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Protocol Title: Phase I / II Adaptive Randomized Trial of Vorinostat, Isotretinoin and Temozolomide in Adults with Recurrent Glioblastoma Multiforme

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1.0 OBJECTIVES

1.1 Phase I

To determine the maximum tolerated dose (MTD) of vorinostat + isotretinoin (cRA), temozolomide (TMZ) + cRA, and vorinostat + cRA + TMZ combinations in adult patients with recurrent glioblastoma multiforme (GBM) and anaplastic gliomas.

1.2 Phase II

Primary endpoint:

1. To determine the efficacy of vorinostat + cRA, versus TMZ + cRA, versus vorinostat + cRA + TMZ in patients with recurrent glioblastoma multiforme as determined by Progression Free Survival (PFS) using an adaptive randomization phase II trial design.

Secondary endpoints:

2. To determine the radiological response, progression free survival at 6 months, overall survival and unexpected toxicity in the three treatment arms.

3. To obtain exploratory data regarding histone 3 and 4 acetylation and p21 levels in tumor tissue and peripheral monocytes in a subset of surgical patients and in non-surgical patients with available tissue from prior surgical interventions.

4. To evaluate the occurrence of symptoms and correlate to disease progression and tolerance to treatment using the MD Anderson Symptom Inventory-Brain Tumor Module (MDASI-BT) self-reporting tool.

2.0 BACKGROUND

2.1 The Disease

GBMs are the commonest of adult gliomas and are notorious for their intrinsic heterogeneity, high morbidity and mortality. Management of these tumors requires multimodality treatment with maximum feasible surgery, radiation therapy and in some instances chemotherapy. However, despite aggressive measures, median survival is about 12-18 months reflecting the grim prognosis of patients with this disease. In patients >50 years of age, median survival is dismal with values of 5 - 7 months from diagnosis. These data have spurred the development of new approaches against these tumors that seek to target their biologic behavior. Several novel agents have emerged in the search for effective therapies against these malignancies. Most of them have been tried as single agents and several have failed to demonstrate statistically significant activity. Some treatments delineate subsets of

patients who respond to the therapy, the reasons for which are unclear. A recent Phase III trial showed that combining continuous low dose temozolomide (75 mg/m2/day x 42 days) with conventional radiation therapy followed by standard dose temozolomide therapy (200 mg/m2/day x 5 days every 28 days) for 6 months significantly prolonged survival in adult patients with GBM [1]. These data have led to widespread use of temozolomide as first line therapy for patients with GBM. However, when the tumor recurs, there is no established second line therapy that has shown benefit. This has generated an area unmet need for which several treatment options are currently being explored. 13-cis retinoic acid (cRA) has shown activity in this population in early phase II trials [2,3]. However, patients fail this treatment possibly because of development of retinoid resistance. Overcoming such resistance by use of agents such as vorinostat (SAHA), a histone deacetylase (HDAC) inhibitor, could potentially enhance the activity of retinoids against recurrent GBM. Additionally, the activity of retinoids in such a setting could also be enhanced by the use of cytotoxic agents such as temozolomide. Evidence of the potential benefit of such an approach was seen in a phase II trial combining temozolomide and cRA against recurrent GBM in which the progression free survival was better with the combination compared with historical controls treated with temozolomide alone [4]. These data lead us to propose the present study which aims to determine the potential of vorinostat + cRA, versus temozolomide + cRA, versus vorinostat + cRA + temozolomide, in an attempt to delineate the best combination against recurrent GBM.

2.2 Retinoids

Retinoids, the natural and synthetic derivatives of vitamin A, are clinically active in diverse premalignant and malignant conditions, such as acute promyelocytic leukemia, cutaneous T-cell lymphomas, leukoplakia, squamous cell carcinomas of the skin, and basal cell carcinomas. Although modest activity was observed in a randomized phase II trial in head and neck cancer [5], 13-cis-retinoic acid (cRA) has limited activity as a single agent in common advanced solid tumors, such as non-small cell lung, breast and colon cancer.

2.2.1 Retinoids and gliomas

The rationale for the use of retinoids in human gliomas comes from *in vitro* studies demonstrating differentiating and growth inhibitory effects of retinoic acid on glioma cells. The *in vitro* response among various cell lines studies is heterogeneous. The growth inhibitory effect in human glioma cells is related to decrease in EGF receptor mediated phosphorylation activity. A phase II study was conducted in MDACC to determine the efficacy of cRA in patients with recurrent malignant brain tumors [2]. Forty-three patients were treated with cRA at a starting dose of 80 - 120 mg/m2 per day for 3 weeks followed by one week without treatment, then repeated to comprise an 8-week course of therapy. Overall, 23 (53%) patients had either response or stable disease to this treatment; three (7%) patients had achieved partial response, 7 (16%) achieved minor response and 13 (30%) patients remained stable. Twenty patients had disease progression. The median time to tumor progression for these

patients was 16 weeks. Recent data from a second study also supports a role for retinoids in treatment of gliomas [3]. A phase II trial utilizing 13 cRA in combination with temozolomide in 98 patients with malignant gliomas showed that the combination was associated with 43% 6-month progression free survival compared with database survival rate of 21% with temozolomide as a single agent [4]. These preliminary results suggest that cRA alone or in combination with cytotoxic agents is active against malignant gliomas and more studies are warranted. In addition, these data suggest a role for retinoids in treatment of gliomas.

2.3 HDAC inhibitors

Chromatin is a highly specialized structure composed of tightly compacted chromosomal DNA. Gene expression within the nucleus is controlled, in part, by a host of protein complexes which continuously pack and unpack the chromosomal DNA from the inaccessible, tightly packed nucleosomal particles (heterochromatin) to the accessible, unwound nucleosomal particles (euchromatin). One of the known mechanisms of this packing and unpacking process involves the acetylation and deacetylation of histone proteins comprising the nucleosomal core.

