TITLE: A PHASE I STUDY OF SAFETY AND TOLERABILITY OF ACETAZOLAMIDE WITH TEMOZOLOMIDE IN ADULTS WITH NEWLY DIAGNOSED MGMT PROMOTER-METHYLATED MALIGNANT GLIOMA

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Intervention: Oral acetazolamide

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This study is being conducted by institutional members of the Personalized Cancer Care Consortium (PCCC), as well as additional sites.

STUDY SUMMARY

SCHEMA

Patient population:

- Adults (≥18 y/o) with histologically diagnosed *MGMT* promoter methylated grade III or IV astrocytoma
- Newly diagnosed tumors
- Karnofsky performance status ≥60
- Standard backbone therapy includes temozolomide (TMZ) and radiation therapy (RT)

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Treatment:

- 24 patients will be enrolled (12 + 12).
- The study will be discontinued if there are 4 regimen limiting toxicity (RLT) events in the first 12 patients or 8 in 24 patients.
- Acetazolamide (ACZ) will be administered twice daily following concomitant TMZ/IR, starting on the day of adjuvant TMZ initiation for the length of TMZ treatment
- For all patients, ACZ will be initiated at 250 mg BID and after 1 week escalated to 500 mg BID.
- During this period TMZ is given on days 1-5, of a 28 day cycle, and ACZ will be given on days 1-21.
- Subjects will receive a total of 6 adjuvant TMZ cycles if not limited by either tumor progression or RLT.

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Follow-up:

- Clinical follow up to assess toxicity and disease control during each treatment cycle and q2 months thereafter until progression
- MRI brain q2 months

Required Sample Size: 24 patients

Study Center: The University of Chicago Medical Center, NorthShore University HealthSystem, Northwestern University, Northwestern Medicine Regional Medical Group Warrenville and University of Illinois Chicago Medical Center, Rush University Medical center, the Illinois Cancer Care (Peoria) and the Decatur Memorial Hospital.

Concept and Rationale: Malignant astrocytomas are a diverse group of tumors that encompass WHO grades III, anaplastic astrocytoma (AA), and WHO grade IV, glioblastoma (GBM), tumors. Alkylating chemotherapy is central to the management of these tumors and temozolomide (TMZ) is now the most commonly used chemotherapeutic [1]. Standard treatment for newly diagnosed malignant astrocytoma uses combined TMZ and radiation therapy (RT). Despite its routine use, many patients experience minimal benefit from the addition of TMZ, such as patients with tumors that have high O^6-methylguanine-DNA methyltransferase (MGMT) expression or low levels of *MGMT* promoter methylation [2]. Given that no new chemotherapeutics have been approved for GBM in over a decade, identification of strategies to enhance the efficacy of TMZ is important. In preliminary work, we identified the proto-oncogene, Bcl-3, as

a biomarker in glioma that can predict response to TMZ. In examining the mechanism by which Bcl-3 promotes resistance to therapy, we identified carbonic anhydrase II (CAII) as a unique factor that is both Bcl-3-dependent and induced by TMZ. CAII is potently inhibited by the oral CA inhibitor, acetazolamide (ACZ, Diamox), an FDA-approved agent used for a variety of medical conditions seen in patients with glioma including epilepsy and raised intracranial pressure. Importantly, using patient-derived GBM cells and xenografts we find that ACZ sensitizes GBM to TMZ. Specifically, our pre-clinical studies show that daily ACZ given in combination with TMZ and extended for 21 days after TMZ initiation significantly prolongs survival of animals bearing GBM xenografts compared to TMZ alone. Moreover, the pre-clinical data indicate that ACZ is specifically effective in tumors that have low MGMT expression.

Based on the above, we hypothesize that ACZ can be safely administered concomitantly with TMZ in patients with malignant astrocytoma and that this regimen improves patient survival without increasing toxicity to an unacceptable level. Information gathered from this study will contribute towards the development of a future larger phase II study in these tumors.

Primary Objective: To determine the safety, tolerability and adverse event profile of adding acetazolamide to temozolomide in patients with newly diagnosed malignant astrocytoma.

Secondary Objectives:

1. To describe objective response rate (ORR), progression free survival (PFS) and overall survival (OS).
2. To determine the feasibility of cooperative interaction between The University of Chicago Medical Center, NorthShore University HealthSystem, Northwestern University, Northwestern Medicine Regional Medical Group Warrenville, University of Illinois Chicago Medical Center, Rush University Medical center, the Illinois Cancer Care (Peoria) and the Decatur Memorial Hospital.
3. To evaluate Bcl-3 expression level within each tumor and preliminarily examine the ability of Bcl-3 to predict response to TMZ and the efficacy of adding ACZ.

Study Design: This is a Phase I study that examines the rate of regimen limiting toxicity (RLT, see below for definition) in patients with malignant astrocytoma treated with combination ACZ and TMZ. Eligible patients must have histologically proven newly diagnosed, *MGMT* promoter methylated WHO grade III or IV astrocytoma and be planning to undergo treatment with standard adjuvant TMZ (after completing concomitant TMZ/IR). During this adjuvant phase, patients will receive daily oral ACZ with TMZ. ACZ will be initiated at 250 mg twice a day (BID) and then escalated to 500 mg BID after one week. During each cycle, ACZ will be started on the day of TMZ initiation and continued for a total of 21 days.

Any RLT will be attributed to the combination regimen of ACZ and TMZ. RLT will be used because any toxicity that stops the backbone regimen of TMZ is considered detrimental for the patient.

This is not a dose escalation trial to determine the maximum tolerable dose (MTD), but rather a study to determine whether a specific regimen consisting of TMZ combined with ACZ at 250 mg BID escalated after 1 week (within patient) to 500 mg BID is safe and tolerable.

Methods:

Definition of primary outcome/endpoint: RLT will be determined on a case-by-case basis by the study group taking into consideration the timing and nature of the toxicity. RLT will be defined as any of the following if they occur within 35 days of treatment initiation.

- Any grade 4 non-hematological toxicity that is treatment-related with the exception of alopecia, nausea and vomiting.
- Any grade 3 non-hematological toxicity that is treatment related that results in delay of backbone regimen (TMZ) by greater than 4 weeks.
- Grade 4 thrombocytopenia (<25,000/ mm^3) that results in a delay of backbone chemotherapy for greater than 4 weeks.

- Grade 4 (<500/ mm^3) neutropenia lasting more than 7 days, or Grade 3 (<1000/ mm^3) febrile neutropenia.

- Any delay in starting the next cycle by more than 4 weeks.

Toxicities will be graded as per Common Terminology Criteria for Adverse Events v4.03 (CTCAE) criteria.

Definition of secondary outcomes/endpoints: ORR will be determined at 6 months and is based on the change in tumor size (as determined by Response Assessment in Neuro-Oncology Criteria (RANO) criteria [3]) at the indicated time relative to the pre-treatment scan (i.e. the scan performed after the concomitant TMZ/IR phase). RANO criteria will also be used to define disease status (CR, PR, etc.). PFS will be defined as progression and OS will be based on death from any cause. Secondary endpoints will be stratified by *IDH* mutation status. Bcl-3 expression will be determined by an independent neuro-pathologist following immunohistochemical analysis of formalin fixed paraffin embedded (FFPE) surgical specimens.

Analytic plan for primary objective: Adverse events will be determined at each follow up visit and the rate of RLT recorded. Freedom from RLT will be determined using the Kaplan-Meier method.

Analytic plan for secondary objectives: ORR and PFS will be estimated at 6 months. An exact two-sided 90% confidence interval will be determined for ORR using the binomial distribution. PFS and OS will be determined using the Kaplan-Meier method. Bcl-3 expression level will be graded on a 4-tier scale that will then be simplified into a binary grade (high or low). The staining grade will be related to ORR, PFS and OS using Fisher's exact test and Cox regression modeling.

Sample size justification: The sample size of 24 patients was determined based on a target RLT rate of < 33%. An initial cohort of 12 patients will be enrolled and if fewer than 4 patients (<33%) have RLT, an additional 12 patients will be recruited. The regimen will be considered tolerable if less than 8 of 24 experience RLT.

TABLE OF CONTENTS

1. OBJECTIVES

1.1. Primary Objective

To determine the safety, tolerability and adverse event profile of adding acetazolamide to temozolomide in patients with newly diagnosed malignant astrocytoma.

1.2. Secondary Objectives

1.2.1 To describe the objective response rate (ORR), progression free survival (PFS) and overall survival (OS).

1.2.2 To determine the feasibility of the cooperative interaction between BrainUp, the University of Chicago Medical Center, NorthShore University HealthSystem, Northwestern University, Northwestern Medicine Regional Medical Group Warrenville, University of Illinois Chicago Medical Center, Rush University Medical center, the Illinois Cancer Care (Peoria) and the Decatur Memorial Hospital.

1.2.3 To evaluate Bcl-3 expression level within each tumor and to preliminarily examine the ability of Bcl-3 to predict response to TMZ and the efficacy of adding ACZ.

2. INTRODUCTION

2.1 Disease Background

Malignant astrocytoma, comprised of World Health Organization (WHO) grade III and IV gliomas, account for almost 85% of newly diagnosed malignant brain tumors annually. Glioblastoma (GBM, WHO grade IV tumor) is the most common primary malignant brain tumor. Current standard treatment for malignant astrocytoma involves maximal surgical debulking followed by concomitant radiation therapy (RT) and the alkylating agent, temozolomide (TMZ), followed by at least 6-12 months of adjuvant TMZ [4]. Despite the virtually universal use of TMZ, significant numbers of GBM patients have minimal benefit from its use [2]. In addition, as with any alkylator, TMZ not only has significant toxicity but can also induce cellular hypermutation that can be detrimental to overall patient outcome [5].

TMZ is a cornerstone of the multimodal management of malignant gliomas and, given that no new chemotherapeutics have been approved for the treatment of malignant astrocytoma in over a decade, strategies that enhance the efficacy of TMZ can significantly improve overall patient prognosis.

One potential strategy to improve treatment response and patient outcome is through identification of biomarkers. While *prognostic* biomarkers are important in understanding the intrinsic nature of tumors, a *predictive* biomarker is more therapeutically relevant as it can reveal the response to a specific treatment. In addition to providing information on patient outcome, biomarkers can also identify novel mechanisms to improve treatment efficacy.

