumor-associated macrophages/microglia in inflammatory lesions (Figure 10). Our animal models suggest that the enhancement seen on T1 weighted images at >24 hr time point after ferumoxytol administration is believed to reflect the extent of local inflammation. This study will provide preliminary data regarding the significance of volume and extent of delayed ferumoxytol enhancement after immunotherapy.

FIGURE 9. 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)



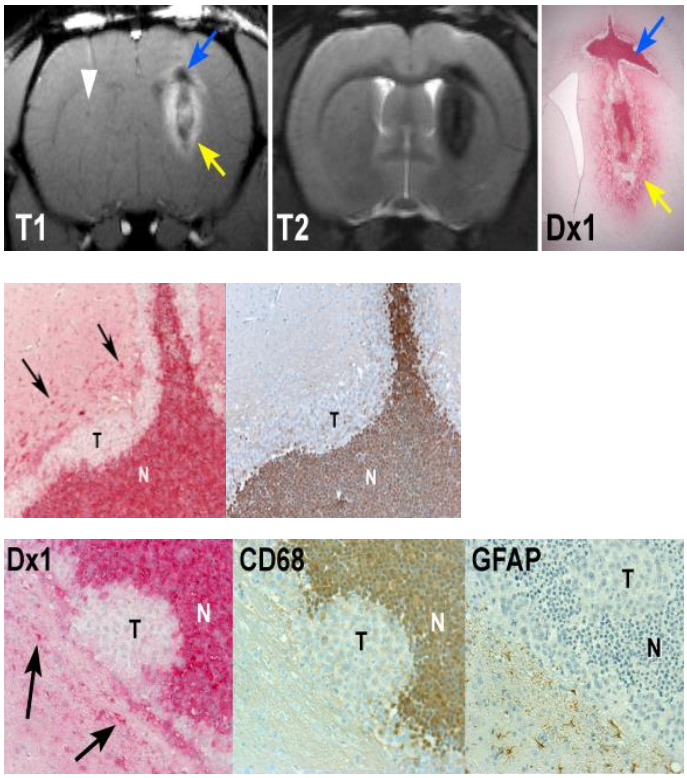


Figure: 10 (TOP) delayed MRI sequences of rat brain after ferumoxytol injection with corresponding histopathology stained for the dextran coating (Dx1) on ferumoxytol. (Bottom) Immunohistochemistry demonstrates ferumoxytol uptake and immune cell infiltration at inflammatory lesions. Twenty-four hours 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).

Our publications indicate that ferumoxytol imaging at early time points (24 hr) provides additional information regarding tumor vascularity and improves visualization of vascular malformations [74] and while at later time points (>24 hrs) (figure 5 and 7), it may help better detect the inflammatory component [77]. Delayed ferumoxytol-enhanced brain MRI was also shown to be useful in the diagnosis of CNS inflammatory disorders and lymphoma and is also useful for patients with renal compromise at risk of nephrogenic systemic fibrosis who are unable to receive GBCA [75].

Ferumoxytol steady state imaging for lesions outside the CNS: differentiating disease progression from flair response.

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 is being evaluated to assess tumor vasculature in a variety of systemic cancers including those in the breast, pancreas and prostate [63, 78, 79]. 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.

OHSU Blood-Brain Barrier (BBB) Program: experience with ferumoxytol

For the past three decades the OHSU Blood-Brain Barrier Program has developed significant laboratory and clinical expertise in imaging the CNS [50, 51]. 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 11). 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). 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 as well. The high volume of patients as well as expertise available at OHSU, including access to advanced imaging capabilities, ensures attainment of anticipated accrual targets and fulfilment of the objectives of this study.

4.1 Trial Description

See section 2.0

4.2 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. Keytruda^{TM} (pembrolizumab) has recently been approved in the United States for the treatment of subjects with advanced melanoma, 1st and 2nd line non-small cell lung carcinoma, head and neck cancer, and classical hodgkins lymphoma [6, 33].

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 and primary brain tumors) 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.

4.3 Preclinical and Clinical Trial Data

Refer to the Investigator's Brochures/Package Inserts for Preclinical and Clinical data (Supplemental Material, section 15).

4.3.1 Rationale for the Trial and Selected Subject Population

Subjects with metastatic brain tumors are frequently excluded from clinical trials of systemic disease. Systemic cytotoxic chemotherapy has consistently failed to show benefit in brain metastases due to the presence of the blood brain barrier, among other things [14, 64]. Similarly, prognosis continues to be poor for glioblastoma patients treated with standard chemo radiation [80].

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. 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. **S**afety and efficacy of pembrolizumab for systemic non–small-cell lung cancer and melanoma is now well described in multiple clinical trials.[5, 7, 34, 81] . There is promising preliminary evidence of efficacy.[35, 82]

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. This pilot study will prospectively evaluate a novel imaging ferumoxytol steady state imaging as a biomarker that can differentiate true from pseudoprogression in the context if immunotherapy with pembrolizumab. This study will also provide valuable pilot data regarding the efficacy of pembrolizumab in brain tumors. Larger studies will be designed using this data.

Significance:

With immunotherapy, a transient increase in size of lesion on imaging called flair response is frequently seen before a response is noted [48]. A similar increase in size of the lesion is noted after radiation therapy in primary brain tumors [46, 47, 63, 83]. 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 [84].

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 [54, 62, 63]. 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.

4.3.2 Rationale for Dose Selection/Regimen/Modification

Pembrolizumab

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.

Ferumoxytol
On June 20, 2009, the FDA approved ferumoxytol (Feraheme™) to treat Iron Deficiency Anemia and Adult Chronic Kidney Disease Patients. The recommended dose is an initial 510 mg IV injection followed by a second 510 mg IV injection three to eight days later. Ferumoxytol is now commercially available in the U.S.A.

Ferumoxytol has provided improved imaging with minimal safety concerns even using extremely high doses in preclinical trials with animals, in vitro using human blood cells, and in multiple IND sponsored studies. Ferumoxytol has few systemic reactions and can be given as a bolus for MRA analysis of tumor vasculature. The ferumoxytol Investigator's brochure 2012 contains detailed information of preclinical and clinical safety studies as summarized below.

Temozolomide for patients with glioblastoma (arm 2) will be as per clinical standard of care.

4.3.3 Preclinical studies

A series of nonclinical toxicity studies were conducted in rats and beagle dogs to assess the safety of ferumoxytol. Repeat-dose toxicity studies with ferumoxytol up to 12 mg Fe/kg/day for 13 weeks in rats (cumulative exposure approximately 12 times the exposure of a human therapeutic course of 1.02 g of ferumoxytol on a mg/m^2 basis) and dogs (cumulative exposure approximately 40 times the exposure of a human therapeutic course of 1.02 g of ferumoxytol on a mg/m^2 basis) demonstrated dose-dependent decreases in body weight gain and food consumption, and increases in pigmentation intensity. No systemic toxicity or immunotoxicity was observed at the relevant clinical doses. Changes in red blood cell (RBC) counts, hemoglobin (Hgb) and serum iron, increases in liver and spleen weight, and the accumulation of iron-positive pigmentation in various organs were observed as expected with the administration of an iron-containing agent.

Ferumoxytol showed no potential for mutagenic or clastogenic activity in several genotoxicity tests. Administration of 100 mg Fe/kg ferumoxytol (the highest dose studied) had minimal potential for inducing an anaphylactic response in a rat model of hypersensitivity and did not appear to cause irritation after IV administration in rabbits or guinea pigs. Ferumoxytol did not cause any visible flocculation, precipitation, or sedimentation when incubated with human serum or plasma (at concentrations up to 15 mg Fe/mL), caused only slight hemolysis in human erythrocytes and did not alter clotting times. No effects on the central nervous, respiratory, and renal systems were observed in mice, rats, and beagle dogs; only a minimal cardiovascular effect was observed in guinea pigs (1of 9 animals had a mild decrease in mean arterial blood pressure).