The enzymes responsible for reversible acetylation-deacetylation of histone proteins are histone acetyltransferases (HATs) and histone deacetylases (HDACs), respectively. Acetylated histone proteins confer accessibility of the DNA template to the transcriptional machinery for expression. HDACs deacetylate histone proteins and thus, may act as transcriptional repressors. There is increasing evidence that HDAC or HAT activity is altered in many cancers, including gliomas [6]. Inhibitors of HDACs induce hyperacetylation of histones that modulate chromatin structure and gene expression. These inhibitors have been shown to inhibit growth, induce differentiation, and induce apoptosis of tumor cells in vitro and in vivo, and represent a promising therapeutic approach to cancer.

Three classes of HDACs have been identified. Class I human HDACs (HDAC1, HDAC2, HDAC3, HDAC8 and HDAC11) have homology to a yeast HDAC called Rpd3. Class II HDACs include HDAC4, HDAC5, HDAC6, HDAC7, HDAC9, and HDAC10. Class II HDACs are homologous to the yeast HDAC Hda1. The key catalytic residues have been conserved in both the Class I and Class II HDACs. The third class of human HDACs consists of homologues of yeast Sir2. The catalytic site of this third class is not similar to that of the Class I and Class II enzymes. Class III HDACs require nicotinamide-adenine dinucleotide (NAD) for activity.

Several HDAC inhibitors from multiple chemical classes have been developed and are currently in clinical trials. Among these, vorinostat (also known as suberoylanilide hydroxamic acid, SAHA, MK-0683 or L-001079038) is a potent HDAC inhibitor that can be administered orally with excellent bioavailability. Vorinostat was synthesized and identified first as a differentiation/apoptosis-inducing agent of transformed cells. Studying its mechanism of action led to the discovery of its HDAC inhibitory activity. Vorinostat inhibits the activity of both Class I (HDAC1 and 3) and Class II (HDAC6) HDACs, but does not inhibit the activity of Class III

HDACs (hSirT1).

Vorinostat binds directly to the catalytic pocket of HDAC enzymes. The ability of vorinostat to inhibit HDAC activity has been assessed in vitro using affinity purified HDAC1. The anti-neoplastic effect of vorinostat is attributed to the inhibition of HDAC activity and subsequent accumulation of acetylated histones, leading to the activation of genes whose expression causes induction of differentiation or apoptosis and the inhibition of tumor growth. Vorinostat causes G1 or G2 phase cell-cycle arrest, apoptosis, or differentiation in cultured transformed cells. The activity of vorinostat was investigated using the 60 cell line screen at National Cancer Institute (NCI) and IC50. Values ranging from approximately 500 nM to 5 μM were observed for growth inhibition activity of vorinostat. Vorinostat has also been shown to have synergetic and additive activity in combination with other cancer therapies (including radiation, kinase inhibitors, cytotoxic agents, and differentiating agents) in a variety of cultured human transformed cell lines [7].

2.3.1 HDAC inhibitors and gliomas

HDAC inhibitors have shown preclinical inhibition of glioma cell lines *in vitro* and *in vivo*. In particular, vorinostat upregulates p21, decreases VEGF levels and increases apoptosis in various glioma cell lines. Also, *in vivo* glioma xenograft studies demonstrate an increased survival time in rats treated with vorinostat. Other HDAC inhibitors, such as trichostatin A, FK228 and sodium butyrate, show similar effects *in vitro*. Ongoing clinical studies are testing anti-tumor activity of vorinostat either as a single agent or in combination with temozolomide in gliomas.

2.3.2 Preclinical experience with vorinostat

Vorinostat has been demonstrated to have biological and anti-neoplastic activity in murine and human xenograft models. Intraperitoneal administration of vorinostat causes significant tumor growth inhibition in human prostate cancer xenografts in mice; tumor regressions were observed at vorinostat doses (50 mg/kg/day) that did not produce toxic side effects. Intraperitoneal administration of vorinostat in combination with retinoic acid induced leukemic remission and prolonged survival in a therapy-resistant transgenic mouse model of acute promyelocytic leukemia (APL). Vorinostat administered to carcinogen-treated rats in feed over a period of 130 days increased tumor latency and reduced mammary tumor incidence, multiplicity, and volume. Vorinostat administered in feed for 5 weeks also inhibited growth of established rat mammary tumors. Vorinostat administered to carcinogen-treated mice in feed over a period of 18 weeks reduced lung tumor multiplicity. Intraperitoneal administration of vorinostat causes significant tumor growth inhibition in both human breast carcinoma and human colon carcinoma cancer xenografts in mice. Tumor growth inhibition was observed at vorinostat doses (100 mg/kg/day) that do not produce toxic side effects.

2.3.3 Pharmacokinetics and toxicity

The pharmacokinetics and toxicokinetics of vorinostat have been evaluated in mice, rats, dogs and humans. Rapid oral absorption has been noted in all species studied. Vorinostat (0.5 to 50 µg/mL) exhibited moderate reversible binding to plasma proteins. In human plasma, vorinostat appears to bind primarily to human serum albumin; however, some binding of vorinostat was also observed in solutions of α1-acid glycoprotein.

Vorinostat was excreted exclusively as metabolites, the major of which were largely attributed to glucuronidation and hydrolysis followed by β-oxidation pathways.

Although pharmacokinetic studies are still ongoing, oral vorinostat is able to inhibit histone deacetylases (target enzymes) in peripheral mononuclear cells at all oral doses starting with the 100 mg dose level. At higher dose levels of 400 mg and 600 mg, duration of target enzyme inhibition lasted at least 10 hours.

The major pathways of metabolism of vorinostat involve glucuronidation and hydrolysis followed by β-oxidation. As vorinostat is not eliminated via cytochrome P450 pathways, it is anticipated that vorinostat will not to be subject to drug-drug interactions when co-administered with drugs that are known to be CYP inhibitors. Although vorinostat was not a potent reversible inhibitor of the cytochrome P450 isozymes (IC50 >75 µM), gene expression studies detected some potential for suppression of CYP2C9 and CYP3A4 activities at ≥10 µM vorinostat; however, these changes were observed at concentrations higher than the pharmacologically relevant serum concentration of 2 µM (Cmax).

