PROTOCOL TITLE: Advanced MRI for Intracranial Metastasis Treated with Stereotactic Radiosurgery.

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1 OBJECTIVES

1.1 Primary Objectives

1.1.1 Conspicuity:

1.1.1.1 Quantitative MRI: To assess the conspicuity of idealized T1 and T2 imaging at detecting brain metastases at baseline.

1.1.1.2 Chemical Exchange Saturation Transfer (CEST) MRI: To assess the conspicuity (CNR) of CEST imaging at detecting brain metastases at baseline.

1.1.1.3 Multiple B Value Diffusion Imaging (Adv Diff): To assess the conspicuity (CNR) of Adv Diff at detecting brain metastases at baseline.

1.1.1.4 Gradient- and Spin-Echo (GESE) Dynamic Susceptibility Contrast (DSC) perfusion imaging: To assess the conspicuity of GESE DSC and standard Gradient Echo (GE) DSC at detecting brain metastases at baseline.

1.2 Secondary Objectives

1.2.1 Conspicuity:

1.2.1.1 Quantitative MRI: To assess the conspicuity of T2 imaging at detecting brain metastases on a per-patient basis at follow-up scans and on a per-lesion basis at baseline and at follow-up time points.

1.2.1.2 Chemical Exchange Saturation Transfer (CEST) MRI: To assess the conspicuity (CNR) of CEST imaging at detecting brain metastases on a per-patient basis at follow-up scans and on a per-lesion basis at baseline and at follow-up time points.

1.2.1.3 Multiple B Value Diffusion Imaging (Adv Diff); To assess the conspicuity (CNR) of Adv Diff at detecting brain metastases on a per-patient basis at follow-up scans and on a per-lesion basis at baseline and at follow-up time points.

1.2.1.4 Gradient- and Spin-Echo (GESE) Dynamic Susceptibility Contrast (DSC) perfusion imaging: To assess the conspicuity of GESE DSC and standard Gradient Echo (GE) DSC at detecting brain metastases on a per-patient basis at follow-up scans and on a per-lesion basis at baseline and at follow-up time points.

1.2.2 Treatment Response:

1.2.2.1 Quantitative MRI: To assess the trend of idealized quantitative T1 and T2 values of brain metastases compared to normal brain parenchyma following treatment with stereotactic radiosurgery.

1.2.2.2 Chemical Exchange Saturation Transfer (CEST) MRI: To assess the ability of CEST imaging to differentiate radiation necrosis from progressive disease in brain metastases following stereotactic radiosurgery.

1.2.2.3 Multiple B Value Diffusion Imaging (Adv Diff): To assess whether Adv Diff can differentiate radiation necrosis from progressive disease in brain metastases following stereotactic radiosurgery.

1.2.2.4 Gradient- and Spin-Echo DSC perfusion imaging: To assess whether GESE DSC improves differentiation of radiation necrosis from progressive disease in brain metastases following stereotactic radiosurgery compared to standard GE DSC.

1.2.2.5 Multiple inversion time (TI) arterial spin labeling (ASL) "Adv ASL" perfusion imaging: To assess the ability of Adv ASL to differentiate radiation necrosis from progressive disease in brain metastases following stereotactic radiosurgery.

2 BACKGROUND

2.1 Brain Metastasis

Brain metastases have been reported to occur in up to 30% of patients with cancer, and treatment options include supportive care, surgery, and radiotherapy. [1] The incidence of brain metastases is increasing, partly because of increase in the incidence of primary cancers and partly because improvements in treatment options have prolonged survival of patients with cancer, which increases the chance of primary tumors metastasizing. [2] There are very few chemotherapy options open to patients with brain metastases. Stereotactic radiosurgery (SRS) has become an increasingly important treatment option for the initial management of patients with brain metastases. Early and accurate detection of small metastases is associated with improved treatment success. [3] At present, gadolinium-enhanced MRI is considered to be the imaging technique of choice in patients suspected of brain metastases. [4]