The toxicity of polyglucose sorbitol carboxymethylether (PSC, the carbohydrate coating of the iron oxide core of ferumoxytol) was evaluated in two repeat-dose studies in rats and dogs, with daily IV administration for 2 weeks. The PSC coating was well tolerated up to the highest tested dose of 1600 mg PSC/kg/day in rats and 750 mg PSC/kg/day in dogs.

The pharmacokinetics (PK) and drug metabolism of ferumoxytol have been evaluated in rats, rabbits, and guinea pigs and these studies demonstrated that the plasma half-life increased with increasing dosage. The highest tissue concentrations of ferumoxytol were found in the liver, spleen, and central lymph node pool. Studies with radiolabeled drug product demonstrated that renal elimination of the iron in ferumoxytol was insignificant, while the carbohydrate coating was significantly excreted in the urine (72% to 78%) and feces (10% to 19%). This processing of ferumoxytol indicates that the iron contained in ferumoxytol is metabolized in tissues with reticuloendothelial cells, which incorporate the administered iron for uptake, storage, and the synthesis of hemoglobin.

4.3.4 Clinical Studies

4.3.4.1 Registrational program

From Ferumoxytol Investigator's Brochure 2009:

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

- ≤4 mg Fe/kg
- 1x 125 mg
- 1x 250 mg
- 1x 510 mg
- 8x 128 mg
- 4x 255 mg
- 2x 510 mg

Approximately 1,740 patients were exposed to ferumoxytol in the entire AMAG clinical development program, including approximately 1,509 patients in the Phase 3 studies. Only one patient (0.06% of all patients exposed to ferumoxytol) experienced an anaphylacoid reaction following treatment with ferumoxytol; this patient had a history of multiple drug allergies and experienced an anaphylactoid reaction (hot flashes and itching, with no respiratory compromise) and severe hypotension a few minutes after receiving ferumoxytol.

There have been no deaths that were considered to be related to ferumoxytol treatment. There were 28 deaths in the clinical program, all of which have occurred in Phase 3 studies, with 16 deaths among the 1,740 patients exposed to ferumoxytol (0.9%) and 8 deaths among the 296 patients exposed to oral iron (2.7%). Four deaths occurred in patients who had signed an informed consent for enrollment but did not receive any iron agents. All deaths in the clinical

program have been in patients with CKD, who have a high risk of death due to cardiovascular disease and other causes. In completed studies that used oral iron as a comparator, there was a lower rate of serious adverse events (SAEs) among patients exposed to ferumoxytol than among patients exposed to oral iron: 4.4% of ferumoxytol treated patients (64 of 1,451 patients) vs. 10.7% of oral iron-treated patients (17 of 159 patients). Patients exposed to placebo had the lowest rate of SAEs (1.7%; 13 of 780 patients). One patient each following ferumoxytol (0.1%), oral iron (0.6%), and placebo (0.1%) experienced a SAE that was considered by the investigator to be related to treatment. In the Phase 3 Study 62,745-5, which enrolled CKD patients undergoing hemodialysis, 31 of 199 (15.6%) patients experienced a SAE following ferumoxytol administration and 8 of 70 (11.4%) patients experienced a SAE following oral iron administration. These data were taken from an unlocked database and therefore are preliminary. In the Phase 3 studies in patients with CKD (62,745-5; 62,745-6; 62,745-7 and 62,745-8, in which approximately 1,509 patients were exposed to ferumoxytol), the most common adverse events following ferumoxytol administration included nausea, diarrhea, dizziness, headache, and peripheral edema. These adverse events were usually more common in the oral iron group than the ferumoxytol group (in Studies 62,745-5; 62,745-6 and 62,745-7, which utilized oral iron as a comparator). Hypotension was one of the more common adverse events in Study 62,745-7 and occurred more frequently in the ferumoxytol group (5.0%) than the oral iron group (1.4%). By contrast, preliminary data showed that there were lower rates of hypotension in Study 62,745-5 (1.8%, 1.7%, and 0% in the ferumoxytol 2x510 mg, ferumoxytol 4x255 mg, and oral iron groups, respectively). Hypotension rates were similar between the ferumoxytol and placebo groups (1.3% vs. 0.8%) in the large safety study, Study 62,745-8; there were no hypotension adverse events in Study 62,745-6.

An independent Data Monitoring Committee met to review the clinical safety data for all Phase 3 studies in October 2005, February 2006, June 2006, October 2006, and March 2007; they identified no safety concerns. The highest dose used was 4mg/kg (280mg for a 70kg patient). If there is no history of iron over load, there is no need to screen for iron over load since 0.5 g will not hurt patients, even if they have asymptomatic iron over load.

4.3.4.2 Post-marketing Program-From 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.

4.3.4.3 Post-Marketing Program- 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 three separate, fractionated doses. The rate of administration of the second and third doses will be slowed down to no faster than 1 mL/s.

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

As shown in Table 1, cumulative postmarketing reporting rates for serious AESIs (adverse events of special interest) with ferumoxytol since product approval (30 June 2009) have remained low. These event reporting rates are rare and very rare using CIOMS standardized

assessment, and no new safety trends or signals have been identified. The reporting rates for all AEs remained low and declined over time. In addition, the reporting rates for all serious events including HSRs have declined following the change in the prescribing information to administration with infusion instead of rapid injection (March 2015). Overall, there is no new safety signal arising from the review of the anaphylactic reactions/shock and hypersensitivity reaction events. The risk of anaphylaxis/hypersensitivity linked to iron agents is well recognized and the need for special caution when administrating ferumoxytol is adequately addressed in the US Prescribing Information. The available information from these events does not change the safety profile of ferumoxytol.

Table 1
Reporting Rates of Serious AESIs by Ferumoxytol Administration

AESI	Rapid Injection (2009-31 Mar 2015) Estimated Exposure: 1,216,518		Diluted infusion (1 Apr 2015-31 Mar 2017) Estimated Exposure: 556,117	
	No of Cases	Reporting Rate (Estimated)	No of Cases	Reporting Rate (Estimated)
Anapyhlaxis	95	0.0078	16	0.0029
Hypersensitivity (severe)	73	0.0060	5	0.00089
Cardiac disorders	37[1]	0.0030	4	0.00071
Hypotension (serious)	72	0.0059	2	0.00035
Syncope, loss of consciousness, unresponsive	44	0.0036	4[2]	0.00071
Fatal	48[1]	0.0040	4[3]	0.00071

1Includes cases received in April 2015 with event onset in March 2015 (AMAG201500330).
2Follow-up received on AEOI reported in Q2, per HCP due to underlying disease (epilepsy) and not ferumoxytol (AMAG201600940).
3Case 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.

4.3.4.4 OHSU Toxicity Data for Ferumoxytol

A total of 800 infusions of ferumoxytol have been completed on 410 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). Toxicities for this group as of May 1, 2017 show no grade 4 or 5 toxicities (CTCAE version 3) that were attributed to ferumoxytol. Three incidents of grade 3 toxicities were possibly attributable to ferumoxytol (one incident of constipation, one of neck pain and one of a rash). The most common adverse events associated with ferumoxytol were 20 (2.5% of total infusions) incidents of mild, transient hypertension and 7 (0.9% of total infusions) incidents of diarrhea. There have been no incidents of hypotension, syncope, unresponsiveness or cardiorespiratory arrest with these 800 administrations of ferumoxytol.