The main toxicities observed in animal models were weight loss and loss of appetite, apparent hemolytic anemia (rats only at 3.8 times the equivalent 400 mg human dose), leukopenia (rats only at 1.3 times the equivalent 400 mg human dose), thrombocytopenia (male rats only, statistically significant change at 0.5 times the equivalent 400 mg human dose but within normal range at all doses), and gastrointestinal tract irritation (dogs only, at 8.5 times the equivalent 400 mg human dose). Although statistically significant and dose-dependent, many of the clinical pathology findings were within normal historical ranges indicating that they that should not have major toxicological consequences. The toxicities appeared to be rapidly reversible within 12 to 14 days. There has been no evidence of cardiac toxicity based on electrocardiogram (ECG, dogs only), blood pressure (dogs only), heart rate (dogs only), creatinine kinase, organ weight, gross pathology, or histopathology assessments in studies up to one month duration. No serious, irreversible damage to any vital organ has been observed. Importantly, toxicities in rats and dogs were predictive of adverse effects in humans (anorexia, weight loss, fatigue). Toxicities present in animals would be manageable in the clinic, and the onset of serious toxicity is readily forecast by prodromal symptoms. The nonclinical toxicity profile of vorinostat is acceptable for an oncology drug.

Vorinostat was evaluated in a panel of genetic toxicity assays; in vivo and in vitro assays were found to be positive. Therefore, vorinostat should not be taken by pregnant women. Pregnancy should be avoided both in female subjects taking

vorinostat, and as data are not yet available to establish the safety of vorinostat ingestion in male patients who impregnate their partners, and in female partners of male subjects taking vorinostat for at least 30 days after last dose of vorinostat.

2.3.4 Clinical experience with vorinostat

As of 24-Oct-2005, 17 Merck, Sharp and Dohme, Corp.,. sponsored clinical trials and 3 Investigator sponsored studies have been initiated with vorinostat [7]. Over 305 patients with advanced malignancies have been treated with vorinostat alone or in combination with other chemotherapeutics. In addition, 29 studies with vorinostat are ongoing, sponsored by the United States National Cancer Institute (NCI) under the Cancer Therapy Evaluation Program (CTEP) with 144 enrolled patients. Therefore, over 449 patients have received at least one dose of vorinostat and additional studies are planned.

2.3.4.1 Safety and Toxicity

The total daily oral dose of vorinostat administered in these studies ranges from 200 mg to 900 mg. The tolerability of oral vorinostat appears to be determined by total daily dose and the length of consecutive days of dosing. The maximum tolerated dose (MTD) for continuous daily dosing without a rest period is 400 mg once a day or 200 mg twice a day (200 mg b.i.d.). The MTD for intermittent dosing is 300 mg b.i.d. x 3 consecutive days per week or 250 mg t.i.d. x 14 consecutive days followed by a 7-day rest. Dose-limiting toxicities (DLTs) are mainly non-hematologic (asthenia and weight loss). Hematologic toxicities were primarily anemia and thrombocytopenia, most of which were mild to moderate in intensity. The majority of DLTs occurred within the first month on oral vorinostat. At continuous daily dosing of 600 mg once daily, 300 mg b.i.d., and 400 mg b.i.d. that exceeded the MTD, the pattern and severity of DLTs were similar. The DLTs were manageable because these toxicities resolved quickly after drug administration was interrupted. The optimal dose, dose frequency, and dose duration remains under active investigation.

The types of adverse experiences seen in clinical trials of vorinostat were those usually associated with chemotherapy, such as fatigue, nausea, vomiting, cytopenias, etc. No new or unique adverse experiences were commonly observed. The three major clinical categories of adverse experiences attributable to vorinostat include a constellation of gastrointestinal symptoms, constitutional complaints, and cytopenias. However, most of the adverse experiences were manageable. In fact, most of the very common adverse experiences were reversible and could be managed using conventional supportive care for chemotherapy. On the whole, treatment with oral vorinostat was well tolerated for use in the outpatient setting.

Adverse experiences considered by the Investigators to be at least possibly related to vorinostat reported as of 24-Oct-2005 by ≥10% of patients across all Merck, Sharp and Dohme, Corp.,. sponsored vorinostat clinical studies are summarized below by medical dictionary for regulatory activities (MedDRA), system organ class (SOC) and preferred term:

SOC	Reported Possible Related Adverse Experiences in ≥10 % Overall Patients
Blood and Lymphatic System Disorders	Thrombocytopenia, anemia
Gastrointestinal disorders	Nausea diarrhea, vomiting, constipation, dry mouth
General disorders and Administration Site Conditions	Fatigue, pyrexia, chills, edema peripheral
Investigations	Blood creatinine increased, weight decreased, hemoglobin decreased, aspartate aminotransferase increased, platelet count increased, prothrombin time prolonged, alanine aminotransferase increase
Metabolism and Nutritional Disorders	Anorexia, hyperglycemia
Musculoskeletal, and Connective Tissue Disorders	Muscle spasm
Nervous System Disorders	Dizziness, dysgeusia
Respiratory, Thoracic and Mediastinal Disorders	Cough, dyspnea
Skin and Subcutaneous Tissue Disorders	Alopecia

Certain classes of patients were excluded from the clinical studies conducted to date, i.e., patients with significant hepatic or renal impairment or other major medical conditions that could have interfered with evaluation of study drug. In addition, patients who were pregnant or nursing were excluded from these studies. No formal studies evaluating the effect of age, gender, race or organ function (such as liver, kidney) on the pharmacokinetics and pharmacodynamics of vorinostat have been conducted. Accordingly, there are no clinical data for these populations.

