A Pilot Study of Using MRI-Guided Laser Heat Ablation to Induce Disruption of the Peritumoral Blood Brain Barrier to Enhance Delivery and Efficacy of Treatment of Pediatric Brain Tumors

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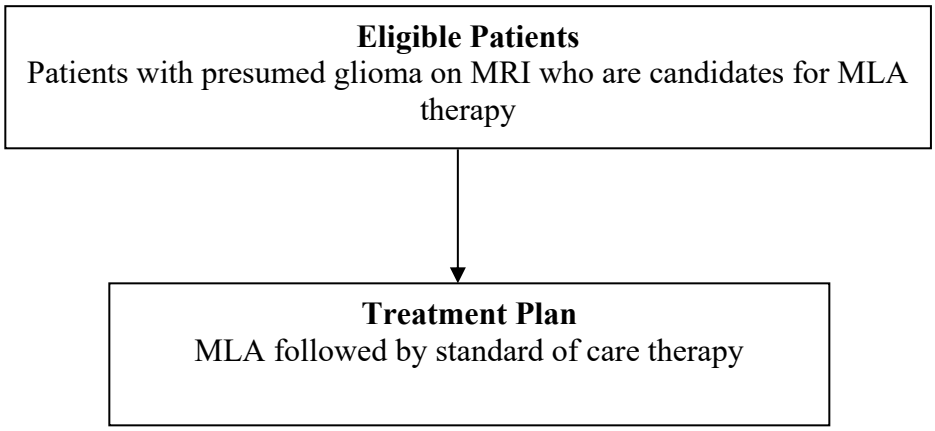
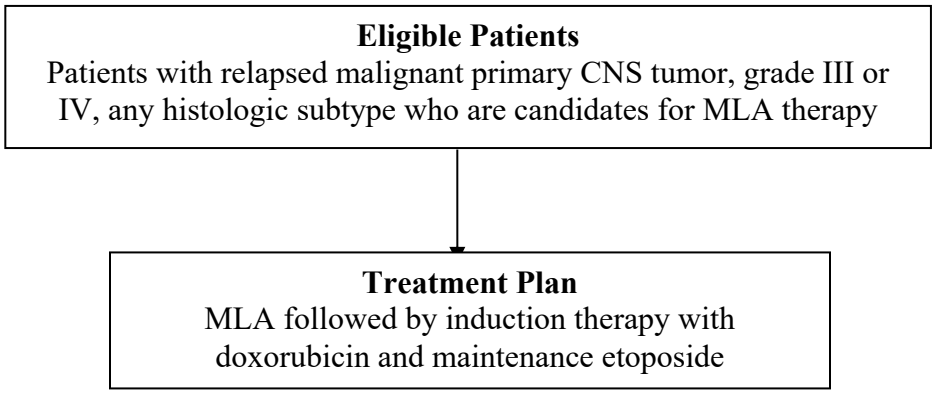
SCHEMA

Arm A



Arm B



Glossary of Abbreviations

AE	Adverse event
ALT (SGPT)	Alanine transaminase (serum glutamate pyruvic transaminase)
ANC	Absolute neutrophil count
AST (SGOT)	Aspartate transaminase (serum glutamic oxaloacetic transaminase)
BBB	Blood brain barrier
B-HCG	Beta human chorionic gonadotropin
BUN	Blood urea nitrogen
CBC	Complete blood count
CNS	Central nervous system
CR	Complete remission
CRF	Case report form
CT	Computed tomography
CTCAE	Common Terminology Criteria for Adverse Events
CTEP	Cancer Therapy Evaluation Program
DNA	deoxyribonucleic acid
DSM	Data and Safety Monitoring
DSMC	Data Safety Monitoring Committee
ECG (or EKG)	Electrocardiogram
EDTA	ethylenediaminetetraacetic acid
EE	Efficacy-Evaluable
EFS	Event free survival
FDA	Food and Drug Administration
G-CSF	Granulocyte colony stimulating factor, filgrastim (Neupogen)
HIV	Human Immunodeficiency Virus
HRPO	Human Research Protection Office (IRB)
IRB	Institutional Review Board
IV	Intravenous (i.v.)
MRI	Magnetic resonance imaging
MTD	Maximum tolerated dose
NCI	National Cancer Institute
OHRP	Office of Human Research Protections
ORR	Overall response rate
OS	Overall survival
PB	Peripheral blood
PBMC	Peripheral blood mononuclear cell
PD	Progressive disease
PFS	Progression free survival
6PFS	Progression Free Survival at 6 months
PI	Principal investigator

PR	Partial response (Partial remission)
QASMC	Quality Assurance and Safety Monitoring Committee
RANO	Response Assessment in Neuro-Oncology
RBC	Red blood cell (count)
RR	Response rate
SAE	Serious adverse event
SCC	Siteman Cancer Center
SD	Stable disease
UPN	Unique patient number
WBC	White blood cell (count)

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1.0 BACKGROUND AND RATIONALE

1.1 Pediatric Brain Tumors

Pediatric brain tumors comprise a heterogeneous group of tumors, including gliomas (high and low grade), medulloblastoma, ependymoma, primitive neuroectodermal tumor (PNET), atypical teratoid/rhabdoid tumor (ATRT), choroid plexus carcinoma, as well as other rarer tumors. Gliomas are the most common tumors in pediatrics, especially grade I (pilocytic astrocytoma) and grade II. High grade gliomas include anaplastic astrocytoma, glioblastoma (GBM), and mixed oligoastrocytomas. As with adults, high grade gliomas in pediatrics confer a poor prognosis. The current standard chemoradiotherapy for newly diagnosed high grade glioma produces only a modest survival benefit in pediatric patients, with less than 20% alive at five years [1]. All recurrent pediatric malignant tumors have a very poor prognosis with no standard and few effective therapeutic options available. Most children with relapsed disease will succumb within 3 years of recurrence with any malignant tumors. One of the challenges in treating brain tumors is the limitation of many agents to penetrate the blood brain barrier (BBB). Several methods to overcome BBB disruption have been attempted and currently undergoing studies, including intra-arterial chemotherapy administration, direct intratumoral/intracavitary placement of chemotherapy agents, and convection-enhanced delivery of agents, although a method with clear survival benefit has yet to be demonstrated [2].

1.2 Penetrating the Blood Brain Barrier (BBB)

Despite the fact that several cytotoxic and targeted agents have shown significant anti-growth activity in cultured cells, when tested in clinical trials there has been with minimal success, particularly in high grade glioma patients [3, 4]. In one study of pediatric high grade glioma using primary tumor cell cultures, it was shown that several classic chemotherapy agents, including anthracyclines, have a high cytotoxicity in vitro [4]. The high failure rate is in part due to the redundancy in key growth pathways and in drug resistance of malignant cells. Another explanation is the poor CNS penetration many of these drugs have due to the blood brain barrier (BBB). As a result, high doses of drugs were used in studies to achieve therapeutic drug concentrations in the CNS, which led to significant systemic toxicities and limited their clinical usefulness [5]. Preclinical models have shown that the delivery across the blood brain barrier depends on the size and molecular weight of the compound, even after blood brain disruption by mannitol [6]. Thus, an outstanding challenge in neuro-oncology has been to generate drugs that have excellent CNS penetration or methods that can compromise the BBB to enhance drug delivery.

The impact of the BBB on primary brain tumors has been most studied in GBM. GBM typically appear on MR imaging as rim-enhancing masses, suggesting that the BBB within the growth-intensive rim is impaired because contrast enhancement reflects increased BBB permeability [7, 8]. Consequently, delivery of cytotoxic agents to the tumor rim is higher than that to normal brain tissue. However, beyond the enhancing rim – the peritumoral

region – where most micrometastatic GBM cells reside, the BBB remains relatively intact as demonstrated by the lack of contrast enhancement. As a result, access of cytotoxic drugs to this region is predicted to be more limited. Similar findings have also been observed in brain metastases arising from extracranial tumors. In many cancers, responses of brain metastases to systemic chemotherapy tend to closely parallel those of extra-cranial tumors [9-14]. Yet brain metastasis still represents a significantly poorer prognostic indicator than extracranial metastasis. This is likely because of the inherent nature of brain metastatic foci compromising critical neurological functions. Another possible explanation for this apparent paradox is that although cytotoxic agents can readily access brain metastases (BBB disrupted), they fail to reach therapeutic levels in the peritumoral or distant foci of brain micrometastases (BBB intact). Evidence supporting this theory came from studies in which drug levels of several cytotoxic agents were sampled in tumors and the surrounding normal brain tissue at the time of surgery or autopsy. Drug concentrations were at the highest in the tumors, and then rapidly decreased up to 40 fold lower within 2 cm distance from the viable tumor edge [15-17]. Evidence also suggests that efflux transporters in the endothelial cells of the BBB, such as multi drug resistance gene (MDR1) and multi drug resistance-related protein gene (MRP1), prevent agents from crossing the BBB [4, 18], and expression of these proteins negatively correlates with achieved concentration of chemotherapy within tumor cells [18]. Overall, these results support two notions: 1) the BBB and its integrity negatively correlate with delivery and therapeutic effect of cytotoxic agents; and 2) if we can disrupt the BBB in the peritumoral region, we could improve cytotoxic chemotherapy delivery in this area. In GBM, most recurrent tumors arise within the peritumoral region – in one series, 90% of GBM relapses occurred within 2-3 cm margin of the primary site [19] - therefore elimination of micrometastatic GBM cells in this area will likely improve long-term disease control.

To circumvent the BBB problem in local drug delivery, recent approaches have focused on bypassing it. A common method is the use of Gliadel wafers, a polymer implant impregnated with the chemotherapeutic agent BCNU and placed intraoperatively in the resection cavity to evade the BBB. This approach resulted in a statistically significant but modest survival advantage in both newly diagnosed and recurrent GBM [20-22]. The modest benefit of Gliadel could be due to the short duration of drug delivery – most BCNU is released over a period of 5 days[23]. However, the fact that direct delivery of a cytotoxic drug into the resection cavity for as little as 5 days could improve survival of GBM patients to a degree approaching that achieved by 8 months of systemic temozolomide chemotherapy is remarkable in itself, supporting the theory that the BBB is critical to cytotoxic chemotherapy effect. Unfortunately, Gliadel is not widely utilized as it requires a major surgery and can impair wound healing. Another approach of bypassing the BBB is the convection enhanced delivery system, in which a catheter is surgically inserted into the tumor to deliver chemotherapy [24]. This invasive procedure requires prolonged hospitalization, meticulous maintenance of the external catheter to prevent serious complications, and as a result remains investigational and is rarely used.