Significant limitations to the utilization of gadolinium-enhanced MRI in the screening for brain metastasis is cost and the requirement of an intravenous injection of a contrast agent. [5] Some patients have claustrophobia requiring sedation. Additionally, underlying conditions, such as back pain, may make it hard for the patients to lie still for the duration of the MRI. Achieving standard-of-care MR imaging in these populations can be challenging. There is a vital clinical necessity for a safe and efficient screening imaging technology to offer patients as risk of brain metastases, who have contraindications to gadolinium as well as the ability to tolerate long examination times. [6] Thus, fruitful diagnostic techniques that do not rely on gadolinium injection would be clinically meaningful by improving cost, time and safety of MR imaging.

Current MR imaging protocols for brain metastasis evaluation rely on contrast-enhanced T1-weighted MRI (CE-T1WI), which has proven itself a valuable tool for the visualization of tumor margins. However, changes in lesion size as determined by CE-T1WI images taken alone are not specific to cell death. There has been more success with quantitative MR methods, such as the apparent diffusion coefficient (ADC) [7], which increases with apoptosis and has been correlated with response to therapy [8]. It has also been shown that low pretreatment ADC values are correlated with a favorable response [8]. However, given that ADC is sensitive to many changes in tissue micro-structure, it is often difficult to distinguish between tumor necrosis, edema, or

radiation-induced inflammation, which inhibits its use in monitoring cancer treatment.

Advanced imaging techniques currently in clinical use such as dynamic contrast enhancement perfusion imaging and dynamic susceptibility contrast perfusion imaging have been shown to improve diagnostic accuracy in the assessment of brain metastasis behavior following stereotactic radiosurgery. [9] However, their diagnostic yield is not ideal (sensitivity and specificity less than 90% in clinical trial settings) and their usage is demonstrated in the reactionary scenario following the development of abnormal conventional imaging. These techniques have not been shown to be able to predict future behavior of lesions prior to the development of anatomic imaging abnormalities.

2.2 Quantitative MRI

Intensity values on routine MR imaging are not defined in terms of any consistent scale since the signal is dependent on many hardware and patient-specific factors. Hence there is very little gained in measuring the “bright” or “dark” pixel values on an MR image, and the images must be interpreted subjectively. [10] This would be analogous to subjectively describing “hot” or “cold” spots on an infrared heat map image when the absolute temperature scale of the pixels is not known. This study proposes to use multi-parameter mapping to quantitatively measure the signal changes in the tissues to address these challenges. In MR imaging, hydrogen nuclei or "spins" are the sources of the signal. In the strong magnetic field of an MR scanner, these spins emit RF signals in response to transmitted RF pulses. Thus contrast mechanisms in MR imaging are highly dependent on the hydrogen spin density or proton density (PD), as well as the longitudinal and transverse relaxation times (T1 and T2, respectively), which tend to vary between different tissues or fluids. However, measurement of relaxation times requires multiple acquisitions, and as a result measurement or quantitation of these parameters can be excessively long for clinical MR exam.

Recent developments in rapid multi-parameter mapping techniques are possible due to advances in accelerated imaging and reconstruction techniques. [10,11] These typically incorporate efficient pulse sequence designs and mathematical constraints to assess the desired information in each pixel. SyntheticMR developed the first clinically available method and released by GE Healthcare as "MAGIC" (MAGnetic resonance Image Compilation), which can achieve whole brain quantitation in 5-6 minutes. [12] The SyntheticMR method assumes the process of T1 and T2 relaxation times to be monoexponential, whereas it may be multi-exponential for many tissues. However, a phantom study has revealed good accuracy and reproducibility for T1, T2, and PD measurements by the SyntheticMR method. These measured parameters can also be applied to an MR spin model produce images with almost any contrast weighting by virtually changing repetition time (TR), echo time (TE), and inversion time (TI). 1 Synthetic MRI is particularly useful when many different contrast settings (for example, T1 weighted imaging (WI), T2WI, proton density WI) are required. Synthetic MRI of the brain without the use of a contrast agent has been reported to produce images that although inferior in image quality the diagnostic power of the images was comparable to that of images obtained via conventional MRI sequences. [13] Therefore, if the diagnostic power of images obtained via synthetic MRI after administration of a contrast agent is also proved to be comparable to that of conventional MRI, synthetic MRI could

be a useful means of screening for brain metastases, significantly reducing scan time and providing quantitative data. [14]