Prolongation of prothrombin time (PT) and International Normalized Ratio (INR) were observed infrequently in patients receiving vorinostat concomitantly with coumarin-derivative anticoagulants. Physicians should carefully monitor PT and INR in patients concurrently administered vorinostat and coumarin derivatives.

Vorinostat should not be administered concomitantly with other HDAC inhibitors (e.g., valproic acid) as class-specific adverse reactions may be additive. Severe (Grade 4) thrombocytopenia with associated gastrointestinal bleeding and anemia has been reported with the concomitant use of vorinostat and valproic acid.

2.3.4.2 Efficacy

Although demonstration of efficacy was not a primary objective in Phase I clinical studies of vorinostat, preliminary anti-tumor activity was observed in patients with acute myeloid leukemia (AML), advanced multiple myeloma, lymphoma, laryngeal carcinoma, thyroid carcinoma, and mesothelioma. Similarly, significant anti-tumor activity was also observed in the Phase II clinical studies of vorinostat in patients with advanced cutaneous T-cell lymphoma (CTCL).

Preliminary observations of anti-tumor response in leukemia, lymphoma, myeloma, mesothelioma, laryngeal carcinoma and stable disease in thyroid carcinoma require confirmation in phase II and III trials. The initial trials of vorinostat activity in combinations with other anti-tumor medications are ongoing.

2.4 Temozolomide and gliomas

Temozolomide, an oral alkylating agent with good penetration of the central nervous system, has been evaluated in patients with glial malignancies. Initial studies evaluated the efficacy of temozolomide in patients with recurrent glioblastoma and anaplastic glioma. A large, randomized phase II study by Yung and colleagues treated patients with recurrent glioblastoma with either temozolomide (200 mg/m^2 days 1-5 of a 28-day cycle) or procarbazine (150 mg/m^2 28 day-on, 28-day off schedule) (Yung, Albright et al. 2000). The study demonstrated only a modest objective response rate for both regimens (approximately 5%), but a superior 6-month progression-free survival rate for temozolomide (21% vs. 9%) was found. In the pre-radiation setting, a phase II study demonstrated a good objective response rate (complete plus partial response = 41%) in patients with glioblastoma during the four monthly cycles of treatment, using 200 mg/m^2 on days 1 to 5 of a 28-day cycle. However, the responses were not durable in many cases and the median progression-free survival rate was 3.8 months. Overall survival for patients on this study was 13.1 months, similar to most reports of treatment in newly diagnosed patients. This suggests that the neoadjuvant temozolomide chemotherapy likely had little overall benefit. However, these results did demonstrate definite activity of temozolomide for glioblastoma.(Gilbert, Friedman et al. 2002)

Stupp and colleagues performed a phase III trial in patients with newly diagnosed glioblastoma, administering a daily lower dose (75 mg/m^2) of temozolomide every day during the course of radiation therapy, followed by 6 months of adjuvant chemotherapy at the standard single-agent dose of 200 mg/m^2 for days 1 to 5 of a 28-day cycle.(Stupp, Mason et al. 2005) This study, performed by the EORTC and the NCIC, randomized patients with newly diagnosed glioblastoma to receive either radiation therapy alone or concurrent radiation and temozolomide followed by 6 months of adjuvant temozolomide. The study demonstrated a statistically significant improvement in median survival for the combination treatment arm (12.1 vs. 14.6 months) as well as a significant increase in 2-year survival (10% vs 26%). Eighty-eight percent of the patients received the full course of concurrent temozolomide with radiation. Approximately 40% of patients received the full 6 cycles of temozolomide after the completion of the radiation (adjuvant therapy). Tumor progression was the most prominent cause of treatment cessation. The chemoradiation treatment was well tolerated, with an incidence of grade 3 or 4 hematologic toxicity of < 4%. This chemoradiation regimen has been widely accepted as the new standard of care for patients with newly diagnosed glioblastoma.

As described above, incorporation of a dose-dense strategy for recurrent primary brain tumors is supported by the results of a recently reported clinical trial that treated patients with recurrent malignant glioma with the week on/week off dosing schedule of temozolomide at a dose of 150 mg/m^2 /day. This study reported a 6-month progression free survival rate of 46%, which compares favorably with the 6-month PFS rate of 21% reported with conventional 5 day of a 28 day cycle of temozolomide (Yung, Albright et al. 2000; Wick, Felsberg et al. 2007).

In this study, we will use this schedule to test if combining temozolomide with vorinostat and retinoids can enhance the activity of the agent against recurrent GBMs.

2.5 Rationale for combining retinoids and HDAC inhibitors

At the molecular level, the biologic effects of retinoids are modulated through nuclear receptors (named RXRs and RARs). The activated nuclear receptors control the expression of genes that mediate retinoid effects, including induction of cell differentiation, inhibition of proliferation by cell cycle arrest, and induction of apoptosis. Retinoid receptors behave as ligand-dependent transcriptional regulators, repressing transcription in the absence of ligand and activating transcription in its presence. The different effects on transcription are carried out through recruitment of co-regulators: unliganded receptors bind corepressors (NCoR and SMRT) that are found within a complex containing histone deacetylase (HDAC) activity, whereas liganded receptors recruit coactivators with histone acetylase activity (HATs).

The mechanisms of retinoid resistance are not well known [23]. Several mechanisms have been proposed, including pharmacologic mechanisms (acceleration of metabolism and decreasing plasma levels), and molecular mechanisms (structural mutations in the retinoid receptor, altered associations with corepressor molecules). Retinoid resistance seems to include increased binding of corepressor/HDAC complexes. Thus, HDAC inhibition may counteract the effect of corepressors, causing a DNA conformation that facilitates retinoid-induced gene transcription and cell differentiation [24].