4.3.5 Pharmacokinetics and Drug Metabolism in Humans

From Ferumoxytol Investigator's brochure dated April 30, 2012:
To evaluate pharmacokinetics of the drug, blood samples were taken at multiple time points to determine blood clearance by MR relaxivity measurements in the Phase 1 study. Statistical analysis indicated that AUC and C_{max} increased with dose, but not with increasing rate of injection. The blood elimination half-life ($t_{½}$) also increased with dose, from 9.7 to 19 hours. Clearance (Cl) and Volume of Distribution (Vd) decreased with dose but not with rate of injection.

Table 2: Summary of Mean Pharmacokinetic Parameters
(From Ferumoxytol Investigator's brochure dated April 30, 2012:)

Parameter	Study 7228-01			Study 62,745-9	
Dose	1 mg/kg (85 mg[a])	2 mg/kg (152 mg[a])	4 mg/kg[b] (316 mg[a])	2 x 510 mg	
Analysis Method	NCA	NCA	NCA	NCA (individual data)	NCA (population-model simulated data)
N	8	8	17	58	1
$AUC_{0-\infty}$ (µg hr/mL)	365 (128)	996 (313)	2930 (685)	15400 (3750)[c]	14800[c]
C_{max} (µg/mL)	27 (8)	62 (12)	138 (34)	Dose 1: 206 (41) Dose 2: 301 (52)	Dose 1: 187 Dose 2: 281
$t_{½}$ (hr)	9.7 (2.0)	11.4 (1.6)	14.9 (2.0)	19.0 (4.6)[d]	15.8[d]
CL/weight (mL/hr/kg)	3.15 (1.52)	2.18 (0.66)	1.44 (0.32)	0.90 (0.16)[d]	0.90[d, e]

a. Mean dose administered
b. Results for all administration rates were combined for 4 mg/kg dose
c. Represents total AUC from time 0 of the first dose to infinity after the second dose
d. Represents time-averaged values
e. Calculated using mean subject weight (76.6 kg)

Abbreviations: $AUC_{0-\infty}$ = area under the curve from time 0 to infinity; Cl = clearance; C_{max} = maximum plasma concentration; NCA = noncompartmental analysis; SD = standard deviation; $t_{1/2}$ = elimination half-life;

The serum iron, ferritin, total iron binding capacity (TIBC) and percent saturation of iron (T_{sat}) were exactly as expected for a metabolized iron oxide drug. The profiles are shown in figure 11. Serum iron rose markedly and in a dose-dependent manner, peaking at one day, then falling back to baseline by day 7. T_{sat} mirrored the serum iron, rising as the serum iron rose. Ferritin began to rise at day one, reflecting some metabolism of the agent, peaked at day three, and fell slowly back toward baseline. Many of the serum iron values were out of normal range, particularly at the higher doses. However, this laboratory test does not reflect true serum iron because it cannot distinguish between intact iron oxide and transferrin-bound physiological iron.

Simple ANOVA analysis (ignoring rate of administration and sex) was performed for iron parameters by day and dose. By dose, there were no differences between two weeks pre, 2 days pre, and 1 day pre. At 8 hours after ferumoxytol administration, serum iron and TIBC varied significantly by dose, and all varied significantly by dose at days 1, 2, and 3. By day 7 after ferumoxytol administration, only serum ferritin varied by dose. ANOVA by time showed no variation for the placebo and significant variation by time for all three dose levels, as expected.

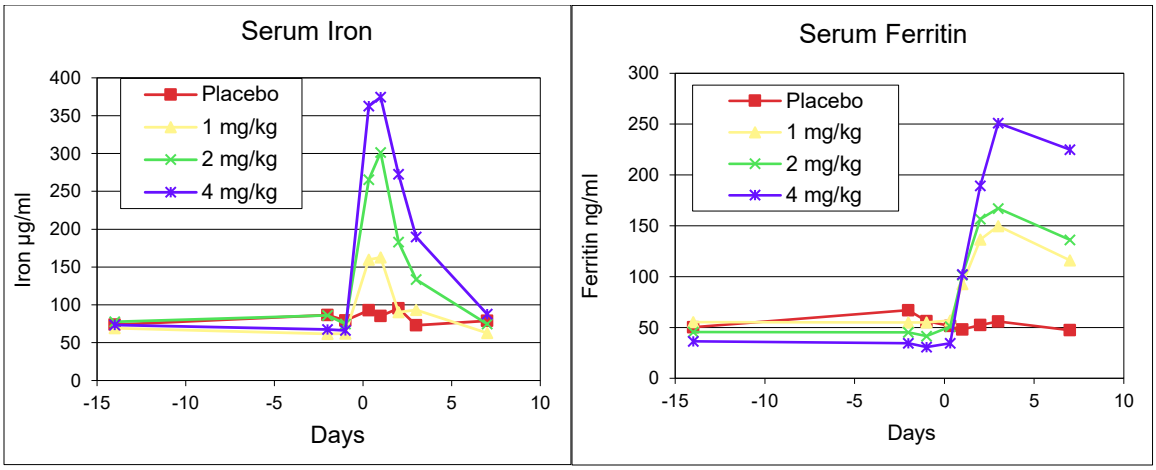


Figure 11: Serum iron and ferritin levels at various ferumoxytol doses

Temozolomide

Rationale for Dose Selection/Regimen/Modification for temozolomide is per the product insert (section 14.5).

4.3.6 Rationale for Endpoints

Efficacy Endpoints

This primary objective of this pilot study is to determine the sensitivity and specificity of ferumoxytol steady state imaging in distinguishing true progression in subjects who will receive standard of care chemoradiation with Immunotherapy (pembrolizumab) for glioblastoma.

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 glioblastoma receiving pembrolizumab in addition to standard of care treatments. Data from this pilot study will be used to power a larger study.

Biomarker Research End Points

Blood Biomarker

The number and type of systemic and tumor infiltrating T cell may suggest response to immunotherapy in various cancers.[39] 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 8.3.1.

Tissue Biomarker

The type and number of tumor infiltrating immune cells including lymphocytes and macrophages correlate with outcomes and prognosis in solid tumors [70]. There is emmerging evedence that there is a correlation between the immunophenotype and PD-L1 expression to treatment response in a variety of solid tumors. [85-88] However, limited data is available regarding brain tumors. 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 in primary and metastatic brain tumors. This is further elaborated on in section 8.3.2.

5.0 METHODOLOGY

5.1 Diagnosis/Condition for Entry into the Trial

Due to the innate differences in the pathology, natural history and differences in current standard of care for primary and metastatic brain tumors this pilot study will have two arms:

ARM 1: Metastatic brain tumors: Melanoma (closed to enrollment)

ARM 2: Primary brain tumor: Glioblastoma multiforme (GBM)

5.2 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 life expectancy of at least 6 months.
4. Have a histologically confirmed diagnosis of: newly diagnosed glioblastoma (WHO Grade IV)
5. Have ECOG performance status of 0 or 1 on stable or reducing dose of steroids for symptom management (not more than 8 mg of dexamethasone or equivalent per day) for 5 days prior to enrollment. Change in glucocorticoid dose for any purpose other than to modulate symptoms from an adverse event. *Note: The use of physiologic doses (e.g., prednisone 10 mg) of corticosteroids may be approved after consultation with Merck & Co. Use of prophylactic corticosteroids to avoid allergic reactions (e.g. IV contrast dye) is permitted.*
6. At least 14 days from any major surgeries including brain biopsy before the start of study drug pembrolizumab.
7. Demonstrate adequate organ function as defined in Table 3.
8. 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.
9. 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, including but not limited to, abstinence from heterosexual activity starting with the first dose of study therapy through 120 days after the last dose of study therapy.
10. Subject is eligible for and agrees to receive standard of care radiation and temozolamide after biopsy or maximum safe surgical resection.