1.3 MRI-guided Laser Ablation (MLA)

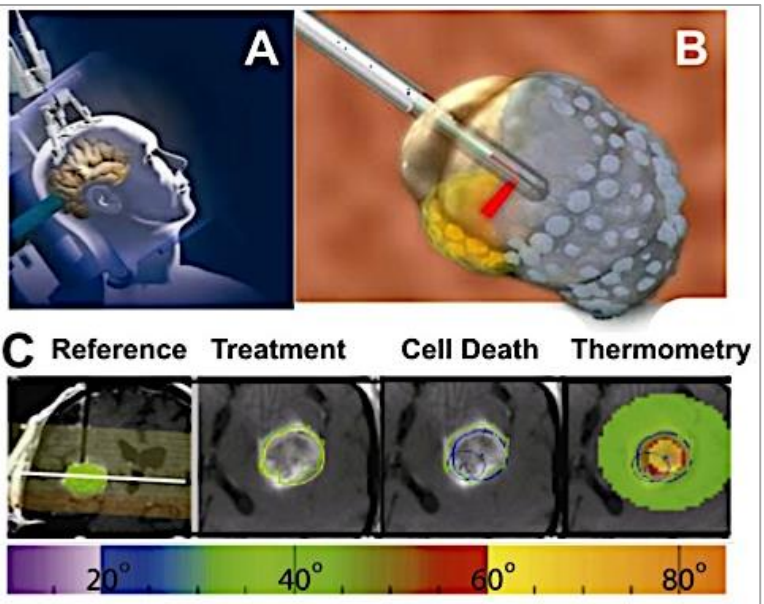
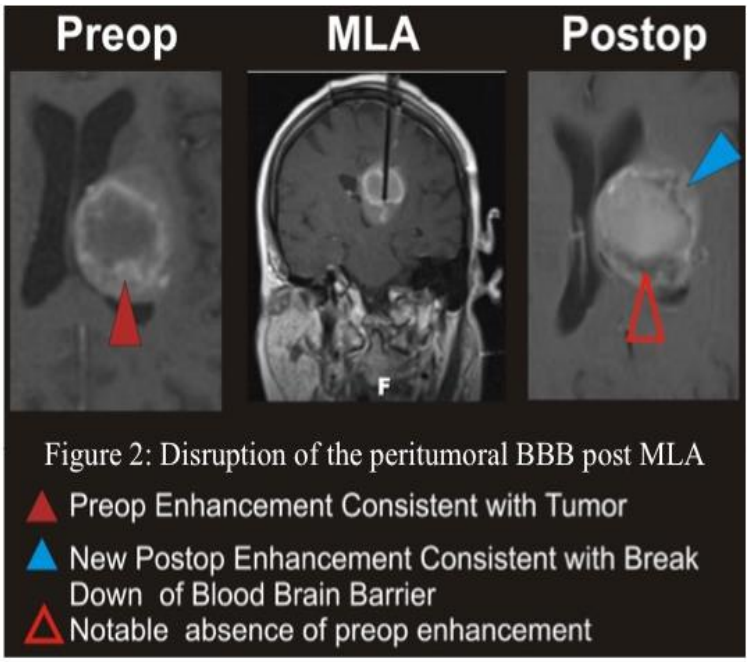


Figure 1: MRI-guided Heat Ablation Therapy (Monteris AutoLITT™). (A) Tripod base affixed to the skull with stereotactic wand visible. (B) Rendering of laser heating tumor from inside out. (C) Targeting strategy, treatment and cell death areas, and thermometry measurements. The target region was defines preoperatively based on MRI images (green sphere and green lines). Treatment areas were defines intraoperatively for each trajectory (yellow lines showing treatment area for trajectory #2). Cell death margins was determined as defined by trajectory and thermometry: thin and thick blue lines indicate cell death margins for trajectories #1 and #2, respectively. Thermometry measurements in degrees Celsius for the total treatment are plotted relative to the MRI.

MLA is a minimally invasive laser surgery currently FDA approved for cytoreductive treatment of brain tumors, both primary and metastatic [25]. Dr. Eric Leuthardt at Washington University performed one of the first MLA procedures in the country and is an international expert in laser ablation treatment of brain tumors. Currently Washington University has one of the largest clinical experiences using this new technology (Monteris, Winnipeg, CA). MLA employs a small incision in the scalp and skull, through which a thin laser probe is inserted and guided by MR imaging to the core of a tumor mass where it delivers hyperthermic ablation from the core to the rim. The maximal temperature in the core can reach greater than 70ºC resulting in coagulative necrosis (Fig. 1). The temperature decreases in the peritumoral region but remains high enough (>40ºC) to induce changes in the BBB as evidenced by new peritumoral contrast enhancement extending several centimeters from the tumor edge, while the original tumor enhancement is lost due to the heat ablation (Figs. 1 & 2). These observations suggest that an interesting side effect of MLA is the disruption of the peritumoral BBB. These changes often persist for several weeks (not shown), providing a rare window of opportunity during which drug delivery can be enhanced to eliminate infiltrative tumor cells residing in this region where most recurrences occur.

Whether MLA indeed causes BBB disruption remains unexplored and is the main focus of this study. Interestingly, the role of hyperthermia in inducing increased BBB permeability has been previously described in several animal models. In a rodent model of human glioma, the global heating of a mouse's head to 42ºC for 30 minutes in a warm water bath

significantly increased the maximal brain concentration of a thermosensitive liposome encapsulated with the chemotherapeutic drug Adriamycin [26]. To effect locoregional hyperthermia in the brain, retrograde infusion of a hyperthermic saline solution at 43ºC into the left external carotid artery in the Wistar rat model reversibly increased BBB permeability to Evans-blue albumin in the left cerebral hemisphere[27]. In the most analogous method to the MLA, Nd:YAG laser-induced thermo-therapy to the left forebrain of Fischer rats resulted in locoregional disruption of the BBB as evidenced by increased locoregional passage of the Evans blue dye, serum proteins (e.g. fibrinogen and IgM), and the cytotoxic drug paclitaxel [28]. Based on these results, we hypothesize that hyperthermia-induced disruption of the peritumoral BBB by MLA (Fig. 2) represents a potentially powerful tool to enhance delivery of chemotherapy to this region to effectively target residual disease in addition to maximal cytoreduction of the tumor.



1.3.1 Prior Clinical Experience with MLA

We have had one of the largest clinical experiences using MLA in the country. To date we have done more than 80 cases, of which approximately two-thirds are high-grade glial neoplasms. In regards to adverse events, patients have experienced transient aphasia, transient hemiparesis, transient hyponatremia, and a documented case of lower extremity deep venous thrombosis. Patients with aphasia or hemiparesis improved over time with steroid therapy. Hyponatremia resolved spontaneously. One patient experienced fatal meningitis. Subsequent in-depth analysis revealed that this patient's meningitis was due to an operating room infrastructure-related contamination and not due to equipment contamination related to performance of the MLA procedure.

1.4 MLA followed by Standard of Care in Newly Diagnosed Glioma Patients (Arm A)

MLA offers an alternative to some patients with tumors that are unresectable. Complete surgical resection of grade I and II gliomas have a >90% overall survival, although complete resection is not always possible due to the location of the tumor. For tumors with less than a complete resection, chemotherapy or radiation therapy is offered for progressive

and/or symptomatic tumors. For high grade gliomas, patients who have a complete resection of the enhancing tumor at time of diagnosis [29, 30] or at relapse have a longer survival compared to those with residual disease [31]. In this arm of the study, patients with any grade glioma who are candidates for MLA will undergo this procedure followed by standard of care treatment.

1.5 MLA followed by Doxorubicin and Etoposide in Relapsed Malignant Tumor Patients (Arm B)

The ideal cytotoxic drugs for the purpose of this proposal should have potent activities against malignant cell lines *in vitro*, and *in vivo* activity that is limited by blood brain barrier function. Doxorubicin has been shown to kill a large number of high grade glioma cell lines *in vitro* [18, 32]. However, it has poor CNS penetration and has not been used extensively in CNS tumors. Doxorubicin and other anthracyclines induce cytotoxicity through intercalating between DNA base pairs, thereby interfering with strand elongation by DNA and RNA polymerase. Doxorubicin also affects topoisomerase II, which creates temporary double-strand DNA breaks during DNA replication. Doxorubicin stabilizes the DNA-topoII complex leading to double-strand DNA breaks and cell death. Doxorubicin has a wide volume of distribution with tissue levels proportional to the DNA content of the tissue. Doxorubicin is 75% bound to plasma proteins. Doxorubicin is mostly metabolized in the liver and eliminated mainly as glucuronide or hydroxylated conjugates in the bile and feces. The half-life of doxorubicin is 1 to 3 hours.

Maintenance chemotherapy using low-dose etoposide (21-day cycles) will be given following the course of doxorubicin. Low-dose etoposide has been found effective in treating relapsed brain tumor patients by extending PFS [33-36]. Etoposide is a topoisomerase II inhibitor and has been shown to inhibit tumor cell proliferation at high doses and to function as an anti-angiogenic agent when administered in low daily doses (metronomic chemotherapy) [37]. *In vitro* and pre-clinical studies demonstrate that low dose daily etoposide inhibits secretion of angiogenic factors, proliferation of endothelial cells, and endothelial tube formation [37]. This agent has been well tolerated in pediatric patients when given as low-dose, metronomic therapy.

In this arm, we are testing the concept that increased delivery of cytotoxic chemotherapy to the peritumoral region after MLA will result in increased peritumoral disease control. Although this approach appears to be similar to the dose escalation method, one clear difference is that in the dose escalation method, increasing systemic doses of drugs are used to achieve adequate drug concentrations in the CNS at the expense of significant systemic and global CNS toxicities, especially at doses near or exceeding the maximal tolerated dose. Therefore, the lack of benefits at high systemic doses may in part be due to excessive toxicities. On the other hand, in the MLA-enhanced drug delivery system, lower doses of drugs will be given more frequently to limit systemic toxicities and to selectively concentrate drugs in the peritumoral region where therapeutic action is desired, thus also reducing CNS toxicities.

In pediatric malignancies, doxorubicin is well tolerated and is given in combination with

other cytotoxic drugs. In high risk acute lymphoblastic leukemia, doxorubicin is given at 25 mg/m^2 IV weekly for 4 weeks in combination with vincristine. In Ewing's sarcoma, doxorubicin is given at 75 mg/m^2 IV in combination with vincristine and cyclophosphamide, alternating with ifosfamide and etoposide every 2 weeks. Similar dosing and schedules are given in other solid tumors, such as neuroblastoma and osteosarcoma. In ATRT, doxorubicin at 60 mg/m^2 (over 2 days) 2-3 weeks apart in combination with multiple other cytoxic agents [38]. We expect doxorubicin 25 mg/m^2/dose weekly for 6 weeks to be well tolerated. This is to minimize systemic toxicity while still achieving adequate drug delivery in the peritumoral region if the BBB is disrupted and to give continuous treatment during the window of MLA-induced peritumoral BBB disruption (6 weeks).