2.3 Multiple B-Value Diffusion Imaging (Adv Diff)

Molecular diffusion is a stochastic process and, as such, it may be described by probability distributions. The most basic of these is the probability of a molecule moving a given displacement over a given time interval. For simple, homogeneous liquids (e.g., a glass of water), this displacement probability distribution function is Gaussian, and the diffusion is referred to as Gaussian diffusion. In conventional MR imaging, diffusion of water molecules in the tissues has a tiny contribution to the MR signal. In diffusion MRI, powerful magnetic gradients with echo planar sequence are used. Thus, the measurement of the molecular diffusion of water has been possible for decades using MRI, and diffusion-weighted imaging (DWI) with MRI has been possible for greater than thirty years. [15]

A diffusion coefficient called apparent diffusion coefficient (ADC) value can be calculated within each image voxel, and ADC maps can be generated on a pixel-by-pixel basis. Because diffusion coefficients are high in fluids where diffusion is free, a low signal is observed on diffusion imaging at b = 1000 mm2/s (high signal on corresponding ADC maps). Normal CSF is an example of this. On the other hand, if the mobility of water molecules is restricted such as in ischemia (cytotoxic edema) high signal is observed on diffusion imaging at b = 1000 mm2/s (low signal on corresponding ADC maps). [16]

Diffusion-weighted imaging has been proposed as a set of tools to improve diagnostic accuracy and achieve a better understanding of the pathophysiology of brain cancer. A specialized form of DWI is *diffusion tensor imaging* (DTI) that provides information not only about the random displacement, or passive diffusion, of water molecules but also about fiber directionality and integrity. DTI can allow visualization of neuronal projections in the central nervous system and estimation of the neuronal changes in the white matter of healthy subjects and patients with various neurological diseases. [17]

Fractional anisotropy (FA) and mean diffusivity (MD) obtained from diffusion tensor imaging (DTI) has been used to assess the nature of brain metastasis and their response to treatment. However, FA and MD have limitations in accurately evaluating brain metastasis. New technologies, such as *diffusion kurtosis imaging* (DKI), can provide a more informative insight into the biology of tissue including brain metastasis. [18] An advantage of DKI is that it is relatively simple to implement for human imaging on conventional MRI clinical scanners. DKI protocols differ from DTI protocols in requiring at least three b-values (as compared to 2 b-values for DTI) and at least 15 independent diffusion gradient directions (as compared to 6 for DTI). Typical protocols for brain have b-values of 0, 1000, 2000 s/mm2 with 30 diffusion directions. The **apparent excess kurtosis coefficient** (AKC) is a dimensionless metric that quantifies the degree of deviation from Gaussian diffusion behavior. [19]

Defining the relationship between DKI and brain metastasis prior, during and following treatment can provide more abundant imaging to guide cancer diagnosis and treatment. [20] DKI

has been used to measure the non-Gaussian nature of water diffusion, which can reveal a more complex microstructure in both normal and pathological tissues compared to DTI alone. Previous studies have demonstrated that there was a significant difference in mean kurtosis (MK) value between high- and low-grade astrocytomas. [21] However, to the best of our knowledge, no comparison of different diffusion imaging approaches for assessing brain metastasis has been performed.