2.6 Rationale for combining temozolomide with retinoids and HDAC inhibitors

We hypothesize that the combination of retinoids with HDAC inhibitors can result in overcoming of retinoid resistance. This would be expected to provide a robust cytostatic effect which may result in prolonged tumor stabilization. We propose to extend this rationale by exploring the potential for a DNA damaging agent such as temozolomide to convert the cytostatic effect of the combination to a cytotoxic response which may potentiate the effects of all agents used in this protocol. Because of non-overlapping toxicities and varied mechanisms of activity of these agents, we hypothesize that this strategy will result in a potent effect against GBMs. In addition, there is a basis for predicting synergy among these three compounds from preclinical and clinical studies - for example, in preclinical studies, HDAC inhibitors such as vorinostat can synergize with temozolomide or carboplatin to increase cell death over that achieved by either agent alone (Puduvalli et al and

Wen et al, unpublished data).. Also, a phase II study of isotretinoin and temozolomide in patients with recurrent GBMs showed an improvement in 6 month PFS compared with historical controls treated with temozolomide alone [4], suggesting that the combination of DNA damaging agents such as temozolomide, and isotretinoin can be active against these tumors. In addition, an ongoing phase I study of temozolomide combined with vorinostat has completed enrollment with the most recent dose level being 500 mg/day on a days 1-7 and 15-21 day dosing of vorinostat and 150 mg/day on days 1-5 of temozolomide on a 28 day schedule. Toxicities seen in this trial included fatigue and thrmobocytopenia. A dose dense schedule of temozolomide has not yet been explored but the data from the ongoing trial will provide a basis for a limited dose level lead-in trial of this combination as proposed in this study. The data presented above also provide support for the approach proposed in this study.

2.7 Rationale for Dosing Schedule

Preclinical studies of combination of the HDAC inhibitor vorinostat (SAHA) and cRA in glioma cells *in vitro* show that simultaneous administration of SAHA and cRA was more potent in inducing apoptosis compared with the schedules of cRA followed by vorinostat or vorinostat followed by cRA (Puduvalli et al, unpublished data). Hence, we have chosen to initiate treatment simultaneously with vorinostat (days 1-14) and cRA (days 1-21) in Arm 1. For Arm 3 which has temozolomide in addition to cRA and vorinostat, we will use a days 1-7 and 15-21 dosing of vorinostat to match with temozolomide dosing schedule (also days 1-7 and 15-21) . HDAC inhibition increases the expression of apoptosis related genes such as caspase 8 and death receptors such as DR5. This may result in the glioma cells being potentially primed to apoptosis in the presence of an appropriate cytotoxic signal. We hypothesize that the addition of a cytotoxic agent such as temozolomide (known to have activity against gliomas) with HDAC inhibition will result in a more robust induction of apoptosis, potentially translating into clinical benefit. In vitro studies have shown that SAHA can potentiate cisplatin induced apoptosis in glioma cells. Based on these data, we propose to administer temozolomide on day 1 -7 and 15-21 of each 28 day cycle along with SAHA.

2.8 Rationale for study design

To assess the activity of a new treatment regimen, conventional phase II designs use either a single arm design compared with historical controls or a randomized design with a control arm of standard treatment, if such a standard is identified. For patients with recurrent GBMs, there are no extant standards of care. In a paradigm such as the one being studied in the proposed trial, testing each combination against historical controls would involve three separate trials, a process that will not provide timely answers to the question at hand nor allow utilization of resources in an efficient manner. To overcome some of these hurdles and test these combinations against each other as well as historical controls, this study proposes to utilize a bayesian adaptive randomization design, which will pit the three treatment regimens against each other. This will result in progressive bias of randomization towards the

best of the three regimens in a single trial. The "winner" of these three regimens will be considered the best regimen for further testing in a larger trial if it compares favorably with historical controls [25, 26, 27].

2.9 Rationale for obtaining exploratory data

The identification of markers that are associated with tumor but not with normal tissue has allowed the development of highly-specific targeted therapies with promising future for treatment of cancer. Targeted therapies are tailored to deregulate the signaling pathways involved in malignant progression. Advances in our understanding of the molecular changes and resultant cellular effects in malignant glioma are expanding the role of targeted therapies for treatment of these tumors. However, due to the complexity of the signaling pathways in gliomas and the inter- and intratumoral heterogeneity of the disease, only a small proportion of patients obtains clinical benefit from these therapies. Further analysis of the signaling pathways in tumor tissue before and after treatment may help to delineate the subset of patients for which these targeted therapies would be of more benefit [28]. For this purpose, we propose to determine the effect of vorinostat on brain tumor tissue by directly assessing the effect of the agent on histone acetylation in tumor specimens. To do this, we will include in our study 10 patients among those recruited for the Phase II trial who have been offered surgical resection of their recurrent tumors because of clinical indications, and who consent to taking vorinostat for 3 days prior to tumor resection, with the last dose to be given on the morning of surgery (see 5.2.2). One important goal of this translational study is to demonstrate that vorinostat can inhibit HDAC activity and cause histone hyperacetylation in tumor tissue. Histone H3 and H4 acetylation in response to vorinostat at a dose of 200 mg in PBMCs is transient and lasts for 4-6 hours. Preliminary data suggest that the duration of hyperacetylation may be prolonged to 8-10 hours if a dose of 400 mg is used. Thus, the dose of the agent and the timing of tissue acquisition and processing will be important determinants for demonstrating the effects of vorinostat on histone acetylation. While we recognize that the biological effects of vorinostat may occur beyond the period of changes in histones, the demonstration of histone hyperactylation is proof of principle of primary target modulation by the agent. Treatment of patients for 3 days (72 h) prior to surgery also allows us to assess the late changes induced by vorinostat such as increased levels of p21 or reduction of cyclin B1. Tissue obtained at surgery will be studied for these effects by immunohistochemistry. Other markers of interest in this context including cell cycle regulatory molecules and apoptosis markers will also be assessed. Non-surgical patients will be asked to participate in this component of the trial provided that tumor tissue is available from prior surgical interventions. In these patients, we will determine the baseline acetylation and p21 levels in tumor tissue and peripheral monocytes. Treatment-related changes in peripheral monocytes after vorinostat administration will be determined as a surrogate of vorinostat effect in tumor tissue. We will also test the same markers in a group of archived tumor tissue from age and relapse-number matched recurrent glioblastoma multiforme patients.