1.6 DCE and DSC-MRI

The measurement of perfusion in the brain using MRI is now commonplace and traces its origin to the seminal paper by Ostergaard et al.[39]. This technique is now referred to as dynamic susceptibility contrast (DSC) and relies on measuring the T2* signal changes that occur in the brain as a bolus of contrast material dynamically passes through the capillary circulation. DSC methods have since proved valuable in the diagnosis of stroke and brain tumors and are routinely used in our radiology practice. The mathematical model that describes DSC assumes that the blood brain barrier (BBB) is intact, but this assumption does not often hold in enhancing high-grade glial tumors, which are often supplied by leaky neo-vascularity. The leakage of contrast into the extracellular space causes changes in the T1 signal [40], which can invalidate uncorrected DSC measurements in tumors. Several methods, such as DCE, have been proposed to correct for this leakage [40, 41], some of which rely on a 2-compartment pharmacokinetic model that can estimate the vascular transfer constant (Ktrans)[41]. Ktrans is a parameter that describes the ability of contrast to move from the intravascular compartment to the extracellular compartment (the 2 compartments in the model) and thus provides a quantitative measure of the degree of BBB leakage. Law et al. used this model to measure Ktrans and cerebral blood volume (CBV)

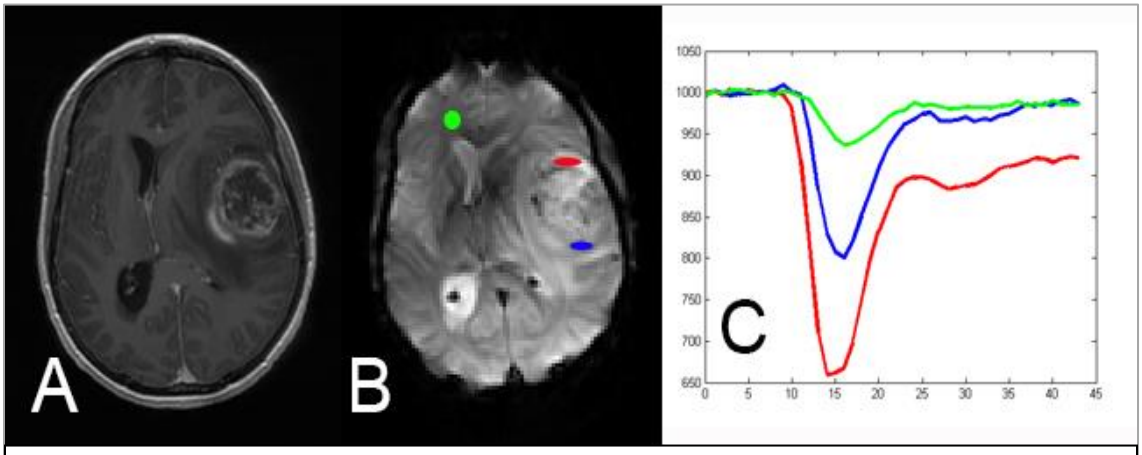


Figure 3. Perfusion measurements in patients with GBM. See text for details.

in 74 patients with glial tumors and demonstrated that the combination of both measures

provided the best discriminator for high-grade gliomas, thus providing validation for this model[42].

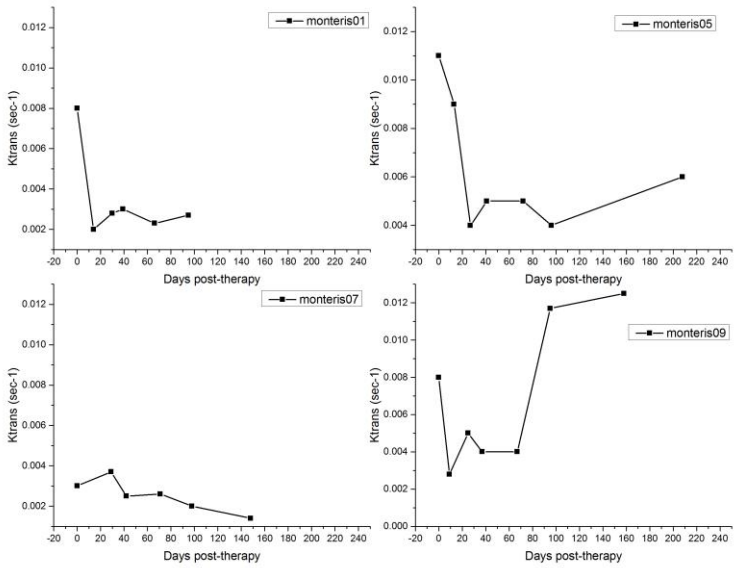


Figure 4: Examples of longitudinal Ktrans measurements in tumor patients follow the monteris procedure using the DCE method. More detail provided in the text

We implemented a modified version of the Johnson model[41] and applied it to several patients with high-grade gliomas as illustrated in Figure 3. Figure 3A is a T1-weighted post contrast image of the tumor, seen as a ring enhancing mass on the left side of the brain (right side of the image). Figure 3B is the same slice in a T2*-weighted image that is used to measure the signal change with the passage of a bolus of contrast material. Several regions of interest (ROI) are drawn on this image where measurements were made. The red ROI was placed in an area of relatively large BBB leakage, the blue ROI in an area of less leakage, and the green ROI in normal white matter. Figure 3C demonstrates the normalized tissue signal curves during the bolus tracking period that correspond to the drawn ROIs. The large signal drop seen centered at time frame 15 is taken at the peak of the contrast bolus transition. The depth and recovery of the signal during and after the bolus passage provides information on the degree of BBB leakage and on the value of Ktrans. In the normal white matter (green) the signal nearly recovers fully to its original value, consistent with the residual contrast left in the blood stream after the passage of the primary bolus. In the enhancing portion of the tumor (red and blue) the signal has a larger drop and does not fully recover providing us with estimates of the Ktrans.

As part of the Monteris study on the adult tumor population we have employed another method to measure the break down of the BBB called dynamic contrast-enhanced MRI (DCE-MRI). DCE-MRI is another common method used to evaluate for permeability of blood vessels in the brain[8]. Similar to DSC-MRI, DCE-MRI consists of injection of an MRI contrast agent followed by multiple T1-weighted images to assess the leakage of this agent into the extracellular space over several minutes. Again, the rate of pooling of the contrast agent is then used to quantify the degree of BBB permeability. Figure 4 demonstrates results from our recent study in 4 sample patients. The horizontal axis represents the number of days post-surgery with each time point representing an MRI

measurement. The vertical axis represents the Ktrans around the tumor, a measure of BBB breakdown. In 3 out of the 4 cases represented there is evidence of BBB breakdown seen as an elevated Ktrans in the first 2 weeks following surgery. In the case of monteris07 there was a smaller shift in the BBB that lasted for a longer period of time. The unusual marked increase in Ktrans seen at the later time points in monteris09 was due to tumor recurrence.

We will employ both methods (DSC and DCE) to determine the degree, extent, and duration of BBB leakage in the peritumoral region after MLA procedure. This will generate a dynamic and temporal map of BBB disruption in the area surrounding the post MLA tumor. Our long-term goal is to correlate these maps with patients' treatment outcome, when we have a larger database of treated patients, to determine whether patients with larger degree and extent of BBB leakage also derive more benefit from early chemotherapy.

1.7 Biomarkers of BBB Disruption

The coagulative necrosis and BBB disruption induced by MLA share several parallels with classical brain injuries (e.g. traumatic, surgical, ischemic, or pathologic brain damage), albeit in a more controlled setting. Several serum biomarkers have been identified, and in some cases extensively validated, in large number of patients with various forms of brain injuries. The compromised BBB after brain injuries allows CNS-specific factors released by damaged CNS cells to escape into the peripheral circulation where they can be detected using highly sensitive and specific detection techniques such as ultrasensitive ELISA, antibody arrays and HPLC/mass spectroscopy. The temporal profile of serum levels of these brain-specific factors (e.g. S100B, GFAP, brain-specific enolase or BSE) can provide information about the duration and degree of BBB disruption[43-53], irrespective of the type of brain injuries.

We will measure serum levels of these 4 brain-specific factors (S100B, GFAP, and BSE) immediately before and after MLA and then during the 6 weeks post-MLA, using ultrasensitive ELISA. If MLA results in sustained BBB disruption, we expect that the level of at least one of these biomarkers will increase precipitously soon after MLA and well above that caused by recurrent GBM, and that the increase will persist for several days to weeks. Antibody microarray can substitute ELISA if higher detection sensitivity is needed. We chose these biomarkers as they have previously been validated in brain trauma, recognizing that other brain specific factors may be more reliable.

1.8 MLA and Immune Activation

One of the most striking findings from the Washington University experience with MLA is the delayed timing, degree, and persistence of peritumoral enhancement following treatment. Specifically, strong enhancement is observed several days after treatment and persists beyond 6 weeks. We hypothesize that these imaging findings are due to persistent disruption of the BBB, which is compounded and maintained by an enhanced immune infiltrate in the peritumoral area. Clinically, we have observed improved patient outcomes in our adult retrospective MLA series (Table 1) and hypothesize that this is in part due to

an MLA-induced anti-tumor immune response. CNS immunosurveillance is likely distinct when compared to other tissues with clearly defined secondary lymphoid structures[54]. However, CNS antigen presentation is thought to occur when antigens drain to the ipsilateral cervical lymph node chain[54]. Thus, it is possible that MLA disrupts the BBB such that tumor antigens, either native or heat denatured, have greater access to cervical draining lymphoid tissue, thereby stimulating an anti-glioma immune response that (a) prolongs patient survival and (b) leads to persistent contrast enhancement characteristic of this treatment. The potential ability of MLA to augment anti-glioma immune responses is particularly compelling because patients with glioma are known to present with a broad range of immunological dysfunction [55].

Table 1
MLA Experience in Patients with GBM at
Siteman Cancer Center in the Past 12 Months

Age at MLA	Diagnosis	Date of MLA	Chemotherapy (<3 wks after MLA)	PFS (months)	Alive
1 73	GBM	11-4-11	Yes	12	Yes
2 64	GBM	12-27-11	Yes	10	Yes
3 34	High grade, likely GBM	01-03-12	Yes	Not yet progressed	Yes
4 46	High grade, likely GBM	01-16-12	Yes	11	Yes
5 72	GBM	02-10-12	Yes	Not yet progressed	Yes
6 68	GBM	07-06-12	Yes	Not yet progressed	Yes

1.9 Study Rationale

By employing a combination of advanced MRI techniques and correlative serum biomarkers of BBB disruption, we plan to develop a powerful, first of its kind clinical algorithm in pediatrics whereby we can measure and identify the window of maximal BBB disruption post MLA to 1) allow for an alternative to surgery in incompletely resected tumors, 2) allow for optimal chemotherapeutic dosing to achieve the greatest benefits and the least systemic side effects and 3) distinguish subsequent tumor progression from long-term MLA treatment effects. Preliminary data in adult imaging studies have shown that the BBB disruption lasts for several weeks following treatment before returning to a low baseline. This pilot therapeutic study will provide preliminary validation in pediatric patients.

Although we have a large armamentarium of cytotoxic drugs with potential activity against brain tumors, the vast majority has poor CNS penetration limiting their usefulness. Our proposed use of MLA to achieve both cytoreduction and increased permeability of the peritumoral BBB has the potential to be practice changing and will allow us to test many drugs that have not shown promise due to poor BBB penetration. The innovative algorithm

to detect and measure MLA-induced peritumoral BBB compromise may also be applied using other treatments.