2.4 Gradient- and Spin-Echo DSC perfusion imaging

The relative cerebral blood volume (rCBV), derived from dynamic susceptibility contrast magnetic resonance imaging (DSC-MRI), is an established biomarker of glioma status that can aid in diagnosis [22], detecting treatment response [23], guiding biopsies [24], and reliable differentiation of post-treatment radiation effects and tumor progression [25]. It is also increasingly leveraged as a biomarker of early therapeutic response in clinical trials [26]. A gradient-echo (GE) echo-planar imaging (EPI) sequence is commonly applied dynamically every 1-1.5 s, during which gadolinium-based contrast agent is injected as a bolus, and the T2*-weighted images are used to calculate tumor blood volume (BV) maps.

The GE-EPI is more commonly used for DSC-MRI because it has high sensitivity to the susceptibility effect caused by the contrast agent. However, it also causes more susceptibility artifact in areas with tissue interface, hemorrhage and is more subject to the susceptibility-blooming near large vessels. When these factors are of concern, spin-echo (SE) EPI can be applied for DSC-MRI as an alternative approach in the clinic, despite its reduced overall sensitivity. In addition to their differences in overall sensitivity, it is well-known that GE-EPI is more sensitive to relaxivity changes caused by contrast agents in larger vessels whereas SE-EPI is more specific to those in vessels with size in the capillary level. [27] When both GE and SE images are acquired following a bolus injection of contrast agent, a vessel size map can be constructed. [28] The voxel value in the vessel size map represents mean vessel caliber of the voxel, which may serve as a useful surrogate for characterizing tumor angiogenesis and monitoring treatment-related changes. [29]

2.5 Chemical Exchange Saturation Transfer (CEST) MRI

Chemical exchange saturation transfer (CEST) is a promising new MRI contrast mechanism that has been used in studying the tumor microenvironment through the detection of mobile proteins and peptides. This technique relies on the labeling of endogenous populations of exchanging protons by a radiofrequency pulse at a specific frequency. These pools can transfer their magnetization to the unbound water (through the exchange), the extent of which constitutes the MRI image contrast, and by varying the radiofrequency pulse frequency, a spectrum is generated. In the absence of the CEST effect, this spectrum is generally considered to be symmetric; however, in its presence, the signal is attenuated at specific frequencies, resulting in chemical-specific negative peaks. Two CEST effects apparent in vivo are attributed to protons of mobile proteins: backbone amide signals with their base catalyzed proton transfer (APT), and Nuclear Overhauser enhancement (NOE) mediated aliphatic proton magnetization transfer (so-called exchange-relayed NOE. [30]

MRI based on the chemical exchange saturation transfer (CEST) effect from amine, amide, sulfhydryl and hydroxyl protons associated with endogenous metabolites has been shown to provide imaging maps of metabolites in tissue noninvasively. [31] Amide proton transfer (APT) imaging has been developed, which detects amide protons of low concentration in endogenous proteins and peptides in tissue. [32] APT has shown promising results in differentiating radiation necrosis from tumor progression in glioma and necrosis models in rats. [33] APT has also been applied to human glioma patients to differentiate high-grade tumors from low-grade ones with encouraging results. [34] Most germane to this project, the NOE immediately surrounding a brain metastasis one-week following treatment with stereotactic radiosurgery was shown to significantly correlate with tumor volume change at one-month post-treatment. [35] Thus, CEST imaging appears to be a promising tool to help inform future treatment decisions in patients with brain metastasis.