2.10 Rationale for Symptom Assessment Evaluation

Glioblastoma multiforme is a highly malignant type of tumor with recognized impact on the patients' physical, and neurologic function [29]. Most patients do succumb to their illness within two years. This study seeks to improve on these clinical results, hypothesizing that using a combination of cytotoxic and cytostatic agents will result in improved survival. However, given the intensive nature of this regimen, it will be important to determine whether any survival benefit is associated with an improvement in symptoms or is the increase in survival offset by a worsening of these parameters. Precedence for measuring "non-therapeutic" endpoints exists in oncology research. For example, Gemcitabine was approved by the FDA partially as a consequence of the decrease in pain reported in pancreatic patients who were treated, not on the basis of survival improvement which was modest, at best [30]. There have been efforts in neuro-oncology to evaluate secondary endpoints using validated instruments as an additional indicator of benefit.

The M.D. Anderson Symptom Inventory-Brain Tumor Module (MDASI-BT) allows the self reporting of symptom severity and interference with daily activities. It has demonstrated reliability and validity in the primary brain tumor patient population [31]. This tool represents a modification of the widely used and validated MDASI, with particular attention to symptoms common in patients with brain tumors. The availability of validated instruments provides an opportunity to prospectively assess the impact of treatment, both positive and negative, on patients. This evaluation of symptom burden in this study will assist in finding the best possible treatment with the least toxicity.

2.11 Preliminary Data from recently completed Phase I trial of vorinostat, isotretinoin and Carboplatin

The proposed trial is a modification of an existing single institution IRB approved trial that is ongoing in MDACC. In the original phase I portion of this trial, we had proposed to test the tolerability and efficacy of the combination of vorinostat, isotretinoin and ***carboplatin.*** An MTD was established for the combination of vorinostat (400 mg/day on days 1-14) and isotretinoin (100 mg/m2/day on days 1-21) on a 28 day schedule (Arm 1). Similarly, the MTD for the combination of carboplatin (AUC 5 on day 1) and isotretinoin (100 mg/m2/day on days 1-21) has also been established (Arm 2). However, when the test combination of vorinostat, isotretinoin and carboplatin were combined in a three drug regimen (Arm 3), the starting dose level and two lower dose levels (dose levels 0, -1 and -2) proved intolerable; this required amending the protocol to introduce a third lower dose level (level - 3) which has currently accrued three patients. A dose level below this is not considered of interest for this tumor type. The overlapping toxicity of carboplatin and vorinostat appears to be the primary reason for the intolerance of this regimen; hence, we propose to explore temozolomide as an alternative cytotoxic agent to carboplatin which has known activity against malignant gliomas and is currently being tested in combination with vorinostat in the upfront setting. The DSMB has been notified of these results and we have received approval for holding accrual until the current amendment is instituted after IRB review.

3.0 DRUG BACKGROUND

3.1 Isotretinoin (13-cis retinoic Acid, cRA)

3.1.1 Therapeutic Classification

Retinoid.

3.1.2 Pharmacologic Data

Isotretinoin is absorbed from the gastrointestinal tract. Due to its high lipophilicity, oral absoprtion is enhanced when given with a high-fat meal. Minimal systemic absorption occurs following topical application. Peak plasma concentrations occur 1 to 4 hours after oral doses. Oral bioavailability is low, possibly due to metabolism in the gut wall and first-pass metabolism in the liver. Isotretinoin is highly bound to plasma proteins (99.9%), primarily albumin. It is metabolized in the liver to its major metabolite 4-oxo-isotretinoin; there is also some isomerisation of isotretinoin to tretinoin. Isotretinoin, tretinoin, and their metabolites undergo enterohepatic recycling. The terminal elimination half-life of isotretinoin is 10 to 20 hours, while that of the 4-oxo metabolite may be up to 50 hours; return to physiological levels of retinoids takes about 2 weeks after stopping therapy. Equal amounts of a dose appear in the feces, mainly as unchanged drug, and in the urine as metabolites.

3.1.3 Pharmaceutical Data

13-cis-Retinoic acid (cRA) is a synthetic analogue of vitamin A. Chemical name: (13Z)-15-Apo- beta- caroten-15-oic acid; (2Z,4E, 6E,8E)-3,7-Dimethyl -9-(2,6,6-trimethyl cyclohex-1-enyl) nona-2,4,6,8- tetraenoic acid. It is a yellow to orange crystalline powder with a molecular weight of 300.44. Isotretinoin is practically insoluble in water; sparingly soluble in alcohol, in isopropyl alcohol, and in macrogol 400; soluble in chloroform.

3.1.4 Usual Dosage Range

Isotretinoin is FDA approved for treatment of severe acne. Isotretinoin inhibits sebaceous gland function and keratinization in pharmacological dosages of 0.5 to 1.0 mg/Kg/day. Isotretinoin has been used in doses up to 100 mg/m2 daily in divided doses for 21 consecutive days, followed by 7 drug-free days for the treatment of recurrent malignant gliomas [2,3].

3.1.5 Route of Administration

Oral.

3.1.6 Supplied

Isotretinoin is commercially available. ACCUTANE® is available in 10, 20 and 40 mg soft gelatin capsules for oral administration. Patients, prescribers, pharmacies and wholesalers must be registered with the iPLEDGE program. This is a computer-based risk management program designed to eliminate fetal exposure to isotretinoin through a special restricted distribution program approved by the FDA. The iPLEDGE program requires that all patients meet qualification criteria and monthly program requirements (see 4.17, 6.4, and Appendix I).