Currently, there are three established molecular biomarkers in glioma: O^6-methylguanine-DNA methyltransferase (*MGMT*) promoter methylation, chromosome 1p/19q co-deletion (del) and isocitrate dehydrogenase 1/2 (*IDH*) mutation. Of the multiple genetic changes in diffuse gliomas, *IDH* mutation is now known to be a defining early feature [6]. While *IDH* mutation status provides mainly prognostic data [7, 8], 1p/19q del is only informative in oligodendroglioma tumors [9, 10]. On the other hand, *MGMT* methylation status has been shown to be both prognostic and predictive in malignant glioma [11, 12]. Despite the ability of *MGMT* promoter methylation to predict response to TMZ, targeting MGMT has not proved to

be a clinically successful strategy [13]. MGMT is particularly important in the setting of alkylating chemotherapy because it is the first line of resistance as this enzyme removes the cytotoxic alkyl adducts that mediate the therapeutic effect. In this regard, patients with a methylated *MGMT* promoter have low MGMT expression and consequently respond better to alkylators like TMZ.

Over the past decade, large-scale systemic analysis has revealed the identity of several novel drivers of glioma formation and identified specific glioma sub-groups that have significantly different clinical behavior and response to therapy [14, 15]. In addition, these systemic studies have uncovered numerous molecular changes whose significance is unknown. This molecular data represent an invaluable resource that can be interrogated to identify potential novel biomarkers in glioma.

2.2 Preclinical Studies

2.2.1 Bcl-3 predicts response to TMZ in GBM

In preliminary studies, we have determined that the oncoprotein Bcl-3, a nuclear protein that modulates NF-κB signaling [16], is a molecular biomarker in GBM whose expression level correlates with patient outcome specifically in patients with a methylated *MGMT* promoter. Initially, we identified Bcl-3 as a factor that modulates signaling in response to TMZ, and subsequently demonstrated that Bcl-3 attenuates cytotoxicity by TMZ in GBM (data not shown).

Having identified the potential of Bcl-3 to attenuate the anti-glioma effect of TMZ, we examined whether Bcl-3 expression is also relevant to patient outcome using publically available GBM databases. In multiple independent mRNA expression datasets, GBM patients with high *BCL3* expression have significantly worse outcome than those with low expression (Figure 1A). Importantly, *BCL3* expression level is significant even on multivariate analysis taking the primary prognostic factors into consideration including: patient age, Karnofsky performance, *IDH1* mutation, *MGMT* promoter methylation, surgery and radiation therapy ($P < 0.001$). Remarkably, while *BCL3* level has no intrinsic prognostic ability (i.e. is unable to separate untreated patients into survival groups) its expression level identifies distinct survival groups in patients who have been treated with TMZ (Figure 1B, similar results obtained using the REMBRANDT database). Notably, this effect is not seen in patients who receive RT alone without TMZ (Figure 1B). Given the importance of genetic factors to glioma biology and classification, we also examined a pan-glioma dataset separated by *IDH* status. *BCL3* level is a significant prognostic factor in both *IDH*-wt *and IDH*-mt tumors (data not shown).

Based on the above, and as immunohistochemical (IHC) staining of surgical specimens is a practical and virtually universally available method to look at expression, we examined whether Bcl-3 protein level is also relevant to glioma patient outcome. 86 independently verified GBM cases were obtained and tissue microarrays (TMAs) constructed. IHC staining for Bcl-3 was performed and graded on a four-tier system (0,1, 2 and 3) that was subsequently converted into a binary grade (0 and 1 = Low; 2 and 3= High) (Figure 1D). Consistent with the mRNA data, patients with high Bcl-3 staining level have significantly worse survival than those with low staining, a finding independent of patient age. These data indicate that Bcl-3 is informative even at the protein level and suggest that Bcl-3 can be rapidly assessed by IHC for practical analysis. In sum, these findings demonstrate that Bcl-3 is a predictive biomarker in GBM.

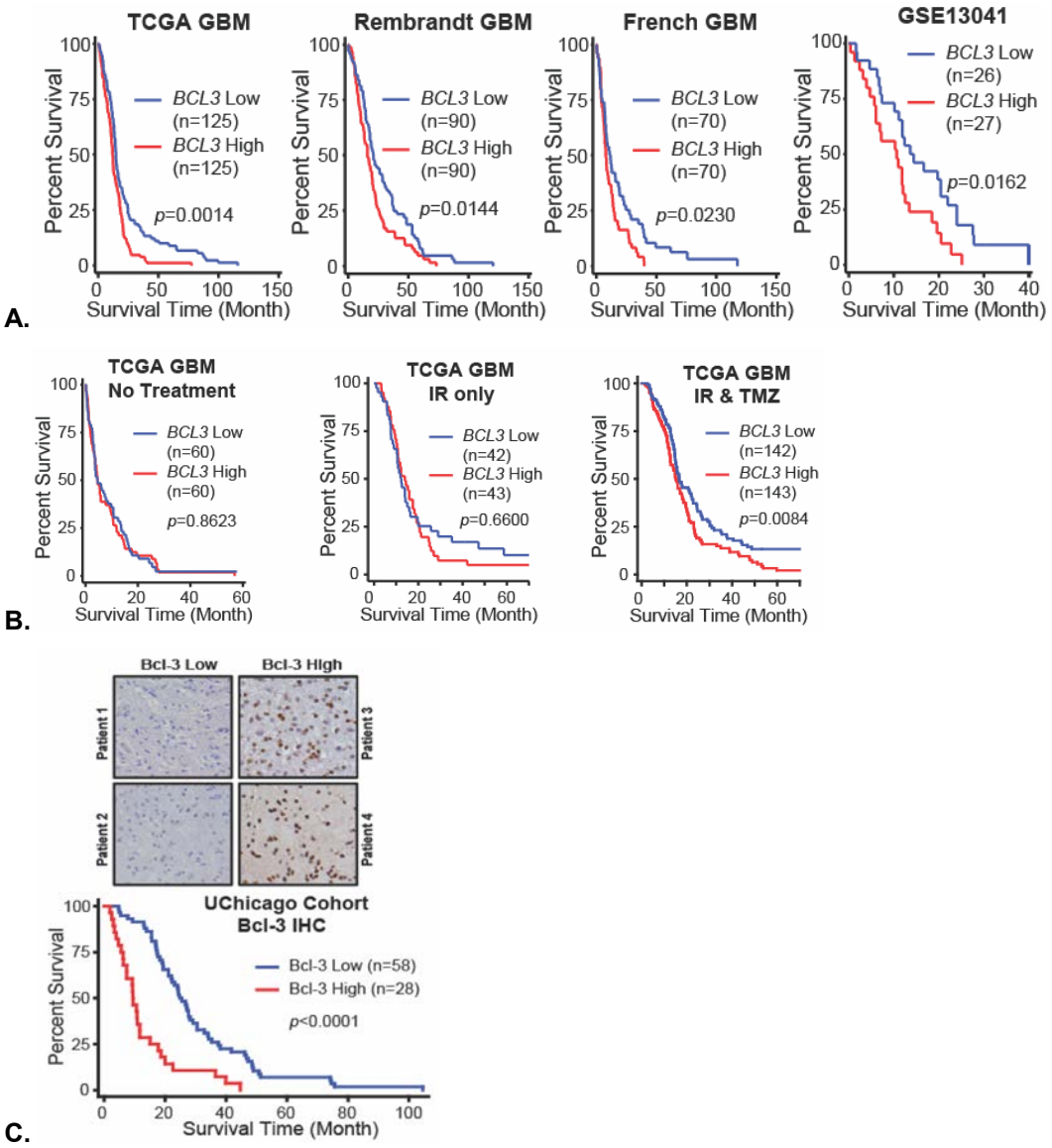

Figure 1. A. Kaplan-Meier survival curves in GBM patients from 4 independent databases based on median *BCL3* mRNA expression level. **B.** Survival curves in GBM patients from The Cancer genome Atlas (TCGA). Patients were separated by median *BCL3* mRNA expression level. Untreated patients (*Left*), ionizing radiation (IR) alone patients (*Center*), TMZ with IR (*Right*). Similar results are seen in REMBRANDT database. **C.** *Upper*- IHC for Bcl-3 in four different GBM specimens showing representative low and high staining. *Lower* – Overall survival curves for GBM patients based on Bcl-3 IHC staining grade. Patient numbers shown for all curves. *P*-values determined by Log-rank analysis.

2.2.2 Carbonic Anhydrase II, Bcl-3 and TMZ

Bcl-3 mediates resistance to TMZ indicating that targeting Bcl-3 is a strategy that can sensitize GBM cells to TMZ. Although loss of Bcl-3 sensitizes patient-derived GBM cells to TMZ, from a clinical perspective such as strategy is impractical as there are no commercially available Bcl-3 inhibitors. However, given that Bcl-3 is a transcriptional co-regulator, targeting a Bcl-3-dependent downstream factor is a potentially fruitful approach. To this end, genome-wide analysis was undertaken to look for Bcl-3-dependent factors modulated by TMZ.

Differential gene expression analysis identified carbonic anhydrase II (CAII) as the sole transcript that fit the criteria of being both Bcl-3-regulated and induced by TMZ. After studying the mechanism by which TMZ induces CAII, we confirmed the ability of CAII to block TMZ-induced cytotoxicity using a series of patient-derived GBM stem-like cells. These results demonstrate that CAII is a bona-fide downstream Bcl-3-dependent factor that can be targeted to enhance the effect of TMZ.

2.2.3 Acetazolamide and Temozolomide

Acetazolamide (ACZ) is an oral sulfonamide carbonic anhydrase inhibitor that is also one of the World Health Organizations List of Essential Medicines. It is used routinely for a number of medical conditions including epilepsy, glaucoma, altitude sickness, intracranial hypertension and as a diuretic. Although ACZ can inhibit many CAs, *in vitro* studies demonstrate that it is >10 times more effective against CAII than other CAs [17]. ACZ has never been used for cancer treatment in humans. However, ACZ was reported to enhance the efficacy of alkylating agents against fibrosarcoma in mice [18], and a previous *in vitro* study indicated that ACZ enhances killing of GBM cells by TMZ [19].