Recent work has collectively demonstrated striking immune dysregulation in patients with GBM, including T cell lymphopenia and anergy, cytokine dysregulation, and increased regulatory T cell (T_{reg}) populations among others, which reflect immunologic compromise and functional impairment[54-57]. However, a growing list of potential tumor antigens has been identified, suggesting that tumor-specific recognition by immune cells may be biologically relevant and therapeutically exploitable[55, 58]. Therefore, given the immune dysfunction characterized in GBM patients, approaches that potentiate the anti-glioma immune response are particularly exciting. A priori, because the immune system has evolved to recognize a tremendous diversity of antigenic epitopes in vertebrates, immune-potentiating efforts – with MLA being one potential approach – may be especially effective at targeting the heterogeneity that defines GBM.

2.0 OBJECTIVES

2.1 Primary Objectives

1. To determine progression-free survival (PFS) and overall survival (OS) of patients with new diagnosis glioma who are candidates for MLA (Arm A).

2. To determine 6-month progression-free survival (6PFS) in patients who receive doxorubicin immediately following MLA and low-dose etoposide as maintenance therapy in patients who are candidates for MLA with any type relapsed malignant brain tumors (Arm B).

3. To evaluate quality of life (QOL) using Karnofsky or Lansky performance status in patients following MLA and in patients who receive doxorubicin and maintenance etoposide after MLA.

2.2 Secondary Objectives

1. To determine MR imaging correlates of peritumoral BBB disruption after MLA.

2. To identify serum biomarkers of peritumoral BBB disruption after MLA, which can be used to establish peritumoral permeability scores.

3. To determine the predictive value of the peritumoral permeability score for patient outcome as measured by 6PFS rate.

2.3 Exploratory Objective

To determine the effects of MLA treatment on patient's tumor-specific immune responses

and immunological correlates.

3.0 PATIENT SELECTION

3.1 Inclusion Criteria – Arm A

1. Presumed pediatric gliomas (grades I-IV) on MRI that are determined to be candidates for MLA by the treating neurosurgeon.

2. Age 3 to ≤ 21

3. Karnofsky/Lansky performance status ≥ 60%, see Appendix 1.

3.2 Inclusion Criteria – Arm B

1. Recurrent pediatric brain tumors determined candidates for MLA as determined by the treating neurosurgeon.

2. Unequivocal evidence of tumor progression by MRI (see Section 12).

3. There must be an interval of at least 12 weeks from the completion of radiotherapy to study registration except if there is unequivocal evidence for tumor recurrence per RANO criteria (see Section 12). When the interval is less than 12 weeks from the completion of radiotherapy, the use of PET scan is allowed to differentiate between evidence of tumor recurrence and pseudoprogression.

4. Recurrent lesions with dimension and contour that are determined by the treating neurosurgeon to be appropriate for MLA.

5. Age 3 to ≤ 21

6. Karnofsky/Lansky performance status ≥ 60%, see Appendix 1.

7. Adequate cardiac function as determined by a shortening fraction ≥ 27% or left ventricular ejection fraction ≥ 50% by echocardiogram within the past 1 year prior to registration.

8. Prior anthracycline therapy does not exceed 200 mg/m^2 total cumulative dose.

9. Adequate bone marrow and hepatic function as defined below (must be within 7 days of MLA):
   a. Absolute neutrophil count (ANC) ≥ 1000/mcl (G-CSF is allowed)
   b. Platelets ≥ 100 K/cumm
   c. Hemoglobin ≥ 9 g/dL (pRBC transfusion +/- ESA are allowed)
   d. ALT ≤ 3 x ULN

e. AST ≤ 3 x ULN
f. ALP ≤ 3 x ULN. If ALP is > 3 x ULN, GGT must be checked and be ≤ 3 x ULN.
g. Bilirubin ≤ 2 x ULN

10. At the time of registration, patient must have recovered from the toxic effects of prior therapy to no more than grade 1 toxicity.

11. At the time of registration, patient must be at least 4 weeks from other prior cytotoxic chemotherapy.

12. Women of childbearing potential and men must agree to use adequate contraception (hormonal or barrier method of birth control, abstinence) prior to study entry and for the duration of study participation. Should a woman become pregnant or suspect she is pregnant while participating in this study, she must inform her treating physician immediately.

13. Ability to understand and willingness to sign an IRB approved written informed consent document (or that of legally authorized representative, if applicable).

3.3 Exclusion Criteria – Arm A

1. Currently receiving or scheduled to receive any other therapies intended to treat the newly diagnosed glioma prior to MLA and the first post-MLA blood collection for correlative studies.

2. Multi-focal or metastatic disease.

3. Pregnant and/or breastfeeding. Premenopausal women must have a negative serum or urine pregnancy test within 14 days of study entry.

4. Inability to undergo MRI due to personal or medical reasons.

5. Known history of HIV or autoimmune diseases requiring immunosuppressant drugs.

3.4 Exclusion Criteria – Arm B

1. Prior treatment with bevacizumab within 12 weeks of study entry.

2. Previous treatment with complete cumulative doses of daunorubicin, idarubicin, and/or other anthracyclines and anthracenediones that is equivalent to a total dose of > 200 mg/m^2 doxorubicin.

3. More than 2 prior relapses (not counting the current relapse being treated on this study).

4. Currently receiving any other investigational agents that are intended as treatments of the relapsed tumor.

5. Multi-focal or metastatic disease.

6. A history of allergic reactions attributed to compounds of similar chemical or biologic composition to doxorubicin or other agents used in the study.

7. Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, recent heart attack within the previous 12 months or severe heart problems, or psychiatric illness/social situations that would limit compliance with study requirements.

8. Pregnant and/or breastfeeding. Premenopausal women must have a negative serum or urine pregnancy test within 14 days of study entry.

9. Inability to undergo MRI due to personal or medical reasons.

10. Known history of HIV or autoimmune diseases requiring immunosuppressant drugs.

3.5 Inclusion of Women and Minorities

Males and females and members of all races and ethnic groups are eligible for this trial.

4.0 REGISTRATION PROCEDURES

Patients must not start any protocol intervention prior to registration through the Siteman Cancer Center.

The following steps must be taken before registering patients to this study:

1. Confirmation of patient eligibility
2. Registration of patient in the Siteman Cancer Center OnCore database
3. Assignment of unique patient number (UPN)

4.1 Confirmation of Patient Eligibility

Confirm patient eligibility by collecting the information listed below:

1. Registering MD's name
2. Patient's race, sex, and DOB
3. Three letters (or two letters and a dash) for the patient's initials
4. Copy of signed consent form
5. Completed eligibility checklist, signed and dated by a member of the study team
6. Copy of appropriate source documentation confirming patient eligibility

4.2 Patient Registration in the Siteman Cancer Center OnCore Database

All patients must be registered through the Siteman Cancer Center OnCore database. Registration in the SCC database will be the last step of the patient registration process. Once the patient has been registered in the SCC database, s/he will be considered registered to the study.

4.3 Assignment of UPN

Each patient will be identified with a unique patient number (UPN) for this study. All data will be recorded with this identification number on the appropriate CRFs.

5.0 TREATMENT PLAN

5.1 Study Summary

5.1.1 Arm A

Six patients enrolled to this study will be in Arm A; patients may be enrolled to Arm A at any time throughout the lifetime of the study. Arm A patients will undergo MLA and will be followed with standard of care therapy at the discretion of the treating physician.

Arm A patients will undergo DSC and DCE-MRI at the following time points along with standard of care imaging:

- no more than 3 weeks prior to MLA (OPTIONAL)
- within approximately 4 days after MLA
- 2-4 weeks after MLA
- Every 12 weeks (+/- 7 days) for the first year or until disease progression (see Section 9.1)

Arm A patients will have 7 ml of blood drawn at the following time points:

- Before MLA; this sample can be collected any time during the 3 days before or the day of the procedure until the start of the procedure
- Within approximately 3 days after MLA
- 2-4 weeks (+/- 3 days) after MLA (at time of DSC/DCE-MRI)
- Every 12 weeks (+/- 7 days) at time points correlating with MRIs for the first year or until disease progression

The purpose of these blood draws is to measure serum levels of brain-specific factors (S100B, GFAP, and BSE) and cytokine levels. PBMC (for the pre- and post-MLA samples) will also be isolated to determine the phenotypes and functions of peripheral blood immune cells following MLA (see Section 9.2-9.4).

5.1.2 Arm B

Six patients enrolled to this study will be in Arm B; patients may be enrolled to Arm B at any time throughout the lifetime of the study. Arm B patients will undergo MLA and will begin doxorubicin (as described in Section 5.4) within approximately 7 days following MLA (range 2-14 days)

Arm B patients will undergo DSC and DCE-MRI at the following time points along with standard of care imaging:

- no more than 3 weeks prior to MLA (OPTIONAL)
- within approximately 4 days after MLA
- 2-4 weeks after MLA
- every 8 weeks (+/- 7 days) until 2 years have elapsed or disease progression (see Section 9.1), whichever comes first

Arm B patients will have 7 mL blood drawn at the following time points:

- before MLA; this sample can be collected any time during the 3 days before or the day of the procedure until the start of the procedure
- within approximately 3 days after MLA
- weekly (+/- 3 days) before (on the same day as) chemotherapy for the 6 weeks post-MLA
- every 8 weeks (+/- 7 days) at time points correlating with MRIs for the first 2 years or until disease progression, whichever comes first

The purpose of these blood draws is to measure serum levels of brain-specific factors (S100B, GFAP, and BSE) and cytokine levels. PBMC (for the pre- and post-MLA samples) will also be isolated to determine the phenotypes and functions of peripheral blood immune cells following MLA (see Section 9.2 -9.4). This is in addition to blood samples for routine laboratory prior to chemotherapy and surgery. During the weeks of DSC/DCE-MRI, biomarker lab must be drawn on the same day as DSC/DCE-MRI (prior to the scan).

5.2 MRI-Guided Laser Ablation

MLA is a minimally invasive laser surgery currently FDA approved for cytoreductive treatment of brain tumors, both primary and metastatic [25]. MLA employs a small incision in the scalp and skull, through which a thin laser probe is inserted and guided by MR imaging to the core of a tumor mass where it delivers hyperthermic ablation from the core to the rim. The maximal temperature in the core can reach greater than 70^{o}C resulting in coagulative necrosis.

5.3 Premedication Administration

Because doxorubicin is emetogenic, prophylactic use of antiemetics will be used.

5.4 Agent Administration

Arm B only: Doxorubicin will be given intravenously on an outpatient basis weekly for 6 weeks at a dose of 25 mg/m^2 over 5-30 minutes. Doxorubicin will be held for an ANC of <500/mcl or platelets <50 K/cumm. Following the 6 weeks of doxorubicin, etoposide at 50 mg/m^2/day will be given orally for 21 days of each 28-day cycle to begin once ANC ≥ 1000/mcl and platelets ≥ 100 K/cumm. Etoposide will be held for ANC of < 500/mcl or platelets < 50 K/cumm. Patients may receive up to 24 cycles of etoposide.

5.5 Evaluability Criteria

All patients are evaluable for toxicity. Patients are evaluated from first receiving study treatment until a 30-day follow up after the conclusion of treatment or death. Patients on Arm A will be followed for a minimum of 6 months or death.

All patients in Arms B are evaluable for efficacy after receiving at least 2 doses of doxorubicin.