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.	

5.3 Subject Exclusion Criteria

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

1. Has a diagnosis of immunodeficiency including Human Immunodeficiency Virus (HIV) (HIV 1/2 antibodies) and is not on continuous daily immunosuppressive therapy within 7 days prior to the first dose of trial treatment. (An exception to this is the use of steroids for brain edema and resulting symptoms. Subjects may receive a stable or reducing dose of steroids (up to 8 mg dexamethasone or equivalent for at least 5 days prior to signing consent) to prevent or manage cerebral edema. Subjects requiring over 8mg of dexamethasone per day on or five days prior to signing consent are excluded).
2. Has a known history of active TB (Bacillus Tuberculosis)
3. Hypersensitivity to pembrolizumab or ferumoxytol or any of their excipients.
4. 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.
5. 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.
6. 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.
7. Has known history of, or any evidence of active, non-infectious pneumonitis.
8. Has an active infection requiring systemic therapy.

9. 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.
10. Has known psychiatric or substance abuse disorders that would interfere with cooperation with the requirements of the trial.
11. 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.
12. Has known active Hepatitis B (e.g., HBsAg reactive) or Hepatitis C (e.g., HCV RNA [qualitative] is detected).
13. Has received a live vaccine within 30 days of planned start of study therapy.
    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.
14. 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.
15. 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.*
16. Subjects who have a contraindication for 3T MRI: metal in their bodies (a cardiac pacemaker or other incompatible device), or are severely agitated.
17. 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.
18. Subject who have received ferumoxytol within 3 weeks of study entry.
19. Subjects with three or more drug allergies from separate drug classes.

5.4 Subject Withdrawal/Discontinuation Criteria

Subjects who complete 2 years of treatment or 35 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.

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 7.11. After discontinuing treatment following assessment of CR, these subjects should return to the site for a Safety Follow-up Visit (described in Section 7.8) and then proceed to the Follow-Up Period of the study (described in Section 7.9).

Subject withdrawal includes patients who wish to withdraw consent for continuing on the study or who were lost to followup. Additionally, subjects may 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.
- Confirmed progression in brain lesions or systemic disease (confirmed by imaging or tissue biopsy)
  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 7.9
- Unacceptable adverse experiences as described in Section 9.0
- Miss two consecutive pembrolizumab treatments for any reason other than surgery.
- Intercurrent illness that prevents further administration of treatment including clinical deterioration at the discretion of the PI.
- 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 negative, a serum pregnancy test will be required.
- Noncompliance with trial treatment or procedure requirements
- Administrative reasons

The end of treatment and follow-up visit procedures are listed in the schedule of events (Sections 6 and 7).

5.4.1 Early stopping rule

This is a pilot study evaluating an imaging biomarker. There is sufficient data regarding the safety and toxicities of ferumoxytol and pembrolizumab as well as the standard of care therapies; safety and toxicity data reading their combination is limited. The study team comprising of the PI and co-investigators, reviewed the toxicity data for arm 2 on March 29, 2019 prior to enrolling the 11th subject and there were no grade 3 or greater toxicities attributed to pembrolizumab, ferumoxytol, or both.

A second toxicity review will be performed prior to starting enrollment of the 18 additional subjects provided from the closing of the melanoma arm of the study, and after every 10 additional subjects are enrolled thereafter. The study will be terminated if more than 50% of the subjects enrolled at the time of the review have a grade 3 or greater toxicity that will be 1) definitely attributed to pembrolizumab, ferumoxytol, or both (attribution code of '3'); or 2) possibly attributed to pembrolizumab, ferumoxytol, or both (attribution code of '2'), unless they are definitely attributed (attribution code of '3') to standard of care therapy. To ensure compliance with the early stopping rule, all adverse events will be monitored and reported continuously by the study team in order to stop treatment as soon as a subject experiences grade 3 or greater AE attributed to pembrolizumab, ferumoxytol, or both.

5.4.2 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 discontinued therapy after 24 months and have attained and maintained a 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 7.9.

5.4.3 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, 4 weeks after the last day of radiation (see section 6.0)

5.4.4 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 28 days of first treatment	Pembro infusion every 3 weeks +15 days	At suspected radiographic progression (±15 days)	At persistent radiographic progression (±15 days)	Safety follow-up: 30 days (±15 days) post discontinuation of pembro	Follow-up: those who complete 2 years of treatment or 35 cycles	Survival: follow-up once off study[8]
Informed Consent	X						
Inclusion/Exclusion Criteria	X						
Archival Tissue Collection (if available)	X						
Standard of Care Biopsy or Resection of Brain Lesion[1]				X			
Demographics and Medical History	X						
Medication, herbal or dietary supplement review	X		X	X	X	X	
Review Adverse Events, include Events of Clinical Interest[10]			X	X	X	X	
Physical Examination[2]	X			X	X	X	
Vital Signs and Weight	X			X	X	X	
ECOG Performance Status	X			X	X	X	
CBC with Differential[3]	X	X			X		
Comprehensive Serum Chemistry Panel (with LDH)[3]	X	X			X		
Urinalysis	X						
FT4 and TSH[4]	X	X			X		
PT/INR, aPTT	X				X		
Pregnancy Test (urine or serumfemales only – see section 7.6)	X						
Clinical Tumor Imaging	X[5]					X[7]	
Ferumoxytol imaging	X[6]						
24 hr post study drug MRI - Optional	X[6]						
Biomarker Collection (serum) Optional	X[6]						
Post-study anticancer therapy status						X	
Survival Status						X	X
Telephone contact							X[8]

[1]Subjects must undergo standard of care biopsy or maximal safe resection as clinically indicated prior to enrollment.

[2]Physical exam required with every pembrolizumab infusion.

[3]CBC and CMP (with LDH) to be done every 3 weeks while on pembrolizumab - labs must be done within 28 days before first pembrolizumab infusion, and within 72 hours of subsequent pembrolizumab infusions. Additional labs may be drawn at treating physician's discretion.

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

[5]Clinical standard of care MRIs are done at baseline, 4 weeks after the last day of radiation and then every 9 weeks (see special instructions below for evaluating persistent radiographic progression). Clinical MRIs may be done more often at physician's discretion.

[6]Ferumoxytol steady state MRIs (within 7 days of clinical standard of care MRIs) are done baseline, 4 weeks after the last day of radiation, anytime there is suspected radiographic progression and 4 weeks after suspected radiographic progression (all timepoints ±5 days). Serum biomarkers will be obtained ±15days of ferumoxytol MRI scan.

[7] Follow-up after completing 2 years of treatment or 35 cycles (whichever comes later): according to standard of care.

[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.

[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
Trial treatment with pembrolizumab will start on the first day of standard of care radiation (±5 days) and thereafter every 3 weeks (+15 days) (dosing delays of up to 7 days are permitted at physician discretion for medical / surgical events or logistical reasons) for up to 2 years or 35 cycles or confirmed radiographic progression, whichever occurs first. 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, unrelated medical events, subject vacation, and/or holidays), not to exceed two consecutive doses or a maximum of 9 weeks.