To overcome the limitations of endogenous CEST-MRI techniques, exogenous molecules have been exploited as extracellular tumor pH reporters for CEST-MRI applications. In the last decade, most work has focused on FDA-approved iodinated contrast agents, considering their high safety profile and translational potential. Due to their hydrophilic chemical structure, iodinated agents remain confined outside the cells and can be visualized as perfusion agents in tumor by CEST-MRI. One of the main advantages of using FDA-approved iodinated contrast agents is their very high safety profile for administration in patients. Consequently, CEST-MRI pH imaging with iopamidol was initially translated for measuring kidney and bladder pH in healthy volunteers. Later on, the capability to provide accurate tumor pH maps was demonstrated with iopamidol in both breast and ovarian cancer patients showing acidic tumor pH values. Their first application as pH CEST-MRI agents involved the use of iopamidol (Isovue®, Bracco Diagnostic), which possesses two amide proton pools that can be saturated at 4.2 and 5.5 ppm. The set-up of a ratiometric procedure allows to accurately measure extracellular tissue pH (pHe) in the pH range of 5.5–7.9, independently of the contrast agent concentration, with an accuracy of 0.1 units at several magnetic fields. CEST-MRI tumor pH imaging was combined to FDG-PET to elucidate the deregulation of tumor metabolism in a breast cancer model. This work evidenced that tumor regions with more acidic pHe show increased FDG uptake and demonstrated in vivo, for the first time, the relationship between tumor acidosis and high glycolytic rate. The combination of CEST pH-imaging and FDG-PET was then exploited for predicting the early therapeutic efficacy of metformin in a preclinical model of pancreatic cancer. In addition, the possibility to measure tumor pHe opened new routes for monitoring the effect of novel anticancer treatments that can reverse the glycolytic tumor phenotype. [36-38]

Based on this prior work, Dr. Pagel (MDACC) has invented and implemented a clinical imaging method that evaluates tumor acidosis, known as “acidoCEST MRI”. Chemical Exchange Saturation Transfer (CEST) MRI is used to detect the two pH- dependent CEST signals of a clinically approved contrast agent (iopamidol), and we have developed advanced analysis methods that use these CEST signals to measure the extracellular pH (pHe). Our pre-clinical studies have shown that an early response to treatment can cause a change in pHe.

2.6 Multiple inversion time (TI) arterial spin labeling (ASL) "Adv ASL" perfusion imaging

Arterial spin labeling (ASL) is an MRI technique that can noninvasively and quantitatively determine cerebral blood flow (CBF) by magnetically labeling the arterial water spins with radiofrequency pulses. [39] This method takes advantage of the fact that water protons of the arterial blood in the feeding vasculature of the brain are magnetically labeled and used as an endogenous tracer. After a specific inversion time, the labeled blood arrives at the image plane in which the image is acquired. In clinical studies, it has been used to assess perfusion in neurodegenerative diseases, epilepsy, central nervous system neoplasms, and vascular malformations. [40] However, a disadvantage of ASL-MRI is that in patients with cerebrovascular disease, the quantification of CBF is hampered by the recruitment of additional blood flow through collateral pathways. [41] These alternative pathways of blood flow lead to delayed arrival of the labeled blood bolus to the brain. [42]

As most ASL-MRI techniques acquire the labeled images at a fixed time after the initial labeling of arterial blood, it is possible that the magnetic label may not have reached the imaging plane, leading to underestimation of CBF. Previous studies reported that tumor-brain blood flow ratios determined by arterial spin-labeling were markedly higher than those obtained with dynamic susceptibility-weighted contrast-enhanced MR imaging. [43] This is probably caused by the underestimation of perfusion in brain regions with long arterial transit times. The use of higher inversion times would overcome these limitations; however, this would conversely lead to a decrease in the SNR caused by the rapid decay of the ASL perfusion signal over time. Recently, ASL-MRI with the acquisition of a series of images at multiple delay times after the initial labeling has been introduced as a method to compensate for these issues and also demonstrated the ability to differentiate low-grade and high-grade astrocytomas. [44]

2.7 Protocol Particular Disease Description

Brain metastasis will be defined as a three-dimensional intraaxial enhancing abnormality on MR imaging in a patient with pathology-proven extracranial non-central nervous system malignancy.

3 ELIGIBILITY

3.1 Inclusion Criteria

- An adult patient with pathology-proven solid organ cancer.
- MRI of the brain with contrast, positive for at least one intra-axial metastatic lesion greater than 5 mm.
- Planned treatment with stereotactic radiation.