Given the above observations, we examined whether adding ACZ enhances the anti-glioma effect of TMZ in preclinical animal models of GBM. An initial pilot study was performed using orthotopic U87 GBM xenografts and ACZ given for a total of 10 days after initiation of TMZ. Using this regimen, although no prolongation of survival was seen, mice treated with the combination regimen appeared healthier than those receiving TMZ alone. What was notable from this initial study was that even 10 days after TMZ treatment, CAII levels remained almost 5-fold higher than at baseline suggesting that ACZ needs to be administered for a longer period of time. Based on this observation, we next administered ACZ at 15 mg/kg/day for a total of 21 days after TMZ initiation. Remarkably, this regimen resulted in a significant increase in animal survival compared to TMZ alone (Figure 2A).

To further examine this combination protocol, the patient-derived GBM xenograft GBM43S was established intracranially and animals treated with TMZ and for a total of 21 days of ACZ. Notably, GBM43S tumors express higher levels of Bcl-3 than U87 cells. Again a significant ($P < 0.02$) increase in survival was seen in combination treated animals relative to TMZ alone with several combination-treated animals noted to be long term survivors (Figure 2B). Importantly, this result was seen in three independent experiments. Notably, ACZ alone has no effect on either animal appearance or overall survival. In addition, we examined this treatment regimen in a PDX GBM that has high Bcl-3 and high MGMT expression (GBM26, (Figure 2D). In this model, ACZ does not augment the effect of TMZ (Figure 2C).

These results indicate that ACZ significantly augments the effect of TMZ in pre-clinical models of GBM. Moreover the data suggest that the effectiveness of ACZ is specifically evident in tumors with low MGMT expression or, from a clinical perspective, patients with a methylated *MGMT* promoter.

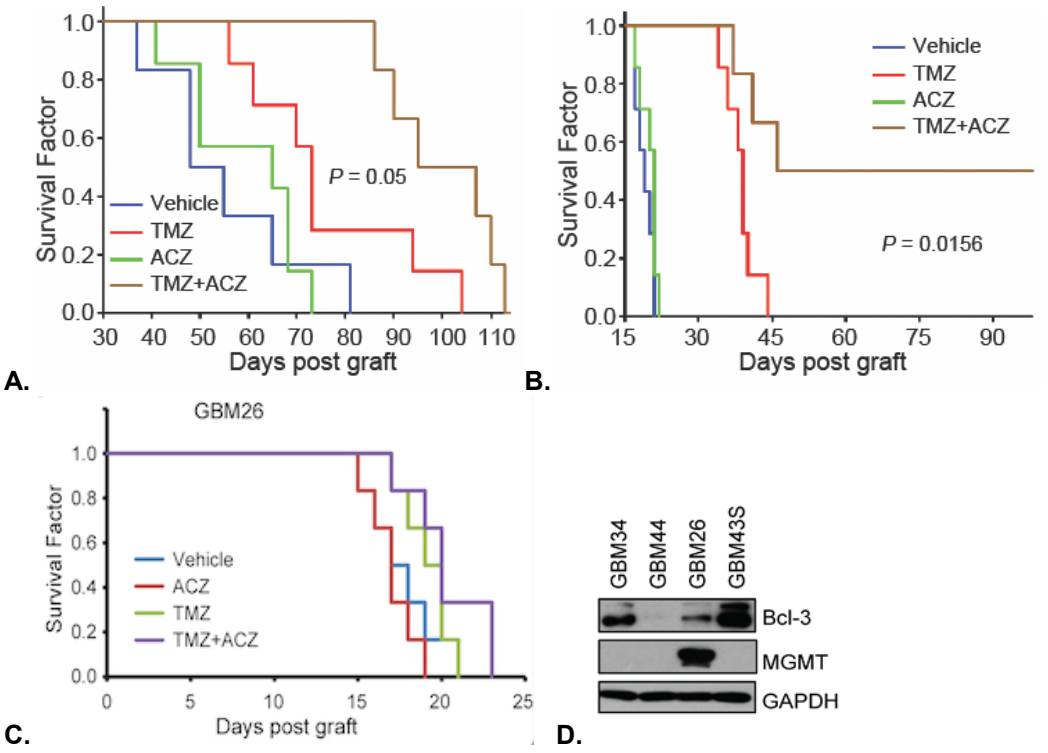

Figure 2. A- C Kaplan-Meier survival curves of nude mice bearing intracranial GBM xenografts. Animals were treated as indicated (TMZ separated into 3 doses on days 1, 3 and 5. ACZ: daily 15 mg/kg/day on days 1-21). **A.** U87. TMZ 5 mg/kg/dose. **B.** GBM43S patient-derived xenografts. TMZ 5 mg/kg/dose (n= 6-7 mice/group, repeated two more times with similar results). **C.** GBM26 PDX. TMZ 20 mg/kg/dose. (n= 5 mice/group, repeated with similar results). **D.** Immunoblot demonstrating Bcl-3 and MGMT expression level in PDX GBMs. *P* –values analyzed by the Log-rank method.

2.3 Rationale for current Study

The above pre-clinical studies suggest that combining TMZ and ACZ is a treatment strategy that can have a significant impact on survival in patients diagnosed with malignant astrocytoma. As TMZ is most often given as the initial backbone chemotherapeutic in combination with RT to patients with newly diagnosed malignant astrocytoma, we propose adding daily ACZ to patients with newly diagnosed tumors. Also, as the preclinical data suggest that the effect of ACZ will be seen primarily in tumors with a methylated *MGMT* promoter, patients with these tumors will be enrolled into the trial. In essence, this protocol will examine the re-purposing of ACZ as a chemo-sensitizing agent in malignant astrocytoma.

We will evaluate the safety of adding ACZ, administered at the standard dose of 250 mg BID and escalated to 500 mg BID, in combination with TMZ to patients with histologically diagnosed, *MGMT* promoter methylated grade III or IV astrocytoma. In this study, ACZ will only be used in the adjuvant TMZ phase, not during the concomitant TMZ/IR phase. This dosing schedule will be used firstly because ACZ was specifically shown to chemosensitize to TMZ and not to IR, and secondly because of potential delays in IR initiation that can occur and cause patient disqualification due to the time constraints associated with trial enrollment. During the maintenance TMZ phase, ACZ will be given throughout the period of TMZ administration and extended for a total of 21 days during each cycle. This Phase I study will evaluate the safety and tolerability of combining ACZ and TMZ. In addition, this study will allow for the collection of data to refine eligibility and treatment for future studies and help

determine sample size calculations for subsequent trials.

While the primary endpoint is analysis of safety and toxicity, an analysis of several secondary endpoints, including ORR, PFS and OS, will give a preliminary understanding of whether ACZ has some efficacy at improving TMZ response. In addition, ancillary studies will be performed on specimens obtained at surgery to examine intratumoral expression of Bcl-3. We will determine the number of patients with low and high Bcl-3 expression and preliminarily correlate Bcl-3 expression level with ORR, PFS and OS in patients treated with TMZ and in response to the addition of ACZ. The results of these secondary endpoints will be important for informing the structure of a future larger trial involving ACZ.

3. PATIENT SELECTION

3.1 Eligibility Criteria

3.1.1 Histologically proven, newly diagnosed WHO grade III or IV astrocytoma that has a methylated *MGMT* promoter as assessed by the standardized institutional analysis.

3.1.2 Patients are eligible if they had a prior low grade astrocytoma that was not previously treated, and there is subsequent histological evidence of a diagnosis of grade III or IV astrocytoma.

3.1.3 Patients are eligible if they are going to receive TMZ as part of the standard adjuvant treatment regimen following concomitant TMZ/RT.

3.1.4 Patients must have a Karnofsky performance ≥60% (see appendix A).

3.1.5 Normal organ activity, which includes adequate bone marrow function as defined by the following laboratory values:

- Absolute Neutrophil Count (ANC) ≥ 1.5 x 10^9/ L
- Platelets ≥ 100 x 10^9 / L
- Hemoglobin ≥ 8.0 g / dL

3.1.6 Age ≥18 years. Because of the risk of adverse events in patients <18 years of age, children are excluded from this study, but will be eligible for future pediatric trials.

3.1.7 Renal function (creatinine level within normal institutional limit, or creatinine clearance ≥ 60 mL/min/1.73 m^2 for patients with creatinine level above institutional normal).

3.1.8 Liver function (AST/ALT <2.5 X institutional upper limit of normal, Total bilirubin ≤ 1.5 times ULN, INR within 1.5 times ULN (or if receiving anticoagulant therapy an INR of ≤ 3.0 is allowed with concomitant increase in PT or an aPTT ≤ 2.5 × control).

3.1.9 Women of childbearing potential must have a negative pregnancy test within 30 days of registration.

3.1.10 Patients must have the ability to understand and the willingness to sign a written informed consent document.

3.2 Exclusion Criteria

3.2.1 Prior invasive malignancy that is not low-grade glioma (except non-melanomatous skin cancer or carcinoma in situ of the cervix) unless the patient has been disease free and off therapy for that disease for a minimum of 3 years.

3.2.2 Active systemic infection requiring treatment, including any HIV infection or toxoplasmosis.

3.2.3 Other severe acute or chronic medical or psychiatric condition, or laboratory abnormality that may increase the risk associated with study participation or study drug administration (e.g. acidosis, adrenocortical insufficiency, cirrhosis)

3.2.4 Systemic corticosteroid therapy, >8 mg of dexamethasone daily (or equivalent) at study enrollment.

3.2.5 Pregnant women are excluded from this study, where pregnancy is confirmed by a positive serum beta-hCG laboratory test. Breast-feeding should be discontinued.

3.2.6 Hypersensitivity to acetazolamide or sulfonamides.

3.3 Inclusion of Women and Minorities

Men and women of all races and ethnic groups are eligible for this trial.

4. REGISTRATION PROCEDURES

4.1 Patient Recruitment

Patients will be identified and recruited through Neurosurgery, Neuro oncology and Radiation Oncology clinic consultations and follow-up visits at the University of Chicago, NorthShore University HealthSystem, Northwestern University, Northwestern Medicine Regional Medical Group Warrenville and University of Illinois Chicago Medical Center, Rush University Medical center, the Illinois Cancer Care (Peoria) and the Decatur Memorial Hospital.