Imaging instructions for evaluating persistent radiographic progression: if there is suspected progression, subjects undergo a ferumoxytol steady state MRI and continue on pembrolizumab for one more cycle (total of 4 weeks). At the 4 week point, clinical standard of care and ferumoxytol MRIs will be repeated to confirm persistent radiographic progression. If the 4 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

7.1 Participant Screening and Enrollment

In order to participate in this study, signed informed consent must be obtained from the participant. The current Institutional Review Board (IRB) approved informed consent must be signed and dated by each participant prior to undergoing any study procedures or before any prohibited medications are withheld from the participant in order to participate in this study.

7.2 Participant Registration

Participants will be required to give written informed consent to participate in the study before any screening tests or evaluations are conducted that are not part of standard care. Registration from all consented participants must be entered into the OHSU electronic Clinical Research Management System (eCRIS). At a minimum, registration of OHSU participants will include:

- Unique study number
- A completed review of eligibility criteria signed by the Principle Investigator
- Signed copies of the most recently IRB-approved, informed consent form and HIPAA authorization

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.

7.3 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
Investigational Agents					
Pembrolizumab	200mg	Every 3 weeks (+15days) (starting on first day of standard of care therapy (+ 15 days)	IV infusion	Day 1 of each 3 week cycle	Experimental
Ferumoxytol	7mg/kg (maximum dose not to exceed 510mg)	Baseline, 4 weeks post radiation, anytime there is	IV infusion	Baseline, 4 weeks post radiation, anytime there is suspected radiographic	Experimental

Drug	Dose/Potency	Dose Frequency	Route of Administration	Regimen/Treatment Period	Use
		suspected radiographic progression and 4 weeks after suspected radiographic progression (all ±15 days).		progression and 4 weeks after suspected radiographic progression (all ±15 days).	
Standard of Care Treatments					
Arm 1 only					
Stereotactic radiosurgery	18-35Gy	See section 7.4	See section 7.4	See section 7.4	Standard of care
Arm 2 only					
Radiation	Up to 60Gy	Daily (Monday-Friday) for 6 weeks	See section 7.4	6 weeks	Standard of care
Concurrent temozolomide	75 mg/m^2	Daily (Monday-Sunday) during radiation	Oral	6 weeks	Standard of care
Adjuvant temozolomide	150-200 mg/m^2	First 5 days of a 4 week cycle	Oral	At least 6 months	Standard of care

Timing of Dose Administration

Pembrolizumab: Trial drug treatment should be administered every 3 weeks. All trial treatments will be administered on an outpatient basis. 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, unrelated 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.

Pembrolizumab 200mg will be administered every 3 weeks as per institutional guidelines. The expiration of the reconstituted pembrolizumab is 4 hours at room temperature and 20 hours refrigerated.

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) per imaging session. 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 20mL 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.

7.4 Study procedures narrative

Due to the innate differences in the pathology, natural history and differences in current standard of care for primary and metastatic brain tumors this pilot study will have two arms:

ARM 1: Metastatic brain tumors - Melanoma

ARM 2: Primary brain tumor - Glioblastoma multiforme (GBM)

All subjects will receive standard of care treatment for their respective arms as described in section 7.6.

All subjects will receive pembrolizumab (200 mg IV every 3 weeks) starting on the first day of radiation until persistent radiographic progression is confirmed, in addition to standard of care for their respective arms. Clinical MRI scans will be performed to assess response to therapy. Clinical standard of care MRIs are done at baseline, 4 weeks after the last day of radiation and then every 9 weeks thereafter until suspected radiographic progression. Early follow up clinical standard of care MRI (and ferumoxytol steady state MRI, see below) will be performed 4 weeks from the date of MRI showing suspected radiographic progression to confirm persistent radiographic progression (all timepoints ±5 days). Patients with imaging evidence of suspected radiographic progression may continue with one additional cycle of pembrolizumab. The decision regarding continuation of pembrolizumab will be made by the principle investigator based on clinical findings that include (1) stable ECOG performance status with no more than 8mg of dexamethasone (or equivalent) a day; (2) absence of new or worsening symptoms not managed with steroids; and (3) absence of progressive tumor at critical anatomical sites (e.g., spinal cord compression) requiring urgent alternative medical intervention.

Additional ferumoxytol steady state MRIs will be done at baseline, 4 weeks after the last day of radiation, at suspected radiographic progression and within 4 weeks from suspected radiographic progression to confirm persistent radiographic progression (all timepoints ±5 days). During this follow up period, subjects will be followed with a clinical exam weekly to monitor for clinical stability.

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.

When there is radiologic progression after radiation, current standard of care includes repeat craniotomy to confirm true vs pseudoprogression. 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.

Standard of care surgery or biopsy is performed only if 1) the lesion is deemed easily accessible 2) surgery is not expected to worsen neurological outcomes and 3) it is in the best clinical interest of the patient. The decision regarding surgery will be consensus based and when possible, will be made after each individual case has been presented at the OHSU multi-disciplinary 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 as described in section 7.11.

In subjects where the lesion remains unchanged or regresses in volume at 4 weeks ($\pm$5 days) 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.

Subjects can restart pembrolizumab within 2 weeks after surgery. If the subject has a surgical complication that will prevent them from starting pembrolizumab within 2 weeks, the PI will discuss these cases with Merck & Co on a case by case basis. However, if pathology suggests pseudoprogression, pembrolizumab will be restarted 30 days ($\pm$ 5 days) after surgery.

7.5 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 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 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.

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.

7.6 Trial Procedures

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

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.
  - 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-L1 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-L1 biomarker assay.
    - At persistent radiographic progression, a repeat biopsy or resection is considered standard of care to distinguish true vs pseudoprogression.
- Standard of care therapy:
    - Arm 1: 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 (5mm 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.
    - Arm 2:
        - All patients in this arm will be treated with standard of care radiotherapy consisting of fractionated focal irradiation to gross tumor volume (GTV) plus a up to 3-cm margin at a dose of up to 2-Gy per fraction given once daily five days per week (Monday through Friday) over a period of six weeks, for a total dose of up to 60 Gy.
        - Additionally, standard of care temozolamide 75 mg/m^2 once daily (Monday through Sunday) over the 6 weeks duration of radiation. After a 4-week break, subjects will then receive up to six cycles of adjuvant temozolomide according to the standard 5-day schedule every 28 days (150 mg/m^2 for the first cycle and then increased to 200 mg/m^2 beginning with the second cycle, as long as there were no hematologic toxic effects as per Stupp et al 2005 [80]. Dose modifications for temozolomide are described in section 7.13.

    - 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 continue with pembrolizumab even if radiation or chemotherapy is discontinued, at the discretion of the PI.

Additionally, subjects may receive prophylaxis against Pneumocystis carinii pneumonia, consisting of either inhaled pentamidine or oral trimethoprim–sulfamethoxazole, at the physician's discretion [89].

Subject may also provide consent/assent for future correlative research (optional). Subjects may participate in the main trial without participating in future correlative research.

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 8.3.1)

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	Lactate dehydrogenase (LDH)	Microscopic exam *(If abnormal)*	Free tyroxine (T4)
Absolute Neutrophil Count	Carbon Dioxide ‡	results are noted	Thyroid stimulating hormone (TSH)
Absolute Lymphocyte Count	*(CO_2 or biocarbonate)*	Urine pregnancy test †	
	Calcium		Blood for correlative studies
	Chloride		
	Glucose		
	Potassium		
	Sodium		
	Total Bilirubin		
	Direct Bilirubin *(If total bilirubin is elevated above the upper limit of normal)*Total Bilirubin		
	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.

Imaging (all timepoints +15 days)
Standard of care clinical brain MRI: Clinical MRIs are done at baseline, 4 weeks after the last day of 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, see section 8.2.2) for gadolinium infusion, but is not required.