3.2 Exclusion Criteria

- Contraindication to MR imaging.

- Known allergy to gadolinium-based contrast agents.
- Renal failure as defined by a GFR less than 30 or the use of hemodialysis.
- Pregnant.
- Patients less than 18 years of age will be excluded.

3.3 NUMBER OF PARTICIPANTS

Fifty (50) patients will be studied.

4 STUDY PLAN/DESIGN

4.1 Patient Enrollment:

Patients scheduled to undergo brain radiation at MD Anderson Cancer Center will be screened for eligibility based on their screening diagnostic MRI scan and available clinical information. Once eligibility is confirmed, patients will be approached for protocol introduction and consenting and enrollment. Enrolled patients will undergo the **baseline** study MRI scan with contrast within the fourteen (14) days before their scheduled brain radiation treatment.

4.2 Procedure to Obtain Consent

The patients will be approached by either the Diagnostic Imaging faculty or Research Staff, including research nurses and research coordinators for their approval before their procedure. During the consenting process, patients will be educated and consented for their research participants in this study.

4.3 MR Scanning:

The study MRI will be performed on a 3.0-T MRI system. All patients will undergo conventional anatomic MR imaging including 3D T1 pre and post-contrast weighted imaging, dynamic contrast-enhanced perfusion imaging, magnetic resonance spectroscopic imaging, as well as the study specific sequences to include:

- Quantitative MRI
- CEST
- Adv Diff
- GESE DSC
- Adv ASL

The study sequences will add approximately thirty (30) minutes to the conventional imaging sequences with the entire scan time to be less than sixty (60) minutes which adheres to a standard UTMDACC MR Operations scanning appointment time slot.

4.4 Contrast Administration

4.4.1 Iodinated contrast for acidoCEST imaging: Up to 120 mL of iopamidol using weight based MDACC scale.

4.4.1.1 Patients with history of allergy or adverse reaction to iodinated contrast will not receive iodinated contrast or acidoCEST imaging. They will receive APT CEST imaging only.

4.4.2 Gadolinium-based contrast: Gadolinium dosing will be performed per MDACC standard-of-care protocol for advanced brain tumor imaging including a unit dose preceding DCE perfusion imaging and a second unit dose preceding DSC perfusion imaging.

4.4.2.1 Patients with history of allergy to gadolinium-based contrast will be excluded from enrollment per section 3.2 of the protocol (Exclusion criteria).

4.5 Imaging Time Points

4.5.1 The baseline study scan will be performed within the fourteen (14) days **before** scheduled brain radiation treatment.

4.5.2 Seven additional study scans will be performed on the schedule of the patient's clinical protocol, but not less than four (4) weeks apart and not more than 16 (sixteen) weeks apart.

4.6 Conclusion of Study Participation:

4.6.1 Completion of eight (8) study scans.

4.6.2 Upon patient request.

4.6.3 A contraindication to further MR imaging.

4.6.4 Death.

5 Image Analysis

5.1 Metastasis size evaluation:

5.1.1 A patient must have a single enhancing intraaxial brain metastasis of 5 mm or greater for study inclusion.

5.1.2 Metastases will be measured on axial T1 post-contrast imaging on a slice displaying the maximal tumor size using the longest diameter after appropriate magnification on the picture archiving and communicating system (PACS) monitors.

5.1.3 Only metastasis planned for radiation therapy will be assessed (for example, lesions present at baseline imaging that are planned for surgical resection will not be assessed).

5.1.4 For patients with more than one metastasis appropriate for study assessment, up to ten (10) lesions will be assessed.

5.1.4.1 If there are more than ten lesions present, the five most caudal (lowest) and the five most cranial (highest) lesions that meet evaluation criteria will be tracked.