3.1.7 Side Effects and Toxicities

Common side effects include arthralgias, back pain, cheilitis, conjunctivits, dry/itchy skin, dry mouth and epistaxis. Serious side effects include birth defects, depression, psychosis and, rarely, suicidal ideation, suicide attempts, suicide, and aggressive and/or violent behavior; pseudotumor cerebri, anaphylactic reactions, elevated triglycerides, pancreatitis, hearing impairment, hepatotoxicity, inflammatory bowel disease, neutropenia, rhabdomyolysis, decrease in bone mineral density and visual disturbances.

3.2 Vorinostat (Suberoylanilide Hydroxamic acid, SAHA)

3.2.1 Therapeutic Classification

Histone deacetylase (HDAC) inhibitor, antineoplastic.

3.2.2 Pharmacologic Data

Plasma samples for pharmacokinetics were collected in early dose-ranging studies with intravenous and oral vorinostat. Exploratory pharmacokinetic analyses of oral vorinostat were conducted in a Phase I trial (Protocol 006). However, the vorinostat clinical assay was not validated at the time of these analyses. Single dose, multiple dose, and fed/fasted pharmacokinetic data were obtained in Protocol 008 using a validated clinical assay. This study was conducted in patients with advanced, refractory cancer using the dose of 400 mg once daily. The apparent t½ of vorinostat was found to be short, on the order of 1.5 hours. In the individual concentration-time profiles (but not the mean profiles), there were distinct secondary peaks (likely due to continuing absorption), which contributed to the variability in Tmax (range ~0.5 to 14 hours). A high-fat meal was associated with a small increase in the extent of absorption of vorinostat; the geometric mean ratio for AUC0-∞ and 90% CI were 1.38 (1.21, 1.57). A high-fat meal was also associated with a modest decrease in the rate of absorption. A lag time of at least 15 minutes was observed before detectable levels of vorinostat were observed in serum in the fed state in most subjects. Additionally, Tmax was modestly delayed (from 1.5 to 4 hours) and Cmax was similar. The mean apparent t½ was similar between the fasted and fed states. The clinical significance of these small effects is uncertain; however, most of the vorinostat studies were conducted in the fed state it is recommended that vorinostat be administered with food.

The pharmacokinetics of the two principal inactive metabolites of vorinostat were also evaluated following vorinostat dosing in Protocol 008, (*O*-glucuronide of vorinostat [L-001302381] and 4-anilino-4-oxobutanoic acid [L-000341257]). The apparent t½ of the *O*-glucuronide of vorinostat (~1.8 hours) was similar to that of vorinostat while that of the 4-anilino-4-oxobutanoic acid was longer (~6 to 9 hours). The mean serum exposures (AUC) of the *O*-glucuronide and 4-anilino-4-oxobutanoic acid metabolites were 3- to 4-fold and 10- to 13-fold higher, respectively, compared to that of vorinostat.

In general, the pharmacokinetics of vorinostat upon multiple dose administration is qualitatively similar to that following single dose administration. The trough concentrations following 8 and 21 days of multiple dosing were generally below the limit of quantification, which is consistent with the short apparent t½. The apparent t½ was similar following a single dose and following 22 days of repeat administration. The geometric mean vorinostat AUC accumulation ratio was 1.21. Based on an apparent t½ of 1.5 hours, no accumulation would be expected with once daily dosing; however, it is possible that intraindividual variability may have contributed to a slightly higher ratio. Because the mechanism of action of HDAC inhibitors like vorinostat may involve changes in gene expression that could persist well after serum concentrations decline, the low trough concentrations do not predict that a once daily regimen would be ineffective.

Pharmacodynamic assessment evaluating histone acetylation in peripheral blood mononuclear cells (PBMC) was performed. Inhibition of histone deacetylases (target enzymes) was achieved in PBMC at the 200 mg dose level. At higher dose levels of 400 mg and 600 mg, duration of target enzyme inhibition lasted at least 10 hours.

The major pathways of vorinostat metabolism involve glucuronidation and hydrolysis followed by beta-oxidation. As vorinostat is not eliminated via cytochrome P450 (CYP) pathways, it is anticipated that vorinostat will not to be subject to drug-drug interactions when co-administered with drugs that are known to be CYP inhibitors or inducers. Vorinostat was not a potent reversible inhibitor of the cytochrome P450 isozymes (IC50 >75 μM). Gene expression studies detected some potential for suppression of CYP2C9 and CYP3A4 activities at ≥ 10 μM vorinostat, however, these changes were observed at concentrations higher than the pharmacologically relevant serum concentration of 2 μM (Cmax).

3.2.3 Pharmaceutical Data

Chemical name: N-hydroxy-N'-phenyl-octane-1,8-diotic acid diamide; N-hydroxyl-N'-phenyl (9CI) octanediamide Suberoylanilide hydroxamic acid. It is a white to light orange powder.Molecular weight is 264.32. Solubility in water is $\leq$ 5 mg/mL.

3.2.4 Usual Dosage Range

The total daily dose administered in vorinostat trials ranges from 200 mg to 900 mg. The tolerability of oral vorinostat appears to be determined by total daily dose and

the length of consecutive days of dosing. The administration frequency across all studies included once daily, (QD continuous dosing), twice daily (BID) continuous dosing, twice daily discontinuous dosing and three times daily (TID) discontinuous dosing. The lowest total daily dose of vorinostat used was 200 mg (once daily) and the highest total daily doses were 600 mg (once daily), 800 mg (400 mg twice daily), and 900 mg (300 mg three times daily). In Protocols 003, 004, 005, 011, 012, 013, 014 015, 029, and 030 on a 14 consecutive day, twice daily schedule followed by a 7-day rest period for each 21-day cycle was tested either alone or in combination with other chemotherapeutics. In protocol 003 and 004, a 200 mg twice daily dosage on this schedule was tolerable. This was anticipated as a 200 mg twice daily dosage was tolerable on a continuous basis in Protocol 006. In Protocols 011 and 013, initial dose levels of 400 mg twice daily for 14 consecutive days, followed by a 7 day rest, and 300 mg twice daily for 14 consecutive days, followed by a 7 day rest period out of 21 days exceeded the MTD due to thrombocytopenia.