4.2 General Guidelines

Prior to registration and any study-specific evaluations being performed, all patients must have given written informed consent for the study and must have completed the pre-treatment evaluations. Patients must meet all of the eligibility requirements listed in Section 3. Eligible patients will be entered on study centrally by the University of Chicago study coordinator. All sites should email PhaseIICRA@medicine.bsd.uchicago.edu to verify availability of a slot.

Following registration, patients should begin protocol treatment within 28 business days or at the time of initiating adjuvant TMZ. Issues that would cause treatment delays should be discussed with the U Chicago Principal Investigator. If a patient does not receive protocol therapy following registration, the patient's registration on the study will be canceled. The U Chicago study coordinator/CRA should be notified of cancellations as soon as possible.

4.3 Registration Process

When a potential patient has been identified, notify the CRA via email to ensure a reservation on the study PhaseIICRA@medicine.bsd.uchicago.edu. Reservations for potential subjects will only be held for subjects who have signed consent for that particular study.

When registering a subject, the following must occur:

- Confirm that the institution has a current IRB approval letter for the correct version of protocol/consent and has an annual update on file, if appropriate.
- Submit all required materials (Eligibility Checklist, Source documentation, & signed consent form) to confirm eligibility and required pre-study procedures to the CRA a minimum of 48 hours prior to the subject's scheduled therapy start date.

- Source documentation includes copies of all original documents that support each inclusion/exclusion criteria. The eligibility checklist does not serve as source documentation but rather as a checklist that original source documentation exists for each criterion.
- Communicate with the CRA to ensure all necessary supporting source documents are received and the potential subject is eligible to start treatment on schedule. If there are questions about eligibility, the CRA will discuss it with the PI. PI may clarify, but not overturn, eligibility criteria.
- Affiliate sites must confirm registration of subjects by obtaining a subject study ID number from the CRA via phone, fax or email.
- If a subject does not start on the scheduled day 1 treatment date, promptly inform the CRA as the delay in start may deem the subject ineligible and/or require further or repeat testing to ensure eligibility.
- The date the patient is randomized if randomization is involved or receives treatment for the first time will be considered the patient's "OnStudy Date." The patient's subject ID will be assigned and a confirmation of registration will be issued by the CRA on this date. Subjects that sign consent and do not go "OnStudy" will be recorded in the database with the date they signed consent and the reason for not going "OnStudy" (e.g., Ineligible, Screen Failure or Withdrawn Consent).

4.4 Patient Remuneration

Patients will receive no payment for participation in this trial.

5. TREATMENT PLAN

5.1 Study Design

This is a Phase I study of the use of ACZ in patients with newly diagnosed, *MGMT* promoter methylated malignant astrocytoma who are being treated with 6 cycles of adjuvant TMZ. Patients will receive ACZ with TMZ throughout their treatment course. During each 28-day TMZ maintenance period, TMZ is given on days 1-5 and ACZ will be started on the day of TMZ initiation and continued for a total of 21 days (based on the data from the preclinical animal studies).

As an FDA-approved agent, the effective concentration of ACZ for inhibition of carbonic anhydrase has been thoroughly established at 250- 1000 mg/day. In addition, in pre-clinical animal studies, ACZ was administered at a concentration equivalent to between 500- 1000 mg daily. Based on the above, we will begin ACZ at 250 mg BID and after 1 week escalate up to 500 mg BID.

Treatment will be administered on an outpatient basis. The details of planning and administration of TMZ and ACZ are discussed in detail in Section 5.3. Reported adverse events (AEs) and potential risks for ACZ are described in Section 7. Appropriate dose modifications for TMZ and ACZ are described in Section 6. No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the patient's malignancy.

The trial will be considered successful if there are <4 RLT events in the first 12 evaluable patients or <8 in the total 24 evaluable patients (<33%) which is considered an acceptable level of toxicity.

5.2 Definition of Regimen Limiting Toxicities (RLT)

The standard treatment regimen for GBM and anaplastic astrocytoma consists of concomitant TMZ and RT followed by TMZ for 6 cycles. TMZ comprises the backbone regimen for these tumors. Although TMZ is associated with RLT itself, given that it s not

possible to differentiate toxicity by TMZ or ACZ, any RLT will be attributed to the combination regimen. Such toxicity will be defined as a toxicity that limits or delays the onset of the next cycle of backbone chemotherapy. Given that delay in the backbone chemotherapy can have a negative impact on overall outcome, any toxicity that delays the subsequent backbone cycle by more than 4 weeks will be considered a RLT. Thus RLT will include all toxicities that are usually dose limiting (i.e. grade ≥4 toxicity) and also less severe toxicities that significantly delay TMZ. The goal of this protocol is to determine whether the addition of ACZ increases RLT beyond that seen with TMZ alone.

RLT will be defined as any of the following if they occur within 35 days of treatment initiation.

- Any grade 4 non-hematological toxicity that is treatment-related with the exception of alopecia, nausea and vomiting,.
- Any grade 3 non-hematological toxicity that is treatment related that results in delay of backbone regimen (TMZ) by greater than 4 weeks.
- Grade 4 thrombocytopenia (<25,000/ mm^3) that results in delay of backbone chemotherapy for greater than 4 weeks.
- Grade 4 (<500/ mm^3) neutropenia lasting more than 7days, or Grade 3 (<1000/ mm^3) febrile neutropenia.
- Delay in starting next cycle by more than 4 weeks.

Toxicity will be defined according to Common Toxicity Criteria version 4.03 (ctep.cancer.gov/reporting/ctc.html). Each investigator will classify the toxicities as likely regimen-related or non-regimen-related based on review of the nature and timing of the toxicity. Any AEs not known to be associated with TMZ or ACZ will mandate a discussion between the PI and investigator to determine the cause and potential need for imaging or treatment cessation.

5.3 Drug administration

TMZ dosing

Temozolomide will be started at bedtime on an empty stomach. An antiemetic (ondansetron 8 mg tablet daily or its equivalent) is recommended approximately 1 hour prior to the oral dose. If vomiting occurs after a dose is administered, the patient will wait until the next scheduled dose. Capsules should not be opened or chewed. TMZ will be administered as standard of care per institutional guidelines.

Patients will receive TMZ per standard Stupp protocol [4]. Four weeks following the initial concomitant TMZ/IR phase, the patient will begin the trial with 28-day maintenance cycles of TMZ. For cycle 1 of the maintenance phase, TMZ will administered at 150 mg/m^2 on days 1-5 followed by 23 days off. For cycles 2- 6, TMZ can be increased to 200 mg/m^2 at the discretion of the investigator if ANC >1.5 X 10^9/L, platelet count >100 X 10^9/L, lymphocyte count > 0.6 X 10^9/L. TMZ can be continued for 6 cycles or until disease progression.

ACZ dosing

Treatment will be on an outpatient basis as is routinely done for ACZ. The standard dosing regimen for other neurological conditions involves a starting dose of 250 mg BID that is then increased to 500 mg BID. For the current study, all patients will receive ACZ starting at 250 mg BID and escalated to 500 mg BID after 1 week. This regimen is adequate to inhibit CA and is consistent with that used in our preclinical animal model. ACZ will be started on the same day as TMZ initiation. No pre-medications are required and tablets should be swallowed whole. ACZ will be continued throughout the period during which TMZ is administered. For each 28-day cycle, ACZ will be administered for a total of 21 days and then

discontinued.

Reported adverse events and potential risks are described in Section 7. Appropriate dose modifications are described in Section 6. No investigational or commercial agents or therapies other than those described above may be administered with the intent to treat the patient's malignancy.

Importantly, unlike other anti-epileptics, there is no indication that ACZ modifies CYP450 activity or is a P450 substrate [20], suggesting that there is no need for TMZ or ACZ drug modification with concomitant use.

Avoid use of high dose aspirin concurrently with ACZ due to possible side effects such as tachypnea, anorexia, lethargy, coma and death.

5.4 General Concomitant Medication and Supportive Care Guidelines

Patients may receive non-chemotherapeutic agents for pre-medication and supportive care as per the standard of care while on study. Patients receiving TMZ may receive pneumocystis carinii (PCP) prophylaxis at the discretion of the investigator.

5.5 Duration of Therapy

Patients will receive ACZ throughout the time they are receiving TMZ, as described above. If patients have TMZ held for any reason, but have completed at least 80% of their treatment regimen, they will remain in the study.

In the absence of treatment delay due to AE, treatment may be continued to a total of 6 adjuvant TMZ cycles until one of the following occurs:

- Disease progression
- Intercurrent illness that prevents further TMZ administration
- Unacceptable AEs
- Patient decides to withdraw from the study
- Patient inability to be compliant with study treatment in the opinion of the PI defined as missing treatments or study visits for non-medical reasons or complying with oral treatments below an 85% threshold on two sequential study visits
- General or specific changes in the patient's condition that render the patient unacceptable for further treatment in the judgment of the PI.

5.6 Duration of Follow Up

After the End-of-Treatment Visit, Follow-up visits will be conducted every 2 months until progression and will include the following:

- Update medical history including current cancer therapy being received every 2 months.
- Physical examination including vital signs, height, and weight, and a review of body systems every 2 months.
- MRI of the brain with and without gadolinium contrast. Done at standard of care timepoints at each institution. No extra MRIs are necessary.

After progression, patients will be followed for survival and possible long-term toxicity from this treatment. These follow-up visits will continue until death and can be performed by telephone, correspondence with treating physicians and death records as necessary to update vital status at least every 6 months until death or loss to follow-up. Also, after progression, further clinic visits, MRI and therapy will be at the discretion of the treating physician.

5.7 Criteria for Removal from Study

Patients will be removed from study when one of the following criteria applies:

- Intercurrent illness that prevents administration of TMZ or ACZ.
- Unacceptable AE(s).
- Death.
- Patient decides to withdraw from the study.
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator/treating physician.

The reason for study removal and the date the patient was removed must be documented in Velos (see section 14).

6. DOSING DELAYS/ MODIFICATIONS

6.1 Temozolomide

Concomitant phase:
Patients will not receive ACZ during this phase.