5.2 Metastasis CNR evaluation:

5.2.1 The largest circular ROI that fits within the brain lesion on axial T1 post contrast imaging will be placed and will define the lesion ROI.

5.2.2 The ROI mean signal intensity will be recorded.

5.2.3 A mirror ROI will be placed in the contralateral normal appearing white matter and used as the “background” mean signal intensity measurement.

5.2.4 An extracranial ROI without visible artifact will be used as the “noise” mean signal intensity measurement.

5.3 Quantitative MRI

5.3.1 The largest circular ROI that fits within the brain lesion on axial T1 post contrast imaging will be placed and will define the lesion ROI.

5.3.2 The T1 and T2 tissue weighting that maximizes the ROI mean signal intensity will be identified.

5.3.3 A mirror ROI will be placed in the contralateral normal appearing white matter and used as the “background” mean signal intensity measurement.

5.3.4 An extracranial ROI without visible artifact will be used as the mean “noise” signal intensity measurement.

5.4 CEST MRI

5.4.1 The largest circular ROI that fits within the brain lesion on axial T1 post contrast imaging will be placed and will define the lesion ROI.

5.4.2 The mean signal intensity for a corresponding ROI location on the processed CEST data will be recorded.

5.4.3 A mirror ROI will be placed in the contralateral normal appearing white matter on the processed CEST data will be used as the “background” mean signal intensity measurement.

5.4.4 An extracranial ROI without visible artifact will be used as the “noise” mean signal intensity measurement.

5.5 Multiple B Value Diffusion Imaging (Adv Diff)

5.5.1 The largest circular ROI that fits within the brain lesion on axial T1 post contrast imaging will be placed and will define the lesion ROI.

5.5.2 The AKC for the lesion will be determined using B-values of 0, 1000, and 2000 mm2/s.

5.5.3 The ADC of the lesion will also be determined using B-values of 0 and 1000 mm2/s.

5.5.4 A mirror ROI will be placed in the contralateral normal appearing white matter, and "background" AKC and ADC measurement will be measured.

5.6 GESE DSC perfusion imaging – GESE vs GE

5.6.1 The largest circular ROI that fits within the brain lesion on axial T1 post contrast imaging will be placed and will define the lesion ROI.

5.6.2 The relative cerebral blood volume (rCBV) will be measured for the lesion on both GESE and GE (alone) processed CBV maps with contralateral normal appearing white matter being used as the background (relative).

5.7 GESE DSC perfusion imaging – vessel size map

5.7.1 The largest circular ROI that fits within the brain lesion on axial T1 post contrast imaging will be placed and will define the lesion ROI.

5.7.2 The mean lesion vessel size will be measured with this ROI from the vessel size map.

5.8 Adv ASL

5.8.1 The largest circular ROI that fits within the brain lesion on axial T1 post contrast imaging will be placed and will define the lesion ROI.

5.8.2 This ROI will be used to assess the cerebral blood flow (CBF) from the processed ASL data.

5.8.3 A mirror ROI will be placed in the contralateral normal-appearing white matter for assessment of the “background” CBF.

5.8.4 This ROI will be used to assess the bolus arrival time (BAT) from the processed ASL data.

6 Patient Information Confidentiality Plan

6.1 Collection of Identifiers:

The patient identifiers that will be collected in this study consist of patient medical record numbers and dates. Only the PI and collaborators at MD Anderson will have access to the identifiable data. Electronic data will be kept securely at MD Anderson (password protected behind the institution firewall).

The study will be performed in accordance with institutional policies for the use of existing medical information for research. Confidentiality will be maintained throughout the study. No identifying information will be used in any publication from this study. Electronic study data will be stored on password protected institution computers behind the institution firewall. Only the PI and collaborators who have completed Human Subject Protection Training (HSPT) will have access to the study data.

6.2 Training of personnel:

Only MDACC personnel designated by the PI who have completed HSPT training will have access to study records. These personnel will be fully trained to maintain patient health information confidentiality.