5.6 General Concomitant Medication and Supportive Care Guidelines

Subjects in Arm B should not receive medications that may interact with doxorubicin or etoposide. Refer to the product labels for details. Therapies excluded during the conduct of this trial include in Arm B include other chemotherapy agents, hormonal therapy for cancer, and other tumor-targeted therapies including but not limited to radiotherapy. Therapeutic use of hematopoietic colony-stimulating factors is permitted following for ANC < 500/mcl and should not be given within 24 hours prior to or following doxorubicin.

5.7 Women of Childbearing Potential

Women of childbearing potential (defined as women with regular menses, women with amenorrhea, women with irregular cycles, women using a contraceptive method that precludes withdrawal bleeding, and women who have had a tubal ligation) are required to have a negative serum pregnancy test within 14 days prior to MLA.

Female and male patients (along with their female partners) are required to use two forms of acceptable contraception, including one barrier method, during participation in the study and for 2 months following the last dose of chemotherapy.

If a patient is suspected to be pregnant, chemotherapy should be immediately discontinued. In addition a positive urine test must be confirmed by a serum pregnancy test. If it is confirmed that the patient is not pregnant, the patient may resume dosing.

If a female patient or female partner of a male patient becomes pregnant during therapy or within 2 months after the last dose of chemotherapy, the investigator must be notified in order to facilitate outcome follow-up.

5.8 Duration of Therapy

If at any time the constraints of this protocol are considered to be detrimental to the patient's health and/or the patient no longer wishes to continue protocol therapy, the protocol therapy should be discontinued and the reason(s) for discontinuation documented in the case report forms.

In the absence of treatment delays due to adverse events, treatment on Arm B with etoposide may continue for 2 years or until one of the following criteria applies:

- Documented and confirmed disease progression
- Death
- Adverse event(s) that, in the judgment of the investigator, may cause severe or permanent harm or which rule out continuation of study drug
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator
- Suspected pregnancy
- Serious non-compliance with the study protocol
- Lost to follow-up
- Patient withdraws consent
- Investigator removes the patient from study
- The Siteman Cancer Center decides to close the study

Patients who prematurely discontinue treatment for any reason will be followed as indicated in the study calendar.

5.9 Duration of Follow-up

Patients in Arm A will be followed every 12 weeks after the 6-week (or 10-week if done) post-MLA scan until 1 year have elapsed since patient registration or until death, whichever occurs first. Following the first year, patients will continue to be followed every 6 months or at event occurrence for 5 years after patient registration or until death, whichever occurs first. Patients in Arm B will be followed every 8 weeks after the 6-week (or 10-week if done) post-MLA scan until 2 years have elapsed since patient registration or until progression, whichever occurs first. Following the first two years, patients will continue to be followed every 6 months or at event occurrence for 5 years after patient registration or until death, whichever occurs first. Follow-up consists of collection of Karnofsky performance status, DSC/DCE-MRI, and blood for biomarkers until disease progression (or 2 years of elapsed since patient registration or death, whichever occurs first). After documented disease progression, vital status and subsequent treatment will be obtained every 12 weeks for the remainder of the 2 years or until death, whichever occurs first. Patients removed from study for unacceptable adverse events that are possibly, probably, or definitely related to study procedures will be followed until resolution or stabilization of the adverse event. "Stabilization" is defined as remaining at a consistent CTCAE version 4.0 grade of the event for two consecutive assessments.

6.0 DOSE DELAYS/DOSE MODIFICATIONS (ARM B)

The following algorithms are meant as guidelines only; adjustments to chemotherapy may be made at the discretion of the treating physician.

Renal Dysfunction:
CrCl 10 to 50 ml/min: administer 75% of etoposide dose
CrCl <10ml/min: administer 50% of etoposide dose

Congestive heart failure:
Given the low dose and short duration of doxorubicin treatment, we do not anticipate significant cardiac toxicity. However, if a prolongation of the QTc interval (>0.48 sec), a decrease in ejection fraction to <50%, or a decrease in left ventricular shortening fraction to <27%, doxorubicin should be omitted one week, any existing electrolyte or micronutrient deficiencies corrected, and the tests repeated. If the abnormalities persist, doxorubicin treatment should be permanently discontinued. Missed doses due to cardiac toxicity will not be made up.

Hematologic abnormalities:
Hold etoposide for ANC <500/mcl or platelet < 50 K/cumm. May restart when ANC ≥ 1000/mcl and platelet > 100 K/cumm. Etoposide may be restarted at full dose or reduced dose at the discretion of the treating physician. Missed doses will not be made up.

Baseline Permissible ANC	**Subsequent ANC**	**Growth Factor Support, Treatment Delay and Follow-up Labs**	**Dose Reduction of Doxorubicin Based on Rates of Abnormal Lab Recovery**
ANC ≥ 1000/mcl	ANC ≥ 1000/mcl	None	None
	ANC < 1000/mcl but > 500/mcl	G-CSF 5 mcg/kg SQ or IV qday x up to 5 days. Check CBC daily. When ANC ≥ 1000/mcl, restart treatment.	None if ANC recovers to ≥ 1000/mcl within 5 doses of G-CSF.
			Decrease to 20 mg/m^2 if ANC recovers to ≥ 1000/mcl after 5 doses of G-CSF.
			Decrease to 15 mg/m^2 if ANC takes > 14 days to become permissible.
	ANC < 500/mcl	G-CSF 5 mcg/kg SQ or IV qday x up to 5 days. Check CBC daily. When ANC ≥ 1000/mcl, restart treatment.	Decrease to 20 mg/m^2 if ANC recovers to ≥ 1000/mcl within 5 doses of G-CSF.
			Decrease to 15 mg/m2 if ANC recovers to ≥ 1000/mcl after 5 doses of G-CSF.
			Discontinue treatment if ANC takes > 21 days to become permissible.

Baseline Permissible Platelets	Subsequent Platelet count	Treatment Delay and Follow-up lab§	Dose Reduction of Doxorubicin Based on Rates of Abnormal Lab Recovery
Platelet ≥ 100 K/cumm	Plt ≥ 50 K/cumm	None	None
	Plt < 50 K/cumm but ≥ 10 K/cumm	Hold treatment. Check CBC twice a week. When plt ≥ 50 K/cumm, restart treatment. Transfuse as needed to keep plt ≥ 20 K/cumm	Decrease to 20 mg/m^2 if plt recovers to ≥ 50 K/cumm within 7 days.
			Decrease to 15 mg/m^2 if plt recovers to ≥ 50 K/cumm within 21 days.
			Discontinue treatment if plt takes > 21 days to recover to ≥ 50 K/cumm
	Plt ≤ 10 K/cumm	Hold treatment. Check CBC twice a week. Transfuse as needed to keep plt ≥ 20,000. When plt ≥ 100 K/cumm without transfusion, restart treatment.	Decrease to 15 mg/m^2 if plt recovers to ≥ 50 K/cumm within 21 days.
			Discontinue treatment if plt takes > 21 days to recover to ≥ 50 K/cumm

§ All follow-up labs for dose modifications are +/- 3 days.

Hyperbilirubinemia:

Direct Bilirubin:	2 – 2.99 mg/dL	Reduce etoposide and doxorubicin dose by 50%
	3 – 4.99 mg/dL	Reduce etoposide and doxorubicin dose by 75%
	≥ 5 mg/dL	Hold etoposide and doxorubicin

7.0 REGULATORY AND REPORTING REQUIREMENTS

7.1 Adverse Events (AEs)

Definition: any unfavorable medical occurrence in a human subject including any abnormal sign, symptom, or disease.

Grading: the descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for all toxicity reporting. A copy of the CTCAE version 4.0 can be downloaded from the CTEP website.

Attribution (relatedness), Expectedness, and Seriousness: the definitions for the terms listed that should be used are those provided by the Department of Health and Human Services' Office for Human Research Protections (OHRP). A copy of this guidance can be found on OHRP's website: http://www.hhs.gov/ohrp/policy/advevntguid.html

7.2 Unanticipated Problems

Definition:

- unexpected (in terms of nature, severity, or frequency) given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the subject population being studied;
- related or possibly related to participation in the research ("possibly related" means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
- suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

7.3 Noncompliance

Definition: failure to follow any applicable regulation or institutional policies that govern human subjects research or failure to follow the determinations of the IRB. Noncompliance may occur due to lack of knowledge or due to deliberate choice to ignore regulations, institutional policies, or determinations of the IRB.

7.4 Serious Noncompliance

Definition: noncompliance that materially increases risks, that results in substantial harm to subjects or others, or that materially compromises the rights or welfare of participants.

7.5 Protocol Exceptions

Definition: A planned deviation from the approved protocol that are under the research team's control. Exceptions apply only to a single participant or a singular situation.

Pre-approval of all protocol exceptions must be obtained prior to the event.

7.6 Reporting to the Human Research Protection Office (HRPO) and the Quality Assurance and Safety Monitoring Committee (QASMC) at Washington University

The PI is required to promptly notify the IRB of the following events:

- Any unanticipated problems involving risks to participants or others which occur at WU, any BJH or SLCH institution, or that impacts participants or the conduct of the study.
- Noncompliance with federal regulations or the requirements or determinations of the IRB.

- Receipt of new information that may impact the willingness of participants to participate or continue participation in the research study.

These events must be reported to the IRB within **10 working days** of the occurrence of the event or notification to the PI of the event. The death of a research participant that qualifies as a reportable event should be reported within **1 working day** of the occurrence of the event or notification to the PI of the event.

7.7 Timeframe for Reporting Required Events

Reportable adverse events will be tracked for 30 days following the last day of study treatment for patients in Arm B and for 30 days following the MLA for patients in Arm A. For patients in Arm A, adverse events (of any grade) considered possibly, probably, or definitely related to the MLA need be reported. Adverse events that are possibly, probably, or definitely related to study procedures will be followed until resolution or stabilization of the event. "Stabilization" is defined as remaining at a consistent CTCAE version 4.0 grade of the event for two consecutive assessments. For Arm B, all grade 3 and higher events regardless of attribution are to be reported.

8.0 PHARMACEUTICAL INFORMATION

8.1 Doxorubicin (Adriamycin)

8.1.1 Doxorubicin Description

Doxorubicin is a cytotoxic anthracycline antibiotic isolated from cultures of Streptomyces peucetius var. caesius. Doxorubicin consists of a naphthacenequinone nucleus linked through a glycosidic bond at ring atom 7 to amino sugar, daunosamine. Chemically, doxorubicin hydrochloride is (8S,10S)-10-[(3-Amino-2,3,6-tirdeoxy-a-L-lyxo-hexopyranosyl)-oxy]-8-glycoloyl-7,8,9,10-tetrahydro-6,8,11-trihydroxy-1-methoxy-5,12-naphthacenedione hydrochloride.

Chemical name: $C_{27}H_{29}NO_{11}\bullet HCl$
Molecular weight: 579.99

8.1.2 Clinical Pharmacology

The cytotoxic effect of doxorubicin on malignant cells and its toxic effects on various organs are thought to be related to nucleotide base intercalation and cell membrane lipid binding activities of doxorubicin. Intercalation inhibits nucleotide replication and action of DNA and RNA polymerases. The interaction of doxorubicin with topoisomerase II to form DNA-cleavable complexes appears to be an important mechanism of doxorubicin cytocidal activity.