TMZ will be administered at 75 mg/m^2/day for 42 days concomitant with focal radiotherapy. No dose reductions are recommended during this phase; however, dose interruptions or discontinuation may occur based on toxicity. The TMZ dose should be continued throughout the 42-day period up to 49 days if all of the following conditions are met: ANC ≥1.5 x 10^9/L, platelet count ≥100 x 10^9/L, common toxicity criteria (CTC) nonhematological toxicity ≤ Grade 1 (except for alopecia, nausea, and vomiting). During treatment a complete blood count should be obtained every two weeks. TMZ dosing should be interrupted or discontinued during concomitant phase according to the hematological and nonhematological toxicity criteria as noted in Table 1. Pneumocystis pneumonia (PCP) prophylaxis can be given per the discretion of the treating physician.

TOXICITY	TMZ INTERRUPTION*	TMZ DISCONTINUATION
Absolute Neutrophil Count	greater than or equal to 0.5 and less than 1.5 x 10^9/L	less than 0.5 x 10^9/L
Platelet Count	greater than or equal to 10 and less than 100 x 10^9/L	less than 10 x 10^9/L
CTC Nonhematological Toxicity (except for alopecia, nausea, vomiting)	CTC Grade 2	CTC Grade 3 or 4

Table 1. TMZ dosing interruption or discontinuation during concomitant TMZ/RT phase

Maintenance phase:
First Cycle: TMZ will be started at a dose of 150 mg/m^2/day.

Second Cycle: If patients tolerate the first cycle of combination therapy without any TMZ related toxicities, the patient may be dose escalated up to 200 mg/m^2/day at the discretion of the treating physician. The dose of TMZ will be modified according to: (1) non-hematologic AE during the preceding treatment cycle, as well as (2) the worst ANC and platelet counts.

Recalculation of BSA and TMZ doses is required if the patient has a 10% or greater weight change (+/-) from baseline or from the last weight used to calculate BSA and drug doses.

Delay: On day 1 of each cycle (within the prior 72 hours), ANC $\leq$1.5 x 10^9/L, platelet count <100 x 10^9/L and all grade 3 or 4 non-hematologic AEs (except for alopecia, nausea, and vomiting) must have resolved (to grade 1).

If AEs persists, treatment should be delayed by 1 week for up to 4 consecutive weeks. If, after 4 weeks of delay, all AEs have still not resolved: then any further treatment with TMZ should be stopped.

Dose reductions: If, during the first cycle, all non-hematologic AEs observed were grade 2 (except alopecia, nausea and vomiting) and with platelets >100 x 10^9/L and ANC >1.5 x10^9/L: then the TMZ dose should remain the same. If any non-hematologic AE observed was grade >2 (except alopecia, nausea and vomiting) and/or if platelets <50 x 10^9/L and/or ANC <1 x 10^9/L, then the dose should be reduced by one dose level (Table 2). Patients who require more than two dose reductions will have treatment stopped.

If any treatment-related non-hematologic AE observed was grade 4 (except alopecia, nausea and vomiting) then TMZ treatment should be stopped.

Subsequent cycles: Any dose reductions of TMZ will be determined according to: (1) non-hematologic AE during the preceding treatment cycle, as well as (2) the lowest ANC and platelets observed. No dose escalation should be attempted. The same dose reductions as for the second cycle should be applied. Important: If the dose was reduced or delayed for AEs, there will be no dose escalation in subsequent treatment cycles.

Dose Reduction or Discontinuation During Maintenance: Dose reductions during the maintenance phase should be applied according to Tables 2 and 3. In addition, a dose reduction to 50 mg/m^2 for 28/28 days per cycle (i.e. metronomic therapy) is allowable in patient who do not tolerate a dose of 100 mg/m^2.

DOSE LEVEL	DOSE (MG/M²/DAY)	REMARKS
-1	100	Reduction for prior toxicity
0	150	Dose during Cycle 1
1	200	Dose during Cycles 2-6 in absence of toxicity

Table 2. TMZ dose level for maintenance treatment

TOXICITY	REDUCE TMZ BY 1 DOSE LEVEL*	DISCONTINUE TMZ
Absolute Neutrophil Count	less than 1.0 x 10^9/L	See footnote†
Platelet Count	less than 50 x 10^9/L	See footnote†
CTC Nonhematological Toxicity (except for alopecia, nausea, vomiting)	CTC Grade 3	CTC Grade 4†

*TMZ dose levels are listed in Table 2.
†TMZ is to be discontinued if dose reduction to less than 100 mg/m² is required or if the same Grade 3 nonhematological toxicity (except for alopecia, nausea, vomiting) recurs after dose reduction.
TMZ=temozolomide; CTC=Common Toxicity Criteria.

Table 3. TMZ dose reduction or discontinuation during maintenance treatment

6.2 Acetazolamide

Acetazolamide will only be administered during the maintenance phase of TMZ treatment.

Acetazolamide is an FDA-approved agent and is in general well tolerated. The most commonly mentioned side-effects include dysesthesias and a metallic taste especially on drinking carbonated drinks. Rare but serious side effects include metabolic acidosis and hepatotoxicity. If a dose reduction is necessary for grade 1 or 2 dysesthesias in the initial phase (250 mg BID), one dose reduction to 125 mg BID is permitted. If this reduction is not tolerated then discontinuation of the study is necessary. If a dose reduction is necessary in the escalation phase (500 mg BID), dose reduction back to 250 mg BID is permitted. Grade 3 dysesthesias will require discontinuation of ACZ. Patients who are not dose escalated will not be considered failure and will remain on their tolerated dose. Symptomatic metabolic acidosis is rarely associated with ACZ at the doses proposed in this study. No dose reduction will be required for asymptomatic grade 1 acidosis. Grade 3 acidosis (there is no Grade 2) will require temporary discontinuation of ACZ until metabolic function normalizes. Re-challenge at one of the allowed lower doses will be permitted at the investigator's discretion. Hepatic toxicity will be monitored through routine testing of liver function tests and dose delays, reductions, and discontinuation from study will follow the guidelines listed in section 5.2, above.
If TMZ is discontinued, ACZ will also be stopped as ACZ has no anti-glioma effect on its own. If TMZ is held for <7 days for vomiting, ACZ will be continued.

7.0 ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

Adverse event (AE) monitoring and reporting is a routine part of every clinical trial. The following list of AEs and the characteristics of an observed AE will determine whether the event requires expedited reporting in addition to routine reporting.

7.1 Most Common Adverse Events

7.1.1 Adverse Events for ACZ

- The most common adverse reactions (> 10% incidence) are: paresthesia, metallic taste especially with carbonated drinks, dizziness, lightheadedness, increased urination, dry mouth, loss of appetite, nausea, vomiting, diarrhea, headache, fatigue

and tinnitus.

7.1.2 Rare Adverse Events for ACZ

- Reported Grade 3 to 4 hematologic laboratory abnormalities that have developed during treatment with ACZ are: thrombocytopenia, leukopenia, agranulocytosis, aplastic anemia (there are no estimates of incidence and they are thought to be rare events).

- Rare reports of fatalities have occurred due to severe reactions to sulfonamides including Stevens- Johnson syndrome, toxic epidermal necrolysis, fulminant hepatic necrosis, agranulocytosis, aplastic anemia, and other blood dyscrasias.

7.1.3 Adverse Events for TMZ

- The most common adverse reactions (≥ 10% incidence) are: alopecia, fatigue, nausea, vomiting, headache, constipation, anorexia, convulsions, rash, hemiparesis, diarrhea, asthenia, fever, dizziness, coordination abnormal, viral infection, amnesia, and insomnia.

- The most common Grade 3 to 4 hematologic laboratory abnormalities (≥ 10% incidence) that have developed during treatment with temozolomide are: lymphopenia, thrombocytopenia, neutropenia, and leukopenia.

- Allergic reactions have also been reported.

7.2 Adverse Event Reporting

Subject data accrued on this study will be reported in accordance with Code of Federal Regulations Title 21 (21CFR) 312.32.

This study will utilize the descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.03 for grading all adverse events. All appropriate treatment areas should have access to a copy of the CTCAE version 4.03. A copy of the CTCAE version 4.03 can be downloaded from the CTEP web site (http://ctep.cancer.gov).

The PI will notify the IRB of all events in accordance with their policies and procedures. Serious Adverse Event Reporting will be done as outlined below.

An adverse event (AE) is any symptom, sign, illness or experience that develops or worsens in severity during the course of the study. Intercurrent illnesses or injuries should be regarded as adverse events. Abnormal results of diagnostic procedures are considered to be adverse events if the abnormality:

- results in study withdrawal
- is associated with a serious adverse event
- is associated with clinical signs or symptoms
- leads to additional treatment or to further diagnostic tests
- is considered by the investigator to be of clinical significance

Related/Attribution to the use of the drug: There is a reasonable possibility (more likely than not) that the experience may have been caused by the investigational drug.

Attribution Categories:

Unrelated	The AE *is clearly NOT related* to the intervention.
Unlikely	The AE *is doubtfully related* to the intervention.
Possible	The AE *may be related* to the intervention.
Probable	The AE *is likely related* to the intervention.
Definite	The AE *is clearly related* to the intervention.

7.3 Determination of Severity and Causality

Serious Adverse Event (SAE)
An AE is considered an SAE if at least 1 of the following conditions applies:

- *Death*: An AE that results in death during the active study period or within 30 days following study drug administration. In addition, a reported death at any time post-study that is thought to be related to study drug administration.

- *Life-threatening adverse event*: An AE that places the subject, in the view of the investigator, at immediate risk of death from the event as it occurred (i.e. this does not include a reaction that, had it occurred in a more severe form, might have caused death).

- *Permanent, persistent, or significant disability*: A disability is defined as any substantial disruption of a person's ability to conduct normal life functions.

- *Inpatient hospitalization or prolongation of existing hospitalization*: In general, hospitalization refers to admission of a subject into a hospital for at least a 24-hour stay. Hospitalizations for routine blood transfusions, hospitalization for an elective or diagnostic procedure, or surgery for a pre-existing condition that has not worsened, are not considered SAEs. (Emergency room visits that do not result with admission are not considered as SAEs).

- *A congenital anomaly/birth defect*: A fixed, permanent impairment established at or before birth.

- *Important medical event*: Events that may not result in death, be life-threatening, or require hospitalization may be considered SAEs when, based upon appropriate medical judgment, they jeopardize the subject and require medical or surgical intervention to prevent a life-threatening situation, hospitalization or death.