Ferumoxtyol MR (FeMRI): ferumoxytol steady state MRIs of the brain are done baseline, 4 weeks after the last day of radiation, anytime there is suspected radiographic progression and 4 weeks after suspected radiographic progression. Additional optional MR imaging of systemic lesions for subjects in Arm 1 may be performed in the same setting and time points as the brain imaging. Details are explained in section 8.2.3.

Evaluation of systemic disease burden (only for Arm 1): 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 systemic cancer. Stable systemic disease burden should be demonstrated at enrollment and for continuation of study drug.

Imaging Schedule (all timepoints +15 days)
Arm 1: All patients will be scanned with clinical MRI at baseline prior to radiation, 4 weeks after the last day of radiation and there after every 9 weeks. Ferumoxytol steady state MRI (FeMRI) will be performed in the same sitting at baseline and 4 weeks after the last day of radiation. Thereafter, FeMRI may be performed independent of the clinical MRI if there is suspected radiographic progression or to confirm radiographic progression. Patients enrolled in ARM 1 may be concomitantly enrolled into additional brain tumor imaging studies conducted at OHSU.

Arm 2: All patients will be scanned with clinical MRI at baseline prior to radiation, 4 weeks after the last day of radiation and there after every 9 weeks. FeMRI will be performed in the same sitting at baseline and 4 weeks after the last day of radiation. Thereafter, FeMRI may be performed independent of the clinical MRI if there is suspected radiographic progression or to confirm radiographic progression. If there is suspected progression, subjects undergo a ferumoxytol steady state MRI and continue on pembrolizumab for one more cycle (total of 4 weeks). At the 4 week point, clinical standard of care and ferumoxytol MRIs will be repeated to confirm persistent radiographic progression. If the 4 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.

Contrast agents dosing and administration

Clinical Standard of Care MRI: Gadoteridol enhanced MRI will be used per institutional guidelines (see section 8.2.2). 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 study schema.

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 7^{th} 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.

Delayed (18-72h) parenchymal phase ferumoxytol scan - Optional

No contrast agent will be given during this MRI. This scan is optional. Every effort will be made to schedule the scan as close to 24 hours after ferumoxytol as possible.

Image processing

Image processing will be done at OHSU. 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. When gadolinium is given, 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. The coregistration and overlay of CBV maps on T1-w MPRAGE will be done using NordicICE as well [90, 91]. 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

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); see the separate ECI guidance document regarding the identification, evaluation and management of potential irAEs.

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

7.7 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.

7.8 Safety Follow-Up Visit: 30 days (±15 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 attributed to pembrolizumab or ferumoxytol 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 7.9) may have up to two safety follow-up visits, one after the Treatment Period and one after the Second Course Phase.

7.9 Follow-up

Subjects who complete 2 years of treatment or 35 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.

7.10 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.

7.11 Second Course Phase (Retreatment Period)

Subjects who stop pembrolizumab with CR in the brain and systemically may be eligible for up to one year of additional pembrolizumab therapy if they progress systemically, but maintain a CR in the brain. 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 systemically 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.
- 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 including but not limited to abstinence from heterosexual activity 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.

7.12 Dose modification and toxicity management for immune-related AEs associated with pembrolizumab

Pembrolizumab

AEs associated with pembrolizumab exposure may represent an immunologic etiology. These immune-related AEs (irAEs) may occur shortly after the first dose or several months after the last dose of pembrolizumab treatment and may affect more than on body system simultaneously. Therefore, early recognition and initiation of treatment is critical to reduce complications. Based on existing clinical trial data, most irAEs were reversible and could be managed with interruptions of pembrolizumab, administration of corticosteroids and/or other supportive care. For suspected irAEs, ensure adequate evaluation to confirm etiology or exclude other causes. Additional procedures or tests such as bronchoscopy, endoscopy, skin biopsy may be included as part of the evaluation. Based on the severity of irAEs, withhold or permanently discontinue pembrolizumab and administer corticosteroids. Dose modification and toxicity management guidelines for irAEs associated with pembrolizumab are provided in Table 6.

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

General instructions:

1. Corticosteroid taper should be initiated upon AE improving to Grade 1 or less and continue to taper over at least 4 weeks.
2. 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.
3. 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

	Grade 4	Permanently discontinue		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.
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).				

Table 7 below shows treatment guidelines for subjects who experience an infusion reaction associated with administration of pembrolizumab.

Table 7: 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 therapy or infusion interruption but responds promptly to symptomatic treatment (e.g., antihistamines, NSAIDs, narcotics, IV fluids); prophylactic medications indicated for ≤24 hrs	**Stop Infusion.** Additional appropriate medical therapy may include but is not limited to: IV fluids Antihistamines NSAIDs 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 1 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 study drug treatment**	Subject may be premedicated 1.5h (± 30 minutes) prior to infusion of pembrolizumab with: Diphenhydramine 50 mg po (or equivalent dose of antihistamine). Acetaminophen 500-1000 mg po (or equivalent dose of analgesic).
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: Epinephrine** IV fluids Antihistamines NSAIDs Acetaminophen Narcotics Oxygen Pressors Corticosteroids 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. **In cases of anaphylaxis, epinephrine should be used immediately. **Subject is permanently discontinued from further study drug treatment.**	No subsequent dosing
Appropriate resuscitation equipment should be available at the bedside and a physician readily available during the period of drug administration. For further information, please refer to the Common Terminology Criteria for Adverse Events v4.0 (CTCAE) at http://ctep.cancer.gov		

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, unrelated 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.

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 as per treating physician's discretion in consultation with the pembrolizumab clinical team at 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.

7.13 Dose Modifications for Ferumoxytol and Temozolomide

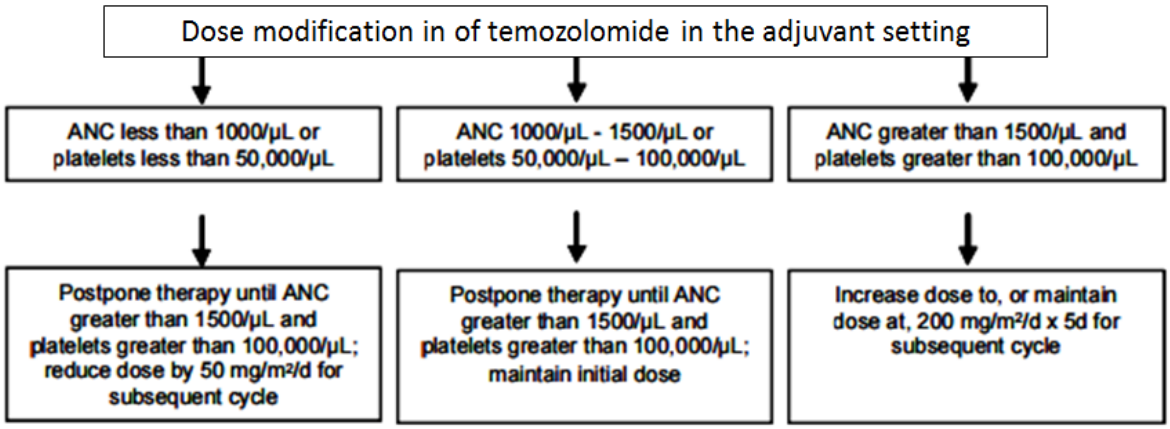
Ferumoxytol

There will be no dose limitations or modifications to ferumoxytol. If subject experiences a grade 2 or greater allergic or anaphylactic reaction (CTCAE v 4.0) that is directly attributable to the ferumoxytol, the subject will be removed from study.