8.1.3 Pharmacokinetics and Drug Metabolism

Pharmacokinetic studies, determined in patients with various types of tumors undergoing either single or multi-agent therapy, have shown that doxorubicin follows a mulitphasic disposition after intravenous injection. The initial distributive half-life of approximately 5 minutes suggests rapid tissue uptake of doxorubicin, while its slow elimination from tissues is reflected by a terminal half-life of 20 to 48 hours.

8.1.4 Supplier(s)

Doxorubicin is commercially available.

8.1.5 Dosage Form and Preparation

Doxorubicin for Injection is supplied as a sterile red-orange lyophilized powder in single dose flip-top vials in the following package strengths: 10 mg vial, 20 mg vial, 50 mg vial.

8.1.6 Storage and Stability

Store unreconstituted vial at 20º to 25º C. Retain in carton until time of use. Discard unused portion.

After adding the diluent, the vial should be shaken and the contents allowed to dissolve. The reconstituted solution is stable for 7 days at room temperature and under normal room light and 15 days under refrigeration (2º to 8º C). It should be protected from exposure to sunlight.

8.1.7 Administration

When possible, to reduce the risk of developing cardiotoxicity in patients receiving doxorubicin after stopping treatment with other cardiotoxic agents, especially those with long half-lives such as trastuzumab, doxorubicin-based therapy should be delayed until the other agents have cleared from the circulation.

Care in the administration of doxorubicin will reduce the chance of perivenous infiltration. It may also decrease the chance of local reactions such as urticaria and erythematous streaking. On intravenous administration of doxorubicin, extravasation may occur with or without an accompanying burning or stinging sensation, even if blood returns well on aspiration of the infusion needle. If any signs or symptoms of extravasation have occurred, the injection or infusion should be immediately terminated and restarted in another vein.

Doxorubicin will be administered during this study at a dose of 25 mg/m^2 weekly for 6 weeks.

8.1.8 Special Handling Instructions

Caregivers should be counseled to take precautions (such as wearing latex gloves) to prevent contact with the patient's urine and other body fluids for at least 5 days after each treatment.

8.2 Etoposide (VP-16)

8.2.1 Clinical Pharmacology

A semisynthetic derivative of podophyllotoxin that forms a complex with topoisomerase II and DNA which results in single and double strand DNA breaks. Its main effect appears to be in the S and G2 phase of the cell cycle.

8.2.2 Pharmacokinetics and Drug Metabolism

The initial t½ is 1.5 hours and the mean terminal half-life is 4 to 11 hours. It is primarily excreted in the urine. In children, approximately 55% of the dose is excreted in the urine as etoposide in 24 hours. The mean renal clearance of etoposide is 7 to 10 mL/min/m² or about 35% of the total body clearance over a dose range of 80 to 600 mg/m². Etoposide, therefore, is cleared by both renal and non renal processes, i.e., metabolism and biliary excretion. The effect of renal disease on plasma etoposide clearance is not known. Biliary excretion appears to be a minor route of etoposide elimination. Only 6% or less of an intravenous dose is recovered in the bile as etoposide. Metabolism accounts for most of the non renal clearance of etoposide. The maximum plasma concentration and area under the concentration time curve (AUC) exhibit a high degree of patient variability. Etoposide is highly bound to plasma proteins (~94%), primarily serum albumin. Pharmacodynamic studies have shown that etoposide systemic exposure is related to toxicity. Preliminary data suggests that systemic exposure for unbound etoposide correlates better than total (bound and unbound) etoposide. There is poor diffusion into the CSF < 5%. Cmax and AUC values for orally administered etoposide capsules consistently fall in the same range as the Cmax and AUC values for an intravenous dose of one-half the size of the oral dose. The overall mean value of oral capsule bioavailability is approximately 50% (range 25-75%).

8.2.3 Supplier(s)

Etoposide is commercially available.

8.2.4 Dosage Form and Preparation

Gelcaps: Etoposide is available as 50 mg pink capsules. Each liquid filled, soft gelatin capsule contains 50 mg of Etoposide in a vehicle consisting of citric acid, glycerin, purified water, and polyethylene glycol 400. The soft gelatin capsules

contain gelatin, glycerin, sorbitol, purified water, and parabens (ethyl and propyl) with the following dye system: iron oxide (red) and titanium dioxide; the capsules are printed with edible ink.

Injection for oral use: The Injection may be used orally for those children too young or unable to swallow capsules:
Etoposide for Injection is available in sterile multiple dose vials. The pH of the clear, nearly colorless to yellow liquid is 3 to 4. Each mL contains 20 mg etoposide, 2 mg citric acid, 30mg benzyl alcohol, 80 mg modified polysorbate 80/tween 80, 650 mg polyethylene glycol 300, and 30.5 percent (v/v) alcohol. Vial headspace contains nitrogen.

8.2.5 Storage and Stability

Etoposide capsules must be stored under refrigeration (2°-8°C or 36°- 46°F).The capsules are stable until the expiration date on the package.

Etoposide phosphate must be stored under refrigeration 2°-8°C (36°-46°F).
Unopened vials of etoposide phosphate are stable until the expiration date on the package when stored at controlled room temperature (20˚-25˚C or 68˚-77˚F).

8.2.6 Administration

Gelcaps: Doses (up to 400 mg/day) can be given as a single once daily dose; doses >400 mg should be given in 2-4 divided doses. For the purposes of this protocol, dosing will be once daily as the 400 mg/day limit will not be exceeded.

Injection for oral use: For oral administration in children too young to take the capsules, the parenteral product can be used orally. A 1:1 dilution can be made by adding equal parts of normal saline and etoposide injection for a 10 mg/mL concentration which is stable for 22 days in Burron plastic oral syringes (polypropylene) either exposed or protected from light (McLeod HL, Relling MV. Stability of etoposide solution for oral use. Am J Hosp Pharm. 1992:49;2784-5). This dilution can be administered directly to be followed by sour candy or gum, or can be further diluted immediately prior to administration with fruit juice. Etoposide injection can be mixed in orange juice, apple juice, or lemonade. Etoposide injection diluted this way in fruit juice must be consumed immediately as it tends to precipitate.

Undiluted etoposide injection (20 mg/mL) has been tested and is stable in a plastic syringe for 5 days when stored at room temperature under fluorescent light.

When etoposide is stored in plastic, there is potential for cracking of rigid plastic containers and of leaching of diethylhexyl phthalate (DEHP) from polyvinyl chloride (PVC) containers. Based on information provided by Bristol-Meyers Squibb Division of Oncology, undiluted etoposide appears to crack syringes made

of an acrylic-based plastic, polycarbonates, polyethylene glycol 300 or ABS (polymer produced by combining acrylonitrile, butadiene and styrene). Storing etoposide 10 mg/mL in polypropylene syringes should not pose a cracking risk or result in leaching of DEHP.

Concentrations need to be 0.4 mg/mL or less to substantially enhance taste. Etoposide stays in solution at 0.4 mg/mL for up to 3 hours in one of the above juices. If storage is required add 1 mL of undiluted etoposide injection (20 mg/mL) or 2 mL of the 10 mg/mL dilution above to 50 mL- of juice (1 & 2/3 ounces) for a concentration of 0.4 mg/ml, which is stable for up to 3 hours but should be consumed as soon as possible after mixing.

8.2.7 Special Handling Instructions

Use proper chemotherapy handling techniques.

9.0 CORRELATIVE STUDIES

9.1 DCE and DSC-MRI

All patients will undergo DSC and DCE-MRI at the following time points:

- no more than 3 weeks prior to MLA (OPTIONAL)
- within approximately 4 days after MLA
- 2-4 weeks (+/- 3 days) after MLA
- Every 12 weeks (+/- 7 days) following the last MRI for the first year or until disease progression for Arm A; every 8 weeks (+/- 7 days) until 2 years have elapsed or until disease progression, whichever comes first, for Arm B.

9.2 Blood for Correlative Studies

Patients will have 7 mL blood for serum drawn at the following time points:

- before MLA; this sample can be collected any time during the 3 day before or the day of the procedure until the start of the procedure
- within approximately 3 days after MLA
- weeks 2-4 post-MLA (Arm A only, at time of DSC/DCE-MRI)
- weekly (+/- 3 days) prior to (on the same day as) doxorubicin (Arm B only)
- Every 12 weeks (+/- 7 days) for the first year or until disease progression for Arm A; every 8 weeks (+/- 7 days) until 2 years have elapsed or disease progression, whichever comes first, for Arm B. Blood draws will be drawn one same days as MRIs, prior to scan.

A minimum of 2 ml will be drawn and placed in a red top tube and a minimum of 5 ml will be collected into a green top (heparin) tube. Specimens should be immediately transported to the Tissue Procurement Core (TPC) for processing.

9.3 Blood for Serum Biomarkers of BBB Disruption

For protein-based serum biomarker assays 0.5 to 1 ml serum will be to be aliquoted and stored at -80ºC until used for ELISA analysis or for plasma microRNA-based assays in the Tran lab.

9.4 Blood for Phenotyping of Peripheral Blood Immune Cells

The remainder of serum will stored at -80ºC until used for the Meso Scale Discovery (MSD)-based cytokine quantitation, which will be performed in the Immune Monitoring Core of the Center for Human Immunology and Immunotherapy Programs. Serum will be used in the MSD immunoassays to assess immunoregulatory cytokine and chemokine profiles pre- and post-treatment over time.

Blood obtained and stored for comparison of immune profiles of peripheral blood cells before and after MLA, provided there is no evidence of significant treatment-induced cytopenias. Blood will be Ficoll separated and the mononuclear cell population (PBMC) isolated per standard protocol through the TPC [59, 60]. The PBMC isolated from blood obtained prior to MLA will be cryopreserved for later analysis. The isolated monocytes will be incubated with IL-4 and GM-CSF to produce dendritic cells (DC)[60] which will then be cryopreserved for analysis of tumor-specific functional activity of peripheral blood immune cells detailed in section 9.4 below. To determine the phenotype of peripheral blood immune cells, following the 4-week interval, cryopreserved PBMC will be thawed and stained with labeled antibodies to CD4, CD8, CD45, CCR7, CD27, CD28, and CD62, as well as to markers of T cell hypofunctionality (PD-1, CTLA-4, LAG-3, TIM3, and ICOS). Specifically, we will compare levels of $CD8^{+}PD\text{-}1^{+}LAG\text{-}3^{+}$ T cells between MLA-treated and untreated patients, as this cell population has been shown to mark tumor-specific $CD8^{+}$ T cells. Cells will be assessed by flow cytometry in the CHiiPs immune monitoring core.

Due to the limited blood volumes that may be available for some patients, our ability to conduct these cellular analyses may be limited. In this event, we will substitute quantitative PCR for lymphocyte cytokines—such as IFN-γ and TNF-α--from mRNA generated from PBMC to assess lymphocyte functional status.