- *New cancer*: Occurrence or diagnosis of a new cancer during the trial is considered an SAE (This does not pertain to metastasis of current disease).

- Any AE associated with an overdose of the study drug: An overdose of study drug is defined as an occurrence of administered dose exceeding that which is prescribed by the investigator per protocol.

Non-Serious Adverse Event
An AE that does not fulfill the criteria for an SAE is classified as a non-serious AE.

Determination of Severity

The severity of AEs will be assessed according to the NCI CTCAE, v. 4.03. If the AE is not defined in the NCI CTCAE, v. 4.03, the investigator will determine the severity of an AE based on the following definitions:

- **Mild (Grade 1)**: The AE is noticeable to the subject, but does not interfere with routine activity. The AE does not require discontinuing administration or reducing the dose of the study drug.

- **Moderate (Grade 2)**: The AE interferes with routine activity, but responds to Symptomatic therapy or rest. The AE may require reducing the dose, but not discontinuing administration of the study drug.

- **Severe (Grade 3)**: The AE significantly limits the subject's ability to perform routine

activities despite symptomatic therapy. In addition, the AE leads to discontinuing administration or reducing the dose of the study drug.

- **Life Threatening (Grade 4)**: The AE requires discontinuing administration of the study drug. The subject is at immediate risk of death.

- **Death (Grade 5)**: The subject dies as a direct result of the complication or condition.

Determination of Causality

The investigator will use medical consideration to determine the potential relationship of the AE to the study drug based on his/her clinical judgment. Assessment of causality will be based upon the following:

- Alternative possible causes of the AE, including the subject's underlying disease or co-morbid conditions, other drugs, other host and environmental factors.
- The temporal sequence between the exposure to the study drug and the AE.
- Whether the clinical or laboratory manifestations of the AE are consistent with known actions or previously reported toxicity of the study drug (or similar drugs).
- Whether the AE resolved or improved with decreasing the dose or stopping the study drug (i.e. de-challenge); or recurred or worsened with re-exposure to the drug (i.e. re-challenge).

Relationship assessments that indicate "Not Related" to investigational product:

- **None**: The event is related to an etiology other than the investigational product (the alternative etiology must be documented in the study subject's medical record and/or SAE form).

- **Unlikely or Remote**: The event is unlikely to be related to the investigational product and likely to be related to factors other than investigational product.

Relationship assessments that indicate "Related" to investigational product:

- **Possible**: There is an association between the event and the administration of the investigational product and there is a plausible mechanism for the event to be related to the investigational product; but there may also be alternative etiologies, such as characteristics of the subject's clinical status or underlying disease.

- **Probable**: There is an association between the event and the administration of the investigational product, a plausible mechanism for the event to be related to the investigational product exists and this could not be reasonably explained by known characteristics of the subject's clinical status or an alternative etiology is not apparent.

- **Definite**: There is an association between the event and the administration of the investigational product, a plausible mechanism is evident for the event to be related to the investigational product and causes other than the investigational product have been ruled out and/or the event re-appeared on re-exposure to the investigational product.

7.4 Routine Adverse Event Reporting

All Adverse Events **must** be reported in routine study data submissions. **AEs reported using the Serious Event Reporting Form and/or MedWatch Form discussed below must also be reported in routine study data submissions.**

All adverse events (except grade 1 and 2 laboratory abnormalities that do not require an intervention), regardless of causal relationship, are to be recorded in the case report form and source documentation. The Investigator must determine the intensity of any adverse events according to the NCI Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0 and their causal relationship.

7.4.1.1 Serious Adverse Event Reporting **to** the Coordinating Center

Use the UC CCC protocol number and the protocol-specific patient ID assigned during trial registration on all reports.

All serious adverse events (as defined in sections 7.3.2 and 7.3.3) and all adverse events that have been specified to require expedited reporting in the section 7.1 table occurring on this study require expedited reporting to the University of Chicago Comprehensive Cancer Center (UC CCC). The responsible Research Nurse or other designated individual at the treating site should report the SAE to the Study Lead Investigator, the University of Chicago CRA and the CCTO by the end of the business day when s/he becomes aware of the event. Events occurring after business hours should be reported to the CCTO by 12pm (noon) the next business day. Reports should be made using the 'Serious Event Report' Form. Please scan and send via email (preferred) or fax to the following:

University of Chicago Phase II CRA General:
PhaseIICRA@medicine.bsd.uchicago.edu
Phone: 773-834-1746; 773-702-9879
Fax: 773-702-4889

UC CCC Cancer Clinical Trials Office Quality Assurance:
qaccto@bsd.uchicago.edu

All serious adverse events should also be reported to the local IRB of record according to their policies and procedures.

7.4.1.2 Serious and Unexpected Adverse Event reporting **by** the Coordinating Center

The designated UC CCC Regulatory Manager will notify all participating sites of all unexpected and serious adverse reactions that occur on this clinical trial and which are reported to the FDA and/or UC Institutional Review Board (IRB).

8. STUDY CALENDAR

Data entry begins at the start of the maintenance phase. Baseline CBC, serum chemistry, and pregnancy test (if indicated) are to be conducted within 30 days prior to study registration (within 4 weeks of starting maintenance phase). Baseline MRI and ECG must be performed within 4 weeks prior to start of maintenance therapy. If the patient's condition is deteriorating, laboratory evaluations should be repeated within 48 hours prior to initiation of the next cycle of therapy.

Study calendar

*** All clinic visits are per standard of care at the institution, no extra clinic visits are required for this study. Consent is valid for 30 days. If patient does not begin maintenance phase within 30 days of signing consent, patient must be re-consented.**

	Pre-study[#] (This includes time during Concomitant TMZ/IR phase)	4 Week TMZ break (post concomitant TMZ/IR)	Maintenance Phase (All cycles)				End of Therapy visit	Follow up visits until progression	Follow up until death
			wk1 d1	wk2 d8	wk3 d15	wk4 d22			
Informed Consent	X								
Vital Signs	X				X	X			
Medical History	X	X			X	X	X	q2 mo	
Concurrent meds	X	X			X	X	X	q2 mo	X
Adverse Events evaluation	X	X			X	X	X		
Clinic Visit[d]	X	X				X	X	q2 mo	X*
Phone call									X$
CBC/diff/plts	X	X			X	X			
Serum chemistry[a]	X	X			X	X			
Radiographic imaging[b]	X	MRI after concomitant TMZ/IR^ and Baseline ECG						q2 mo MRI	M*
Beta-HCG[c]	X								
Temozolomide			T						
Acetazolamide			A	A	A				

#: Patients can be enrolled either prior to or during concomitant TMZ/IR phase.
a: Albumin, alkaline phosphatase, total bilirubin, bicarbonate, calcium, chloride, creatinine, glucose, LDH, phosphorus, potassium, total protein, SGOT [AST], SGPT [ALT], sodium.
b: MRI of the brain with and without contrast.
c: Serum pregnancy test (women of childbearing potential)
d: Will include physical exam and neuro check.
^: This is the official pre-study MRI that will be used to calculate ORR.
*: After progression clinic visits/MRI of the brain are per the discretion of the treating physician.
$: After progression, follow up as to overall survival analysis can be via phone call.

9. MEASUREMENT OF EFFECT

9.1 Antitumor Effects and Endpoints

Antitumor response including Investigator assessment of Objective Response Rate (ORR), Progression Free Survival (PFS) and Overall Survival (OS) are secondary endpoints. Tumor response and regrowth can be difficult to measure directly. Serial neurological exams and MRI scans can provide a guide to the actual course. Time interval to progression will be measured from study enrollment until documentation of deterioration using these guides:

- **Toxicities** - measured using the CTCAE criteria, version 4.03
- **Objective Response Rate** – measured at 6 months is based on the change in tumor size (by RANO criteria –see below [3]) at the indicated time relative to the pre-treatment scan (i.e the scan following the concomitant TMZ/IR phase).
- **Overall Survival** - measured from the start of treatment on protocol until death.
- **Progression Free Survival** - measured from the start of treatment on protocol until the first occurrence of progression on protocol or death.

9.2 Response Criteria

For this study we will be utilizing the RANO criteria (Table 4):

9.2.1 Complete Response

Complete response requires all of the following: complete disappearance of all T1 gadolinium enhancing measurable and nonmeasurable disease sustained for at least 8 weeks; no new lesions; stable or improved nonenhancing (T2/FLAIR) lesions; and patient must be off corticosteroids or on physiologic replacement doses only, and stable or improved clinically. In the absence of a confirming scan at least 8 weeks after first indication of an objective response is noted, this response will be considered only stable disease.

9.2.2 Partial Response

Partial response requires all of the following: > 50% decrease, compared with baseline, in the sum of products of perpendicular diameters of all measurable T1 gadolinium enhancing lesions sustained for at least 4 weeks; no progression of nonmeasurable disease; no new lesions; stable or improved nonenhancing (T2/FLAIR) lesions on same or lower dose of corticosteroids compared with baseline scan; and patient must be on a corticosteroid dose not greater than the dose at time of baseline scan and is stable or improved clinically. In the absence of a confirming scan at least 8 weeks after first indication of an objective response is noted, this response will be considered only stable disease.

9.2.3 Stable Disease

Stable disease occurs if the patient does not qualify for complete response, partial response, or progression (see next section) and requires the following: stable nonenhancing (T2/FLAIR) lesions on same or lower dose of corticosteroids compared with baseline scan and clinically stable status. 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, considered to show stable disease will be the scan obtained when the corticosteroid dose was equivalent to the baseline dose.

9.2.4 Progression

Progression is defined by any of the following: >25% increase in sum of the products of perpendicular diameters of enhancing lesions (compared with baseline if no decrease) on stable or increasing doses of corticosteroids; a significant increase in T2/FLAIR nonenhancing lesions on stable or increasing doses of corticosteroids compared with baseline scan or best response after initiation of therapy, not due to comorbid events; the appearance of any new lesions; clear progression of nonmeasurable lesions; or definite clinical deterioration not attributable to other causes apart from the tumor, or to decrease in corticosteroid dose. Failure to return for evaluation as a result of death or deteriorating condition should also be considered as progression.