6.3 Data Storage:

Electronic study data will be kept on password protected computers behind the institution firewall. Study data will be maintained indefinitely in the future and not be shared, reused or disclosed to anyone or any entity outside of the investigators nor will it be used for other or future research without prior IRB approval. Paper records (data forms, and unique identifiers, etc.) will be kept in a locked file cabinet with access granted only to study investigators and research staff.

6.4 Data Sharing:

Study data will not be shared with any individuals or entities that are not involved in the study without prior IRB approval. Study collaborators will utilize REDCap and Box to store and share data.

6.5 Final Disposition of study records:

After publication, any printed paper records will be placed into the Shred-it confidential waste bins for disposition. The study will be performed in accordance with current institutional standards for the use of existing medical information, banking the data and identifiers for research. There will be no additional use of the study data without prior review and approval by the IRB.

7 STATISTICAL CONSIDERATIONS

7.1 Definitions

Brain metastases "**treatment response**" will be defined by size and contrast to noise ratio (CNR).

Size – treatment response in a lesion will be considered as a decrease in size of 30%. (see section 5.1)

CNR—treatment response will be considered as a decrease in T1 post-contrast CNR of at least 20% (see section 5.2) by standard T1 post contrast imaging for a lesion.

Metastases that do not meet these criteria for treatment response will be considered treatment failure.

Disease **recurrence** will be determined by surgical pathology (if available) or at least three months of imaging follow-up. Imaging findings will be considered recurrent disease in a lesion if:

1. The lesion was originally considered to be a treatment responder.
2. The lesion showed a successive increase in the size of 25% from nadir or a single imaging time point increase of 40%. (see section 5.1)

Lesions that show an increase in size, but do not meet these criteria will be considered radiation necrosis.

7.2 Sample Size

For each of the primary conspicuity endpoints, the objective is to estimate the ability of each imaging method (T2 weighted MRI, CEST MRI, Adv Diff, and DESE DSC) at identifying brain metastases at baseline. Standard of care qualitative T1 post-contrast imaging will be considered the gold standard. Each patient will have between 1 and 10 lesions, with an expected average of 4 lesions per patient. (For patients with more than 10 lesions, 10 will be chosen as described in Section 5.1.4.1.) The primary analysis will consider the largest lesion within each patient, and the analysis will be repeated considering all lesions. The sensitivity will be calculated along with the corresponding 95% confidence interval. With 50 patients, this confidence interval will be no wider than 14.5% on either side of the estimated proportion. The confidence interval around the proportion of total lesions detected will be tighter, depending on the total number of lesions.

7.3 Analysis Plan

Patients are expected to have on average four brain lesions each. The primary analysis will be performed on the largest lesion within patients at baseline, and secondary analyses will consider all lesions. The sensitivity of each method at detecting lesions as determined by T1 weighting will be reported with 95% confidence intervals. Analyses will also be performed at the subsequent scans as numbers permit.

For the secondary endpoints that consider treatment response, for each of the imaging methods, at each time point, we will calculate the contrast to noise ratio (CNR) in patients with lesions that respond to treatment and in patients with local failure and will compare these by using a Wilcoxon rank-sum test. If numbers permit, analyses may be repeated using all lesions. Additionally, of the lesions that initially respond, some will subsequently grow in size after the initial response. Of these, some will be considered treatment change and some residual disease. The CNR will be compared between lesions with treatment change and those that are progressive disease using a Wilcoxon rank-sum test.

All statistical tests will be performed with two-sided tests with 5% Type I error rates. No adjustment will be made for the multiplicity of testing. We recognize that the Type I error rate overall will be greater than 5%.

8 PROTOCOL MONITORING PLAN

This is not a treatment study, and no adverse effects are anticipated related to contrast-enhanced MR imaging. There is a tiny risk of an allergic reaction to the gadolinium-based and iodinated contrast agent for which the patient will go through regular Departmental screening.

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