9.5 OPTIONAL Biopsy for Tumor-Specific Functional Activity of Peripheral Blood Immune Cells

The patients in both arms may have a biopsy immediately before MLA. Specimens not used for histologic diagnosis will be cryopreserved in the tumor bank for later processing. Should our studies by either flow cytometry or qRT-PCR described in Section 9.4 show evidence of lymphocyte activation, then after PBMC samples have been obtained, we will test T cell reactivity in an in vitro stimulation assay. Dendritic cells matured from monocytes as described in Section 9.3 above will be incubated with freeze-thawed biopsy specimen lysate for 2 days and subsequently incubated with T cells purified from PBMC

using CD3/CD8-positive microbeads. Five days after stimulation, T cell reactivity will be assessed by ELISPOT assay for IFN-γ stimulation. This study will test whether MLA stimulates increased tumor-specific T cell recognition and activation. As in Section 9.4, the amount of blood drawn from some patients may be limited and could limit or ability to conduct these functional assays.

9.6 Quality of Life Assessments

Patients in both arms will undergo QOL assessments in the form of evaluation of Karnofsky/Lansky performance status at each physician visit and documented in the note.

10.0 STUDY CALENDAR

10.1 Arm A Study Calendar

	Baseline[1]	MLA	3 Days Post-MLA	2-4 Weeks Post-MLA	Every 12 Wks Thereafter for 12 months Post-MLA[4]	F/U[10]
Informed consent	X					
Medical history	X					
Physical exam incl. wt	X		X[3]	X	X[9]	X[11]
Karnofsky/Lansky	X		X	X	X[9]	X[11]
CBC	X					
CMP	X					
Urine or serum βHCG	X[7]					
MRI with DCE/DSC	X[8]		X[2]	X[2]	X[9]	X[11]
Biopsy		X[8]				
Blood to measure brain-specific factors (Sections 9.2-9.4)	X		X[2]	X[2]	X[9]	X[11]
Adverse event assessment[5]		X	X	X	X	X

1. No more than 21 days prior to MLA.
2. +/3 days
3. Weight not required
4. Patients will be followed every 12 weeks from the Week 4 MRI until 1year has elapsed since MLA or progression, whichever occurs first. After progression, vital status and subsequent treatment will be recorded.
5. Through one year or off protocol.
6. To be obtained with MRI.
7. Females of childbearing potential
8. Optional
9. +/- 7 days
10. F/U every 6 months or at event occurrence for 5 years or until death, whichever occurs first
11. +/- 2 weeks

10.2 Arm B Study Calendar

	B/L[1]	MLA	3 Days Post-MLA	Wk 1[4]	Wk 2[4]	Wk 3[4]	Wk 4[4]	Wk 5[4]	Wk 6[4]	Monthly[5]	F/U[10]
Informed consent	X										
Medical history	X									X	
Physical exam incl. wt	X			X	X	X	X	X	X	X	X[15]
Karnofsky/Lansky	X			X	X	X	X	X	X	X	X[15]
CBC	X			X	X	X	X	X	X	X	
CMP	X			X			X			X	
Urine or serum βHCG	X[12]										
Echocardiogram	X[2]										
MRI with DCE/DSC	X[14]		X		X[11]					X[5,6]	
Biopsy		X[14]									
Blood to measure brain-specific factors (Section 9.2-9.4)		X[3]	X	X[7]	X[7]	X[7]	X[7]	X[7]	X[7]	X[5,6]	
Doxorubicin[8]				X	X	X	X	X	X		
Etoposide[13]										X	
AE assessment[9]		X ---------- X									

1. No more than 21 days prior to MLA.
2. No more than 1 year prior to registration.
3. May be collected any time during the 3 days before or the day of the procedure until the start of the procedure.
4. +/- 3 days
5. +/- 7 days
6. Every 8 weeks for the first 2 years or until disease progression, whichever occurs first. To coincide with MRI. After progression, vital status and subsequent treatment will be recorded.
7. Same day as chemotherapy.
8. To begin approximately 7 days after MLA.
9. Through one year or off protocol
10. Patients will be followed every 6 months or at event occurrence for 5 years or until death, whichever occurs first.
11. To be obtained 2-4 weeks post-MLA
12. Females of childbearing potential
13. To be started at time of count recovery after the 6 weeks of treatment with doxorubicin has concluded.
14. Optional
15. +/- 2 weeks

11.0 DATA SUBMISSION SCHEDULE

Case report forms with appropriate source documentation will be completed according to the schedule listed in this section.

Case Report Form	Submission Schedule
Original Consent Form	Prior to registration
On-Study Form	Prior to starting treatment
MLA Form	After procedure
Biopsy Form	After biopsy
QOL Form (documentation of performance status)	Weeks 1, 3, and 5 of doxorubicin With each cycle of etoposide Every 8 weeks for one year post-MLA
Doxorubicin Form (Arm B)	At the end of treatment with doxorubicin
Etoposide Form (Arm B)	At the end of each cycle of etoposide
Biomarkers Form	Refer to Section 9.2
Adverse Events Form	Continuous through 30 days after MLA (Arm A) Through 30 days after last dose of chemotherapy (Arm B)
Treatment Summary Form (Arm B)	Completion of treatment
Follow Up Form	Arm A: Every 12 weeks for one year post-MLA Arm B: Every 8 weeks for one year post-MLA
Tumor Response Form	With each MRI
AE Reporting Form	See Section 7.0 for reporting requirements

12.0 MEASUREMENT OF EFFECT

12.1 Antitumor Effect – Solid Tumors

For the purposes of this study, patients should be re-evaluated for response every 12 weeks on Arm A and every 8 weeks on Arm B. The DCE/DSC-MRI scan obtained within approximately 3 days after MLA will be used as the baseline scan for determining response. The DCE/DSC-MRI scans obtained at pre-MLA, post-MLA, and prior to 6 weeks post-MLA will not be assessed for disease response.

Response and progression will be evaluated in this study using the updated response assessment criteria for high-grade gliomas: Response Assessment in Neuro-Oncology (RANO) working group guideline [JCO 28(11): 1963-1972, 2010].

Criteria for Determining First Progression Depending on Time From Initial Chemoradiotherapy

First Progression	Definition
Progressive disease < 12 weeks after completion of chemoradiotherapy	Progression can only be defined using diagnostic imaging if there is new enhancement outside of the radiation field (beyond the high-dose region or 80% isodose line) or if there is unequivocal evidence of viable tumor on histopathologic sampling (eg, solid tumor areas [ie, > 70% tumor cell nuclei in areas], high or progressive increase in MIB-1 proliferation index compared with prior biopsy, or evidence for histologic progression or increased anaplasia in tumor). Note: Given the difficulty of differentiating true progression from pseudoprogression, clinical decline alone, in the absence of radiographic or histologic confirmation of progression, will not be sufficient for definition of progressive disease in the first 12 weeks after completion of concurrent chemoradiotherapy.
Progressive disease ≥ 12 weeks after chemoradiotherapy completion	1. New contrast-enhancing lesion outside of radiation field on decreasing, stable, or increasing doses of corticosteroids. 2. Increase by ≥ 25% in the sum of the products of perpendicular diameters between the first postradiotherapy scan, or a subsequent scan with smaller tumor size, and the scan at 12 weeks or later on stable or increasing doses of corticosteroids. 3. Clinical deterioration not attributable to concurrent medication or comorbid conditions is sufficient to declare progression on current treatment but not for entry onto a clinical trial for recurrence. 4. For patients receiving antiangiogenic therapy, significant increase in T2/FLAIR nonenhancing lesion may also be considered progressive disease. The increased T2/FLAIR must have occurred with the patient on stable or increasing doses of corticosteroids compared with baseline scan or best response after initiation of therapy and not be a result of comorbid events (eg, effects of radiation therapy, demyelination, ischemic injury, infection, seizures, postoperative changes, or other treatment effects).

Criteria for Response Assessment Incorporating MRI and Clinical Factors (Adapted from JCO 2010)

Response	Criteria
Complete response	• Requires all of the following: complete disappearance of all enhancing measurable and nonmeasurable disease sustained for at least 4 weeks. • No new lesions; stable or improved nonenhancing (T2/FLAIR) lesions. • Patients must be off corticosteroids (or on physiologic replacement doses only) and stable or improved clinically. Note: Patients with nonmeasurable disease only cannot have a complete response; the best response possible is stable disease.
Partial response	Requires all of the following: • ≥ 50% decrease compared with baseline in the sum of products of perpendicular diameters of all measurable enhancing lesions sustained for at least 4 weeks. • No progression of nonmeasurable disease. • Any new measureable lesion within the 3 cm radius of the rim of the MLA-treated recurrent tumor. † • Stable or improved nonenhancing (T2/FLAIR) lesions on same or lower dose of corticosteroids compared with baseline scan; the corticosteroid dose at the time of the scan evaluation should be no greater than the dose at time of baseline scan. • Stable or improved clinically. Note: Patients with nonmeasurable disease only cannot have a partial response; the best response possible is stable disease.
Stable disease	Requires all of the following: • Does not qualify for complete response, partial response, or progression. • Stable nonenhancing (T2/FLAIR) lesions on same or lower dose of corticosteroids compared with baseline scan. In the event that the corticosteroid dose was increased for new symptoms and signs without confirmation of disease progression on neuroimaging, and subsequent follow-up imaging shows that this increase in corticosteroids was required because of disease progression, the last scan considered to show stable disease will be the scan obtained when the corticosteroid dose was equivalent to the baseline dose.
Progression	Defined by any of the following: • ≥ 25% increase in sum of the products of perpendicular diameters of enhancing lesions compared with the smallest tumor measurement obtained either at baseline (if no decrease) or best response, on stable or increasing doses of corticosteroids*. • Significant increase in T2/FLAIR nonenhancing lesion on stable or increasing doses of corticosteroids compared with baseline scan or best response after initiation of therapy* not caused by comorbid events (e.g.

Response	Criteria
	radiation therapy, demyelination, ischemic injury, infection, seizures, postoperative changes, or other treatment effects). • Any new measureable lesion within the 3 cm radius of the rim of the MLA-treated recurrent tumor. † • Clear clinical deterioration not attributable to other causes apart from the tumor (e.g. seizures, medication adverse effects, complications of therapy, cerebrovascular events, infection, and so on) or changes in corticosteroid dose. • Failure to return for evaluation as a result of death or deteriorating condition; or clear progression of nonmeasurable disease.

- NOTE. All measurable and nonmeasurable lesions must be assessed using the same techniques as at baseline.
- Abbreviations: MRI, magnetic resonance imaging; FLAIR, fluid-attenuated inversion recovery.
- * Stable doses of corticosteroids include patients not on corticosteroids.
- † If any new measurable lesion is outside the 3 cm radius of the rim of the MLA-treated recurrent tumor or is located in the contralateral hemisphere regardless of its distance from the rim of the MLA-treated tumor AND there is not any measurable lesion within the 3 cm radius, the patient will be considered to have progressive disease but will not be considered evaluable for PFS in this study, in which only local disease control or failure is measured.

12.2 Disease Parameters

Measurable disease: Bi-dimensionally measurable lesions with clearly defined margins by MRI scan. All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

Non-measurable disease: Uni-dimensionally measurable lesions or lesions with margins not clearly defined such as areas of T2/FLAIR signal abnormality or poorly defined enhancing abnormality.