Pseudo-progression

The apparent increase in tumor burden that sometimes precedes response in some

patients may reflect continued tumor growth until a sufficient response develops. In the absence of overt clinical deterioration, patients with apparent radiographic disease progression in the first two months of therapy will be re-scanned 8 weeks later to evaluate progression, as long as they remain clinically stable [21]. In addition, a brain Magnetic Resonance Spectroscopy (MRS) may be performed (per investigator's decision) to differentiate tumor progression versus tumor necrosis/inflammation.

Non-measurable enhancing lesion increased to measurable enhancing disease
Patients with non-measurable enhancing disease whose lesions have significantly increased in size and become measurable (minimal bidirectional diameter of > 10 mm and visible on at least two axial slices that are preferably, at most, 5 mm apart with 0-mm skip) will also be considered to have experienced progression. Ideally, the change should be significant (>5 mm increase in maximal diameter or >25% increase in sum of the products of perpendicular diameters of enhancing lesions). In general, if there is doubt about whether the lesion has progressed, continued treatment and close follow-up evaluation will help clarify whether there is true progression.

9.3 Measurement per RANO criteria

9.3.1 Measurable lesions

- CT or MRI, contrast enhancing with clearly defined margins
- Visible on two or more axial slices, preferably <5 mm thick with 0 mm skip
- Maximal diameter and second perpendicular measurement at least 10 mm in size (if slice thickness <5 mm), 2 times slice thickness (if slice thickness >5 mm)
- Do not measure cystic cavity

9.3.2 Non-Measurable lesions

Non-measurable lesions are those that do not meet the criteria above, a cystic / necrotic tumor or one with a surgical cavity. In such cases only a solid peripheral nodular component should be measured, provided it fulfills the above 'measurable' criteria.

If it is difficult to define a 'nodule', the baseline scan is necessary and the axes of initial measurement to assess response.

Measurements are obtained from axial post contrast T1 images. The maximal diameter is obtained, and then the second diameter is obtained at right angles to the first. The product of these measurements is used for comparison.

9.4 Special circumstances

Non-target lesions

All other lesions (or sites of disease) should be identified as **non-target lesions** and should also be recorded at baseline. Non-target lesions include measurable lesions that exceed the maximum numbers per organ or total of all involved organs as well as non-measurable lesions. Measurements of these lesions are not required, but the presence or absence of each should be noted throughout follow-up.

Steroid dose

Increase in corticosteroid dose alone, in the absence of clinical deterioration related to tumor, will not be used as a determinant of progression. Patients with stable imaging studies whose corticosteroid dose was increased for reasons other than clinical deterioration related to tumor do not qualify for stable disease or progression. They should be observed closely. If their corticosteroid dose can be reduced back to baseline, they will be considered as having stable disease; if further clinical deterioration related to tumor becomes apparent, they will be

considered to have progression. The date of progression should be the first time point at which corticosteroid increase was necessary.

Clinical deterioration
Determination of clinical deterioration is left to the discretion of the treating physician, it is recommended that a decline in the KPS from 100 or 90 to 70 or less, a decline in KPS of at least 20 from 80 or less, or a decline in KPS from any baseline to 50 or less, for at least 7 days, be considered neurologic deterioration unless attributable to comorbid events or changes in corticosteroid dose. Similarly, a decline in the Eastern Cooperative Oncology Group and WHO performance scores from 0 or 1 to 2 or 2 to 3 would be considered neurologic deterioration.

Uncertainty regarding progression
The patient may continue on treatment and remain under close observation (e.g., evaluated at 4- week intervals). If subsequent evaluations suggest that the patient is in fact experiencing progression, then the date of progression should be the time point at which this issue was first raised.

Multifocal Tumors

- **Progressive disease** is defined as >25% increase in the sum of products of perpendicular diameters of all measurable lesions compared with the smallest tumor measurements after initiation of therapy. The appearance of a new lesion or unequivocal progression of nontarget lesions will also be considered progression.

- **Partial response** is defined as >50% decrease, compared with baseline, in the sum of products of perpendicular diameters of all measurable lesions sustained for at least 4 weeks with stable or decreasing corticosteroid doses.

	CR	PR	SD	PD
T1-Gd+	None	≥50%	<50%↓- <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*

CR = Complete Response, PR = Partial Response, SD = Stable Disease, PD = Progressive Disease
*Progression occurs when this is present
NA: An increase in steroid alone will not cause a determination of progression in the absence of clinical deterioration or radiographically documented lesion growth.
Adapted from Wen P. Y. et al. (2010). Updated Response Assessment Criteria for High-Grade Gliomas: Response Assessment in Neuro-Oncology Working Group (4).

Table 4. Summary of RANO (Response Assessment in Neuro-Oncology) criteria.

10. STATISTICAL CONSIDERATIONS

10.1 Study Design/ Endpoints

This is a Phase I study looking at addition of acetazolamide to temozolomide for the treatment of patients with malignant glioma. All patients enrolled in the study will receive oral acetazolamide. ACZ will be initiated at 250 mg BID and escalated to 500 mg BID after 1 week. The treatment will be considered tolerable if there are < 8 RLT events in 24 patients.

10.2 Sample Size/Accrual Rate

The maximum sample size will be 24 patients. This size was selected to provide an early stopping rule after 12 patients (4 or more RLTs).

10.3 Analysis of the Primary Endpoint

The primary endpoint is identification of AEs and determination of the rate of RLT in patients receiving ACZ and TMZ. RLT will be recorded as any AE that results in cessation or delay in the backbone regimen of TMZ for a period of more than 4 weeks.

10.4 Analysis of Secondary Endpoints

The secondary endpoints include:

1. determining objective response rate (ORR), progression free survival (PFS) and overall survival (OS).
2. determining the feasibility of accrual and adequacy of eligibility criteria.
3. evaluating tumoral Bcl-3 expression and preliminarily determining whether Bcl-3 expression is related to the response to TMZ and the addition of ACZ.

ORR, PFS and OS will be calculated for each patient. An exact two-sided 90% confidence interval will be determined for ORR using the binomial distribution. PFS and OS will be estimated using the Kaplan-Meier method [22]. Bcl-3 expression level will be graded on a 4-tier scale that will then be simplified into a binary grade (high or low). The staining grade will be related to ORR, PFS and OS using Fisher's exact test and Cox regression modeling [23]. In addition, for survival analyses, patients will be stratified by *IDH* mutation status. These analyses will be performed as part of the standard of care pathological analysis of GBM.

For determination of ORR, MRIs will be analyzed in a blinded manner by Dr. Collins. For patients enrolled at NorthShore University HealthSystem Northwestern University, Northwestern Medicine Regional Medical Group Warrenville, University of Illinois Chicago Medical Center, Rush University Medical center, the Illinois Cancer Care (Peoria) and the Decatur Memorial Hospital. MRIs will be sent on CD to The University of Chicago for central review by Dr. Collins.

11. CONFIDENTIALITY

11.1 Patient Confidentiality Issues

Study records that identify patients will be kept confidential. Study records will contain patients' name, address, and medical history number and will be available to the study doctor, research nurse, and data coordinator. Data collected in this study will be maintained on a password-protected computer that only the PI, co-investigators, research nurse, and data coordinator will be able to access. Study records will be secured in locked offices in the section of Neurosurgery, Department of Surgery. Neither patient's name nor other personally identifying information will be used in any publication resulting from the research study.

12. TISSUE AND BLOOD BANKING FOR FUTURE RESEARCH

Biomarkers that have prognostic or predictive value are of great interest in the management of patients with glioma. As noted in Section 2.2, based on analysis of retrospective data, Bcl-3 is a molecular biomarker that can predict response to TMZ. Moreover, our preclinical data suggest that ACZ might be particularly effective in tumors that express high levels of Bcl-3 (Section 2.3). Thus, analysis of pre-treatment tumoral Bcl-3 expression is potentially highly relevant for identifying patients that might most benefit from the therapeutic regimen being studied in this protocol. Consent for tissue and blood acquisition will be an optional part of the main consent form. Tissue will be acquired at surgery from the Department of Pathology as per standard Human Tissue Resource Center (HTRC) protocol after adequate specimen has been obtained for pathology. In addition, at the University of Chicago. Blood will be obtained from patients at the same time as a regular phlebotomy at the time of surgery. The tissue and blood will be labeled with the date of collection, the patient's initials, and protocol number. The specimens will not be shared with anyone outside of Dr. Yamini's lab. However, it is anticipated that in the future other research projects might benefit from the use of these samples. Future research projects conducted on these samples will require additional IRB approval to ensure compliance with all relevant regulations**.** Coded frozen tissue samples, cells and other patient derived material will be stored at –80°C in labeled cassettes in secure freezers in Dr. Yamini's laboratory in the KCBD. No blood will be collected at sites other than the University of Chicago.

5-10 FFPE tissue sections will be obtained at University of Chicago for biomarker analysis. Also, 5-10 FFPE tissue sections from other study sites will be shipped to Dr. Yamini and Dr. Pytel at The University of Chicago where biomarker staining will be performed. Any unused tissue sections will be returned to the primary institution.

Address for sending tissue slides to University of Chicago:

Bakhtiar Yamini
MC3026
Neurosurgery
5841 S Maryland Ave
Chicago, IL 60637

Fedex account for shipments: Neurosurgery Department Fedex account: 160-712-139

13. DATA MANAGEMENT AND REPORTING

Data reporting will be performed utilizing the eVelos electronic data capture system. The University of Chicago CRA will provide you with the applicable user registration information. Velos is a secure, web-based application designed to support data capture for research studies, providing: 1) an intuitive interface for validated data entry; 2) audit trails for tracking data manipulation and export procedures; and 3) automated export procedures for seamless data downloads to common statistical packages. Velos is a HIPAA-secure data collection tool that can be used to meet 21 CFR-part 11 requirements. Databases can be quickly developed and customized for studies' needs. Each institution will enter patient information into Velos. No PHI will be publically available.