Temozolomide (arm 2 only)

Temozolomide will be administered as described in section 7.6 with standard dose modifications per the product prescribing information (Section 14.4. Subject may remain on study and continue on pembrolizumab if temozolomide is stopped.

In the concomitant setting (daily during radiation) the dose of temozolomide will be 75mg/m2 per day. Dose will be held for ANC less than 1000/µL or platelet count less than 100,000/µL and the dose will be held until ANC is over 1000/µL or platelet count over 100,000/µL .In the adjuvant setting (monthly after completing radiation), dosage of temozolomide must be adjusted according to nadir neutrophil and platelet counts in the previous cycle and neutrophil and platelet counts at the time of initiating the next cycle. The initial dose is 150 mg/m2 orally once daily for 5 consecutive days per 28-day treatment cycle. Temozolomide dose may be increased to 200 mg/m2 /day for 5 consecutive days per 28-day treatment cycle. The general algorithm for dose adjustment is as follows:



Additionally, for subjects in arm 2 who will receive temozolomide and pembrolizumab, although there is no concern for overlapping toxicities, in cases where attribution to one drug of the other is unclear, the dose of pembrolizumab will be modified or discontinued based on the guildelines in table 6. If the toxicity resolves appropriately, pembrolizumab will be restarted. Temozolomide will be modified or discontinued at the discretion of the treating physician

Radiation

Radiation will be administered as described in section 7.4. Subject may remain on study and continue on pembrolizumab and/or temozolomide if radiation is stopped for toxicities attributed to radiation.

7.14 Trial Blinding/Masking

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

7.15 Randomization or Treatment Allocation

N/A

7.16 Stratification

N/A

7.17 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.

7.17.1 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.

7.17.2 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 supplements that are being used will be recorded at all clinic visits. Natural therapies and herbal supplements may stimulate or suppress subject's 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 8 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 8 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, panax and 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. European mandrake 22. Quillaia 23. Reishi Mushroom (gandoderma) 24. Shitake Mushroom 25. Sweet Annie 26. Terminalia chebula 27. Thunder god vine 28. Wild indigo 29. Zinc *Note:*Can be immune suppressing also*

Immune suppressing agents	
1. Bitter Melon 2. Caramel Color 3. Carrageenan(intravenous only) 4. Echinacea** 5. Indole-3-carbinol 6. Ipriflavone 7. Hydrazine	8. Fish Oils 9. Licorice 10. Periwinkle(vinca) 11. Poria Mushroom *Note : ** Can be immune stimulating 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.

7.18 Pharmacokinetic/Pharmacodynamic Evaluations
NA

7.19 Blood Collection for Anti-Pembrolizumab Antibodies
NA

7.20 Blinding/Unblinding
N/A

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

7.22 Contraception
Both pembrolizumab and ferumoxytol 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 without alternate medical causes will be considered postmenopausal), or 3) not heterosexually active for the duration of the study. In women ≤ 45 years of age a high follicular stimulating hormone (FSH) level in the post-menopausal range may be used to confirm post menopausal state in women not using hormonal contraception or hormonal replacement therapy. 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. Male subjects should agree to use an adequate method of contraception, including but not limited to, abstinence from heterosexual activity, starting with the first dose of study therapy through 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) from study Visit 1 throughout the study period up to 120 days after the last dose of study therapy. 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.

7.23 Use in Pregnancy

If a subject inadvertently becomes pregnant while on treatment with pembrolizumab or ferumoxytol, 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.

7.24 Use in Nursing Women

It is unknown whether pembrolizumab or ferumoxytol 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 RESPONSE ASSESSMENT, IMAGE PROCESSING AND BIOMARKERS

8.1 Definitions for Response assessment:

The study will use the following criteria for the assessment of 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 ($\pm$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.

Systemic Lesions (only for Arm 1):
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.

8.2 Image processing

Image processing will be done at OHSU.

8.2.1 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 (see protocol below) ferumoxytol enhancement will be used). The coregistration and overlay of CBV maps on T1-w MPRAGE will be done using NordicICE as well [27, 33]. 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.

8.2.2 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

8.2.3 For systemic lesions (only Arm 1).

With immunotherapy treatment, an increase in total tumor burden of previously stable systemic lesions (tumor flare reaction) has been described [92]. Based on the location of the systemic lesions, and the feasibility of MR imaging, subjects in Group 1 (melanoma) 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; ΔR_2, ΔR_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, $\Delta\chi$.

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

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

$$\frac{3}{4p}\frac{\Delta R_{2,muscle}^{*}}{fBV_{muscle}\gamma B_0} = \frac{3}{4p}\frac{\Delta R_{2,tumor}^{*}}{fBV_{tumor}\gamma B_0}$$

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

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

Vessel Size Index will be computed using the following equation:

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

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.

8.3 Tumor Tissue Collection and Correlative Blood Biomarker Studies

8.3.1 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 ±15 days of each ferumoxytol MRI scan. These timepoints are: at baseline, at 4 weeks after the last day of radiation, at suspicion of radiographic progression, and 4 weeks after suspected radiographic progression. All timepoints ±15 days.
- Blood samples will be collected at OHSU and processed at the OHSU research laboratory.

8.3.2 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. All adverse events will be monitored continuously by the PI in order to stop treatment as soon as a subject experiences grade 3 or greater AE attributed to pembrolizumab, ferumoxytol, or both. 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 therapies:
    Arm 1: stereotactic radiosurgery
    Arm 2: radiation and temozolomide

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 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.

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.

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 and in greater detail in the ECI guidance document. 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 (as outlined in the ECI guidance document). Refer to Section 7.12 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. Suggested conditional procedures, as appropriate, can be found in the ECI guidance document.

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.

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. There will be no dose limitations or modifications to ferumoxytol. If subject experiences a grade 2 or greater allergic or anaphylactic reaction (CTCAE v 4.0) that is directly attributable to the ferumoxytol, the subject will be removed from study.

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.

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

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].

9.6.1 Expected AEs from Temozolomide

See product insert (section 14.4). Adverse events and toxicities will be managed per institutional and accepted standard of care practices.

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.)	

9.6.2 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).

9.6.3 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).

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

9.6.4.1 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.

9.6.4.2 Events of Clinical Interest

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

Events of clinical interest for this trial include:

1. an overdose of Merck product, as defined in Section 9.6.1 - 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. The trial site guidance for assessment and follow up of these criteria can be found in the Investigator Trial File Binder (or equivalent).

9.6.5 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, PDL-1 expression, clinical responses, radiological responses and other variables will be explored using two sample tests or correlation measures, as appropriate.

Power and sample size calculation for Arm 1

The accrual to arm 1 is stopped with 2 patients. These 2 patients will be described separately and no further analysis will be done.

Power and sample size calculation for Arm 2

For arm 2, we will enroll 43 GBM patients. Here we roughly estimate the precision of sensitivity and specificity in the primary objective based on these 43 patients.

In subjects with GBM, we anticipate to have only one lesion/subject. We assume that all subjects will eventually have disease progression and due to dropout and inoperable cases, a total of n = 32 subjects will undergo surgery to provide histopathological data on disease progression. Further, we assume that 40% of the n = 43 subjects will develop pseudo-progression and be observed based on follow-up scan.

For sensitivity, further assume that out of the n = 32 subjects who will undergo surgery, 26 subjects will have true progression based on pathology (gold standard). Then based on n = 26,

if we observe 22 subjects (lesions) for whom the true progression is correctly identified by Ferumoxytol MRI, then the sensitivity will be 85% with a 95% CI between 65% and 96% (Table XX).