Note: For cystic lesions, the only measurable part is any enhancement area around the cyst that is clearly defined and bi-dimensionally measurable. The cyst itself should not be considered measurable or non-measureable disease.

Target lesions: All measurable lesions that are residual of the lesion treated with MLA or that are located within the 3 cm radius of the rim of the MLA-treated recurrent tumor should be identified as target lesions and recorded and measured. Target lesions should be selected on the basis of their size (lesions with the longest diameter), but in addition should be those that lend themselves to reproducible repeated measurements. It may be the case that, on occasion, the largest lesion does not lend itself to reproducible measurement in which circumstance the next largest lesion, which can be measured reproducibly should be selected. When there are too many measurable lesions, choose the largest 3 lesions as target lesions to follow. The other measurable lesions should be considered evaluable for

the purpose of objective status determination.

Non-target lesions: All non-measurable lesions should be identified as non-target lesions and should also be recorded at baseline. Measurements of these lesions are not required, but the presence, absence, or in rare cases unequivocal progression of each should be noted throughout follow-up.

12.3 Methods for Evaluation of Measurable Disease

All measurements should be taken and recorded in metric notation using a ruler. All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than 2 weeks before the beginning of the treatment.

Clinical lesions: Clinical lesions will only be considered measurable on brain MRI when they are ≥ 5 mm diameter as assessed using a ruler.

Histology: This technique can be used to differentiate between partial responses (PR) and complete responses (CR) in rare cases when biopsy or surgical resection of a measureable lesion is clinically indicated.

Perfusion/CBV: This advanced brain MRI technique can be used as an adjunct test to determine treatment response or disease status. However, it should not be used as the primary or sole method to determine response or disease status.

Brain FDG-PET coupled with head CT or brain MRI: This advanced metabolic imaging technique can be used as an adjunct test to determine response or disease status. However it should be used as the primary or sole method of determining response or disease status.

12.3.1 Evaluation of Target Lesions

Complete Response (CR): Disappearance of all target lesions.

Partial Response (PR): ≥ 50% decrease compared with baseline in the sum of products of perpendicular diameters of all target lesions sustained for at least 4 weeks.

Progressive Disease (PD): At least a 25% increase in the sum of products of perpendicular diameters of at least 1 target lesion, taking as reference the smallest sum of products of perpendicular diameters on study (this includes the baseline sum if that is the smallest on study).

Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum of products of perpendicular diameters while on study.

12.3.2 Evaluation of Non-Target Lesions

Complete Response (CR): Disappearance of all non-target lesions.

Non-CR/Non-PD: Persistence of one or more non-target lesion(s).

Progressive Disease (PD): Appearance of one or more new lesions and/or unequivocal progression of existing non-target lesions on stable or increasing doses of corticosteroids compared with baseline scan or best response after initiation of therapy* not caused by comorbid events (e.g. radiation therapy, demyelination, ischemic injury, infection, seizures, postoperative changes, or other treatment effects). Unequivocal progression should not normally trump target lesion status. It must be representative of overall disease status change, not a single lesion increase.

Although a clear progression of "non-target" lesions only is exceptional, the opinion of the treating physician should prevail in such circumstances, and the progression status should be confirmed at a later time by the review panel (or Principal Investigator).

12.3.3 Evaluation of Best Overall Response

The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence (taking as reference for progressive disease the smallest measurements recorded since the treatment started). The patient's best response assignment will depend on the achievement of both measurement and confirmation criteria.

Summary of the RANO Response Criteria (Adapted from JCO 2010)

Criterion	CR	PR	SD	PD
T1 gadolinium enhancing disease	None	≥ 50% ↓	< 50% ↓ but < 25% ↑	≥ 25% ↑*
T2/FLAIR	Stable or ↓	Stable or ↓	Stable or ↓	↑*
New lesion	None	None	None	Present*
Corticosteroids	None	Stable or ↓	Stable or ↓	NA†
Clinical status	Stable or ↑	Stable or ↑	Stable or ↑	↓*
Requirement for response	All	All	All	Any*

Abbreviations: RANO, Response Assessment in Neuro-Oncology; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; FLAIR, fluid-attenuated inversion recovery; NA, not applicable.

* Progression occurs when this criterion is present.

† Increase in corticosteroids alone will not be taken into account in determining progression in the absence of persistent clinical deterioration.

12.3.4 Duration of Response

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

The duration of overall CR is measured from the time measurement criteria are first met for CR until the first date that progressive disease is objectively documented.

Duration of stable disease: Stable disease is measured from the start of the treatment until the criteria for progression are met, taking as reference the smallest measurements recorded since the treatment started, including the baseline measurements.

12.3.5 Neurological Exam and Performance Status

Patients will be graded using the Karnofsky Performance Status scale and their neurological function evaluated as improved, stable or deteriorated in addition to objective measurement of tumor size. These parameters will be used to determine the overall response assessment.

12.3.6 Progression-Free Survival

PFS is defined as the duration of time from start of treatment to time of progression or death, whichever occurs first.

13.0 DATA AND SAFETY MONITORING

In compliance with the Washington University Institutional Data and Safety Monitoring Plan, the Principal Investigator will provide a Data and Safety Monitoring (DSM) report to the Washington University Quality Assurance and Safety Monitoring Committee (QASMC) semi-annually beginning six months after accrual has opened (if at least five patients have been enrolled) or one year after accrual has opened (if fewer than five patients have been enrolled at the six-month mark).

The Principal Investigator will review all patient data at least every six months, and provide a semi-annual report to the QASMC. This report will include:

- HRPO protocol number, protocol title, Principal Investigator name, data coordinator name, regulatory coordinator name, and statistician
- Date of initial HRPO approval, date of most recent consent HRPO approval/revision, date of HRPO expiration, date of most recent QA audit, study status, and phase of study
- History of study including summary of substantive amendments; summary of accrual suspensions including start/stop dates and reason; and summary of protocol exceptions, error, or breach of confidentiality including start/stop dates and reason

- Study-wide target accrual and study-wide actual accrual
- Protocol activation date
- Average rate of accrual observed in year 1, year 2, and subsequent years
- Expected accrual end date and accrual by arm
- Objectives of protocol with supporting data and list the number of participants who have met each objective
- Measures of efficacy
- Early stopping rules with supporting data and list the number of participants who have met the early stopping rules
- Summary of toxicities separated by arm
- Abstract submissions/publications
- Summary of any recent literature that may affect the safety or ethics of the study

The study principal investigator and Research Patient Coordinator will monitor for serious toxicities on an ongoing basis. Once the principal investigator or Research Patient Coordinator becomes aware of an adverse event, the AE will be reported to the HRPO and QASMC according to institutional guidelines.

14.0 STATISTICAL CONSIDERATIONS

Since we do not have any preliminary data for MR imaging, serum biomarkers, etc, it is not feasible to estimate sample size or provide power estimates. Therefore, this study is considered as a pilot study and the data collected will be used to design future studies. We propose six patients each arm as this is considered to be reasonable to recruit within the available a time frame.

In each arm, we will consider the following statistical analysis:

For the primary endpoint, MR imaging will be collected at the time points: pre-MLA (OPTIONAL), post-MLA, two-four weeks after MLA and every twelve weeks in Arm A; pre-MLA, post-MLA, two-four weeks after MLA and every eight weeks in Arm B. The linear regression model will used to investigate the correlation between MR imaging and peritumoral BBB disruption. To account for correlation among the repeated measures from the same patient, the longitudinal data will be analyzed with the use of linear generalized estimating equation (GEE). Whether the average measurements differ at the multiple time points will be evaluated through GEE model. Least-square means at each time points will be presented and standard errors will be calculated within the use of the GEE sandwich method when accounting for within-patient correlation.

Since we do not know which biomarkers will have better correlation with the Ktrans data from DCE and DSC-MRI and patients' survival outcome, we plan to determine the levels of all 3 biomarkers in a blinded fashion. Once both the Ktrans and biomarker levels are available, we will determine which biomarkers have the closest correlation that is statistically significant with the Ktrans. Pearson correlation coefficient (r) will be determined for each biomarker and Ktrans value. Biomarkers with higher correlation coefficient (r approaching 1) will be given higher priority. A minimum r=0.5 is required for inclusion for further analysis and will be used as a peritumoral

permeability score. This score will then be correlated with the patient outcome data (as measured by 6PFS rate) to determine whether it has a predictive value.

PFS and OS will be estimated by Kaplan-Meier product limit method. The progression-free survival and overall survival probabilities at certain time points (e.g. 3 months, 6 months) will be presented as well. The difference in OS between Arm A and the historical control of bevacizumab alone will be compared by log-rank test. The quality of life (QOL) using Karnofsky or Lansky performance status will be characterized by summary statistics (mean, standard deviation and range).

The tumor-specific immune responses are collected at pre and post-MLA treatment. Paired t-test will be used to determine the effects of MLA treatment on patient's tumor-specific immune responses. Cox proportional hazard model will be used to determine the relationship between the duration of MLA-induced BBB disruption and PFS.

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APPENDIX 1: Karnofsky and Lansky Performance Status Scale

Karnofsky		Lansky	
Score	Description	Score	Description
100	Normal, no complaints, no evidence of disease	100	Fully active, normal.
90	Able to carry on normal activity, minor signs or symptoms of disease.	90	Minor restrictions in physically strenuous activity.
80	Normal activity with effort; some signs or symptoms of disease.	80	Active, but tires more quickly
70	Cares for self, unable to carry on normal activity or do active work.	70	Both greater restriction of and less time spent in play activity.
60	Required occasional assistance, but is able to care for most of his/her needs.	60	Up and around, but minimal active play; keeps busy with quieter activities.
50	Requires considerable assistance and frequent medical care.	50	Gets dressed, but lies around much of the day; no active play, able to participate in all quiet play and activities.
40	Disabled, requires special care and assistance.	40	Mostly in bed; participates in quiet activities.
30	Severely disabled, hospitalization indicated. Death not imminent.	30	In bed; needs assistance even for quiet play.
20	Very sick, hospitalization indicated. Death not imminent.	20	Often sleeping; play entirely limited to very passive activities.
10	Moribund, fatal processes progressing rapidly.	10	No play; does not get out of bed.

APPENDIX 2: PATIENT'S MEDICATION DIARY

Today's Date: _____ Agent: Etoposide Cycle: _____

Patient Name: _____ Study ID#: _____

INSTRUCTIONS TO THE PATIENT:

1. Complete one form for each cycle. Take _____mg (___capsules) of etoposide as directed by your study doctor.
2. Record the date, the number of capsules taken, and when you took them.
3. If you forget to take it before bedtime, then do not take a dose that day. Restart taking it the next day.
4. If you have any questions or notice any side effects, please record them in the comments section. Record the time if you should vomit.
5. Please return the forms to your physician or your study coordinator when you go to your next appointment. Please bring your unused study medications and/or empty bottles with you to each clinic visit so that a pill count can be done.

Day	Date	What time was dose taken?	# of capsules taken	Comments
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2				
3				
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5				
6				
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9				
10				
11				
12				
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