All required data must be recorded in the database at the completion of each cycle. AEs are to be entered in real time. SAEs are also to be reported on the Adverse Event Velos form and separately by email or fax to University of Chicago. The Serious Event Form should be sent within 24 hours of the site's knowledge of the event and sent via email (preferred) or fax to the University of Chicago (PhaseIICRA@medicine.bsd.uchicago.edu or

qaccto@bsd.uchicago.edu; Fax: 773-702-4889). All case report forms must be completed by designated study personnel. Each screened (consented) patient is to be entered into eVelos within 48 hours of patient registration. In addition to direct data entry, you may be required to provide supporting source documentation. Source records are original documents, data, and records (e.g., medical records, raw data collection forms, pharmacy dispensing records, recorded data from automated instruments, laboratory data) that are relevant to the clinical trial. Each site will prepare and maintain adequate and accurate source documents. These documents are designed to record all observations and other pertinent data for each subject enrolled in this clinical trial. Source records must be adequate to reconstruct all data transcribed onto the case report form.

14. STUDY MANAGEMENT AND REGULATORY AFFAIRS

14.1 Multicenter Guidelines

The specific responsibilities of the Principal Investigator and the Coordinating Center are presented in Section 14. Clinical studies coordinated by The University of Chicago must be conducted in accordance with the ethical principles that are consistent with Good Clinical Practices (GCP) and in compliance with other applicable regulatory requirements

The Study Lead PI/Coordinating Center is responsible for distributing all official protocols, amendments, and IND Action Letters or Safety Reports to all participating institutions for submission to their individual IRBs for action as required.

14.2 Institutional Review Board (IRB) Approval and Consent

Unless otherwise specified, each participating institution must obtain its own IRB approval. It is expected that the IRB will have the proper representation and function in accordance with federally mandated regulations. The IRB should approve the consent form and protocol.

In obtaining and documenting informed consent, the investigator should comply with the applicable regulatory requirement(s), and should adhere to Good Clinical Practice (GCP) and to ethical principles that have their origin in the Declaration of Helsinki.

Before recruitment and enrollment onto this study, the patient will be given a full explanation of the study and will be given the opportunity to review the consent form. Each consent form must include all the relevant elements currently required by the FDA Regulations and local or state regulations. Once this essential information has been provided to the patient and the investigator is assured that the patient understands the implications of participating in the study, the patient will be asked to give consent to participate in the study by signing an IRB-approved consent form.

Prior to a patient's participation in the trial, the written informed consent form should be signed and personally dated by the patient and by the person who conducted the informed consent discussion.

14.3 Required Documentation

Before the study can be initiated at any site, the following documentation must be provided to the Cancer Clinical Trials Office (CCTO) at the University of Chicago Comprehensive Cancer Center.

- A copy of the official IRB approval letter for the protocol and informed consent
- IRB membership list
- CVs and medical licensure for the principal investigator and any sub-investigators who will be involved in the study.

- The Investigator's signature documenting understanding of the protocol and providing commitment that this trial will be conducted according to all stipulations of the protocol is sufficient to ensure compliance.
- CAP and CLIA Laboratory certification numbers and institution lab normal values
- Investigational drug accountability standard operating procedures
- Additionally, before the study can be initiated at any site, the required executed research contract/subcontract must be on file with the University of Chicago.

14.4 Data and Safety Monitoring

This study will be remotely monitored by the designated University of Chicago Clinical Research Associate (CRA) in accordance with the University of Chicago, Section of Hematology/Oncology standard operating procedure titled Monitoring of Multi-Institutional Investigator Initiated Clinical Trials.

Prior to subject recruitment, and unless otherwise specified, a participating site will undergo a Site Initiation Teleconference to be conducted by the designated University of Chicago research team. The site's principal investigator and his or her study staff must attend the site initiation meeting.

Monitoring will be conducted to verify the following:

- Adherence to the protocol
- Completeness and accuracy of study data and samples collected
- Compliance with regulations
- Submission of required source documents

Participating sites will also undergo a site close-out teleconference upon completion, termination or cancellation of a study to ensure fulfillment of study obligations during the conduct of the study, and to ensure that the site Investigator is aware of his/her ongoing responsibilities.

Unless otherwise specified, this protocol will undergo weekly review at the multi-institutional data and safety monitoring teleconference as per procedures specified by the UC CCC NCI-approved Data and Safety Monitoring Plan. The conference will review:

- Enrollment rate relative to expectations, characteristics of participants
- Safety of study participants (Serious Adverse Event & Adverse Event reporting)
- Adherence to protocol (protocol deviations)
- Completeness, validity and integrity of study data
- Retention of study participants

Protocol deviations are to be documented using the Protocol Deviation Form and sent via email to PhaseIICRA@medicine.bsd.uchicago.edu. Deviations that are considered major because they impact subject safety or alter the risk/benefit ratio, compromise the integrity of the study data, and/or affect subjects' willingness to participate in the study must be reported within 7 days. Please contact the University of Chicago CRA (PhaseIICRA@medicine.bsd.uchicago.edu) if you have questions about how to report deviations. All major protocol deviations should also be reported to the local IRB of record according to their policies and procedures.

14.5 Auditing

In addition to the clinical monitoring procedures, the University of Chicago Comprehensive Cancer Center will perform routine Quality Assurance Audits of investigator-initiated clinical trials as described in the NCI-approved UC CCC DSM Plan. Audits provide assurance that trials are

conducted and study data are collected, documented and reported in compliance with the protocol. Further, quality assurance audits ensure that study data are collected, documented and reported in compliance with Good Clinical Practices (GCP) Guidelines and regulatory requirements. The audit will review subjects enrolled at the University of Chicago in accordance with audit procedures specified in the UC CCC Data and Safety Monitoring plan. For institutions who are formal members of the Personalized Cancer Care Consortium (PCCC), the UC CCC will conduct on site quality assurance audits on average every two years during the enrollment and treatment phase of the study.

Auditing procedures for participating sites that are not full members of the PCCC must be specified and approved by the UC CCC Clinical Research Advisory Committee. In general, for sites that are not full members of the PCCC, auditing responsibility will be delegated to the participating center, with the annual audit report forwarded to the University of Chicago for review.

A regulatory authority (e.g. FDA) may also wish to conduct an inspection of the study, during its conduct or even after its completion. If an inspection has been requested by a regulatory authority, the site investigator must immediately inform the University of Chicago Cancer Clinical Trials Office and Regulatory Manager that such a request has been made.

14.6 Amendments to the Protocol

All modifications to the protocol, consent form, and/or questionnaires will be submitted to the University of Chicago IRB for review and approval. A list of the proposed modifications or amendments to the protocol and/or an explanation of the need of these modifications will be submitted, along with a revised protocol incorporating the modifications. Only the Study Lead PI can authorize any modifications, amendments, or termination of the protocol. Once a protocol amendment has been approved by the University of Chicago IRB, the Regulatory Manager will send the amended protocol and consent form (if applicable) to the affiliate institutions electronically. Upon receipt of the packet the affiliate institution is expected to do the following:

- The affiliate must reply to the email from the Regulatory Manager indicating that the amendment was received by the institution and that it will be submitted to the local IRB.

- The amendment should be submitted to the affiliate institution's IRB as soon as possible after receipt. The amendment **must** be IRB approved by the institution **within 3 months** from the date that it was received.

- **The University of Chicago version date and/or amendment number must appear on the affiliate consent form and on the affiliate IRB approval letter.** The version dates can be found on the footer of every page of the protocol and consent form. The amendment number can be found on the University of Chicago IRB amendment approval letter that is sent with the protocol/amendment mailing.

- The IRB approval for the amendment and the amended consent form (if amended consent is necessary) for the affiliate institution must be sent to the designated UC Regulatory Manager as soon as it is received.

14.7 Annual IRB Renewals, Continuing Review and Final Reports

A continuing review of the protocol will be completed by the University of Chicago IRB and the participating institutions' IRBs at least once a year for the duration of the study. The annual IRB renewal approvals for participating institutions should be forwarded promptly to the Regulatory Manager. If the institution's IRB requires a new version of the consent form with

the annual renewal, the consent form should be included with the renewal letter.

14.8 Record Retention

Study documentation includes all CRFs, data correction forms or queries, source documents, Sponsor-Investigator correspondence, monitoring logs/letters, and regulatory documents (e.g., protocol and amendments, IRB correspondence and approval, signed patient consent forms).

Source documents include all recordings of observations or notations of clinical activities and all reports and records necessary for the evaluation and reconstruction of the clinical research study.

Government agency regulations and directives require that all study documentation pertaining to the conduct of a clinical trial must be retained by the study investigator. In the case of a study with a drug seeking regulatory approval and marketing, these documents shall be retained for at least two years after the last approval of marketing application in an International Conference on Harmonization (ICH) region. In all other cases, study documents should be kept on file until three years after the completion and final study report of this investigational study.

14.9 Obligations of Study Site Investigators

The Study Site Principal Investigator is responsible for the conduct of the clinical trial at the site in accordance with Title 21 of the Code of Federal Regulations and/or the Declaration of Helsinki. The Study Site Principal Investigator is responsible for personally overseeing the treatment of all study patients. He/she must assure that all study site personnel, including sub-investigators and other study staff members, adhere to the study protocol and all FDA/GCP/NCI regulations and guidelines regarding clinical trials both during and after study completion.

The Study Site Principal Investigator at each institution or site will be responsible for assuring that all the required data will be collected and entered into the CRFs. Periodically, monitoring visits or audits will be conducted and he/she must provide access to original records to permit verification of proper entry of data.

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APPENDIX A

Karnofsky Performance Status Scale Definitions Rating (%) Criteria		
Able to carry on normal activity and work; No special care needed.	100	Normal no complaints; no evidence of disease
	90	Able to carry on normal activity; Minor signs of symptoms of disease.
	80	Normal activity with efforts; some signs of symptoms of disease.
Unable to work; able to live at home and care for most personal needs; Varying amount of assistance needed.	70	Cares for self; unable to carry on normal activity or to do active work.
	60	Requires occasional assistance, but is able to care for most of his personal needs.
	50	Requires considerable assistance and frequent medical care.
Unable to care for self; Requires equivalent of institutional or hospital care; diseases may be progressing rapidly.	40	Disabled; requires special care and assistance.
	30	Severely disabled; hospital admission is indicated although death not imminent.
	20	Very sick; hospital admission necessary; Active support treatment necessary.
	10	Moribund; fatal processes progressing rapidly.
	0	Dead

Oxford Textbook of Palliative Medicine, Oxford University Press. 1993, 109.