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
26	26	100%	87% to 100%
26	24	92%	75% to 99.1%
26	22	85%	65% to 96%
26	20	77%	56% to 91%
26	18	69%	48% to 86%
26	16	62%	41% to 80%

Table 12: Estimated sensitivity and 95% CI based on 26 subjects (lesions).

For specificity, it will be calculated based on patients with pseudo-progression determined by either surgical results or follow-up scan. We assume that 40% of the subjects (n = 17) will develop pseudo-progression, then if we observe 15 subjects (lesions) for whom the pseudo-progression status is correctly identified by ferumoxytol MRI, the specificity will be 88% with a 95% CI between 64% and 99% (Table 13).

Number of lesions with pseudo-progression	Number of Lesions with pseudo-progression detected by Ferumoxytol MRI	Specificity	Exact 95% CI of Specificity
17	17	100%	80% to 100%
17	15	88%	64% to 99%
17	13	76%	50% to 93%
17	11	65%	38% to 86%

Table 13: Estimated specificity and 95% CI based on 17 patients.

Statistical Analysis

For arm 2, sensitivity, specificity, rate of adverse events (safety and tolerability) 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 (<1.75 is pseudoprogression and $\geq$ 1.75 is progression). Analysis of sensitivity will be based on patients with surgical results, and specificity analysis will be based on patients with pseudo-progression determined based on surgical results or follow-up clinical 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 patients 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.

Exploratory objectives 1 to 3 will be analyzed using patients from arm 2. Immune responses will be determined by the volume, pattern and intensity of delayed (24hr) ferumoxytol uptake and compared between patients who develop true vs. pseudoprogression and provided data using Wilcoxon Rank Sum test (volume and intensity) or Fisher's exact test (pattern). Systemic immune responses will be measured by immunological parameters (CD3, CD4, CD8, CD19, CD25, CD27, CD45, CD56, CD45R0, CD45RA, titers of IgD, Tγδ and Tαβ) 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.

For exploratory objective 3, among the 43 patients, 8 to 10 patients are expected to have data on PD-1 receptor expression (Yes vs. No, where Yes is defined as ≥1% PDL-1 expression and No as < 1% based on FDA recommendations https://www.keytruda.com/static/pdf/keytruda-pd-l1-expression-testing-guide.pdf) 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, and use univariate logistic or Cox regression models to correlate PD-L1 expression with response rates and survival. Lastly, in metastatic patients with measurable systemic lesions, vascular volume fraction (VVF), vessel size index (VSI) and vessel density index (VDI) will be measured and compared between patients with true vs. pseudoprogression, if data allow.

In summary, all these proposed analyses will be conducted on the 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 won't 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

Standard of Care Product

Temozolomide will be supplied, stored, handled and distributed per package insert (section 14.5).

Investigational Products

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 for pembrolizumab and ferumoxytol will be provided by Merck and AMAG Pharmaceuticals as summarized in Table 14.

Table 14 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

Packaging and Labeling Information

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

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 physician's IND.

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.

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

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.

12.1 Retention of Records

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

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.

12.2 Data Collection, Storage, Privacy, Confidentiality and Security

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

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

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.

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

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

The study monitor, other authorized representatives of the sponsor, representatives of the IRB or manufacturer supplying study product may inspect all documents and records required to be maintained by the Investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

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

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

12.3 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 and the database, ensuring confidentiality and security of the data. Data will be kept indefinitely.

If the investigator relocates or for any reason withdraws from the study, the study records must be transferred to an agreed upon designee, such as another institution, another investigator, or to OHSU Knight Cancer Institute (KCI) Clinical Trials Office. Records must be maintained according to sponsor or FDA requirements.

Data collection is the responsibility of the clinical trial staff at the site under the supervision of the site Investigator. The Investigator is responsible for ensuring the accuracy, completeness,

legibility, and timeliness of the data reported. Standard institutional practices will be followed as described in the OHSU's Information Security Directives to maintain the confidentiality and security of data collected in this study. Study staff will be trained with regard to these procedures.

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

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

12.4 Future Use of Stored Specimens

Each participant who signs consent will be assigned a unique coded identifier consisting of numbers. This identifier will be associated with the participant throughout the duration of their participation in the trial. The coded identifier will be used to identify any participant specific samples. Blood and tissue samples collected for the purposes of this protocol will be stored until they can be analyzed and will then be destroyed.

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.

12.6 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.

12.7 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)).

13.0 ETHICS/PROTECTION OF HUMAN PARTICIPANTS

13.1 Ethical Standard

The Investigator will ensure that this study is conducted in full conformity with Regulations for the Protection of Human Participants of Research codified in 45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, 21 CFR 312 (for IND studies), 21 CFR 812 (for IDE studies), and/or the ICH E6.

13.2 Institutional Review Board

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the IRB for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. All changes to the consent form will be IRB approved; a determination will be made regarding whether previously consented participants need to be re-consented.

13.3 Informed Consent

Written informed consent will be obtained from all participants participating in this trial, as stated in the Informed Consent section of 21 CFR Part 50. Documentation of the consent process and a copy of the signed consent shall be maintained in the participant's medical record.

Informed consent is a process that is initiated prior to the individual's agreement to participate in the study and continues throughout the individual's study participation. Extensive discussion of risks and possible benefits of participation will be provided to the participants and their families as appropriate. Consent forms will be IRB-approved and the participant will be asked to read and review the document. The Investigator will explain the research study to the participant and answer any questions that may arise. All participants will receive a verbal explanation in terms suited to their comprehension of the purposes, procedures, and potential risks/benefits of the study, alternatives to participation, and of their rights as research participants. Participants will have the opportunity to carefully review the written consent form and ask questions prior to signing. The participants should have the opportunity to discuss the study with their surrogates or think about it prior to agreeing to participate. The participant will sign the informed consent document prior to any procedures being done specifically for the study. The participants may withdraw consent at any time throughout the course of the trial. A copy of the informed consent document will be given to the participants for their records. The

rights and welfare of the participants will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

13.4 Protocol Review

The protocol and informed consent form for this study must be reviewed and approved in writing by the OHSU Knight Cancer Institute's Clinical Research Review Committee (CRRC) and the appropriate IRB prior to any participant being consented on this study.

13.5 Changes to Protocol

Any modification of this protocol must be documented in the form of a protocol revision or amendment submitted by the Investigator and approved by the CRRC 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 participant. In that event, the Investigator must notify the IRB (and sponsor/FDA if under an IND/IDE) within 5 business days after the implementation.

An Investigator who holds an IND or IDE application must also notify the FDA of changes to the protocol per 21 CFR 312 or 21 CFR 812, respectively.

14.0 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. Gender-nonconforming and gender-fluid individuals as members of the general population will also be recruited.

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

Table 15: 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			

Ethnic Category	**Sex/Gender**		
	Females	Males	Total
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 16: Projected Accrual

Ethnic Category	**Sex/Gender**			
	Females	Males	Unknown	Total
Hispanic or Latino	3	2		5
Not Hispanic or Latino	20	20		40
Unknown				
Ethnic Category: Total of all subjects*	23	22		45
Racial Category				
American Indian or Alaskan Native				
Asian	1	1		2
Black or African American	1			1
Native Hawaiian or other Pacific Islander				
White	19	19		38
More than one race	1	1		2
Unknown	1	1		2
Racial Category: Total of all subjects*	23	22		45

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.

15.0 SUPPLEMENTAL MATERIAL

15.1 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.	

15.2 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)

15.3 Ferumoxytol Investigator's Brochure

15.4 Pembrolizumab Package insert

15.5 Temozolomide Package Insert

16.0 APPENDICES

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

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