The maximum tolerated dose (MTD) was determined based upon dose limiting toxicities observed in Protocol 006. The maximum tolerated once daily dose is 400 mg. The 600 mg once daily dose given continuously was not tolerated. The maximum tolerated twice daily dose is 200 mg. The 300 mg twice daily dose given continuously was not tolerated. The maximum tolerated three times per day dose is 200 mg for 14 consecutive days followed by 7 days of rest. The 300 mg dose given three times daily on this schedule was not tolerated. The maximum tolerated twice daily dose for 3 consecutive days per week is 300 mg. The 400 mg twice daily dose on this schedule was not tolerated.

3.2.5 Route of Administration: Oral.

3.2.6 Supplied

Vorinostat is supplied by Merck and Co., Inc. Vorinostat is supplied as a white, opaque gelatin, size 3 capsule, containing 100 mg of vorinostat. The inactive ingredients contained in each capsule are microcrystalline cellulose, sodium croscarmellose, and magnesium stearate. The capsules are supplied in HDPE (high-density polyethylene) bottles. Each bottle contains the protocol specified count of vorinostat capsules. The shelf-life of the 100 mg capsule is 2 years.
Special Handling Requirements: Vorinostat is an anticancer drug. Spills of powder from vorinostat capsules due to damaged or broken capsules should be cleaned up carefully to minimize inhalation of vorinostat. The affected area must be washed at least 3 times with ethyl alcohol, followed by water. Direct contact of the powder in vorinostat capsules with the skin or mucous membranes should be avoided. If such contact occurs, wash thoroughly. Vorinostat capsules should be stored at room temperature (do not store above 30°C) in a dry, limited-access area. Care should be taken to maintain acceptable storage temperature. Vorinostat capsules should not be opened or crushed and must be administered whole.

3.2.7 Side Effects and Toxicities reported in Preclinical and Clinical Studies of Vorinostat (CAEPR version 2.4, June 12, 2006; CIB Version 3.0, June 23, 2006

A. Toxicities in Preclinical Studies: The main toxicities observed in animal models were weight loss and inappetence, apparent hemolytic anemia (rats only at 3.8 times the equivalent 400 mg human dose), leukopenia (rats only at 1.3 times the equivalent 400 mg human dose), thrombocytopenia (male rats only, statistically significant change at 0.5 times the equivalent 400 mg human dose but within normal range at all doses), and gastrointestinal tract irritation (dogs only, at 8.5 times the equivalent 400 mg human dose). Although statistically significant and dose-dependent, many of the clinical pathology findings were within normal historical ranges indicating that they that should not have major toxicological consequences. The toxicities appeared to be rapidly reversible within 12 to 14 days. There has been no evidence of cardiac toxicity based on electrocardiogram (ECG, dogs only), blood pressure (dogs only), heart rate (dogs only), creatinine kinase, organ weight, gross pathology, or histopathology assessments in studies up to one month duration. No serious, irreversible damage to any vital organ has been observed. Importantly, toxicities in rats and dogs were predictive of adverse effects in humans (anorexia, weight loss, fatigue).

B. Toxicities in Clinical Trials: Among the 101 patients with **solid tumor patients** treated with vorinostat, the most commonly reported adverse experiences were fatigue (81.2%), nausea (70.3%), anorexia (64.4%), vomiting (53.5%), hyperglycemia (52.5%) diarrhea (40.6%), and blood creatinine increased (40.6%). Fatigue, the most commonly reported adverse experience was considered by the Investigator to be at least possibly related to study drug for 71 (70.3%) patients with an intensity of Grade 3 or higher in 16 (15.8%) patient. Other than fatigue, the most commonly reported adverse experiences assessed by the Investigator as at least possibly related to vorinostat were nausea (65.3%), anorexia (62.4%), vomiting (45.5%), hyperglycemia (41.6%) and blood creatinine increased (37.6.%) and diarrhea (34.7%).

Overall, among the 305 patients with **all advanced malignancies** treated with vorinostat in, the most commonly reported adverse experiences were fatigue (68.9%), nausea (60%), diarrhea (55.1%), anorexia (47.9%), and hyperglycemia (38.7%). Fatigue, the most commonly reported adverse experience was considered by the Investigator to be at least possibly reported to study drug for 192 (63.0%) patients with an intensity of Grade 3 or higher for 42(13.8%) patients. Other than fatigue, the most commonly reported adverse experiences assessed by the Investigator as at least possibly related to vorinostat were nausea (55.4%), anorexia (46.2%), diarrhea (49.5%), and hyperglycemia (25.2%).

As of 30-Nov-2005, of the 305 patients exposed to vorinostat, there were 24 deaths reported. Most of these deaths (87.5%) were considered by the Investigator to be not drug-related, of which 14 (58.3%) were attributed to progression of underlying disease. The remaining 3 deaths (AN1008 and AN1048 in Protocol 001 and AN003 in Protocol 011) were due to single cases of unknown cause, ischemic stroke, and tumor hemorrhage, which were considered by the Investigator to be related to study drug. A total of 5 participants in the CTCL trials (3 in the 400-mg daily treatment

group) died during the study observation period. Ten solid tumor patients (one in the 400-mg daily treatment group) and 9 non-CTCL hematologic malignancy patients (none in the 400-mg daily treatment group) died during the study observation period.

Likely (>20%) adverse events also included constipation, dry mouth (xerostomia), heartburn/dydpepsia, taste alteration (dysgeusia), increase in ALT and AST, changes in creatinine, hypophosphatemia, muscle weakness, dizziness and cough.

Less likely adverse effects (< 20%) are leukopenia, neutropenia, , abdominal pain, and mucositis/stomatitis. Also reported on vorinostat trials, but with the relationship to vorinostat still undetermined: allergy/immunology (vasculitis), cardiovascular (palpitations, tachycardia, hypertension, hypotension), coagulation (INR, PTT alterations), constitutional symptoms (fever, insomnia, rigors/chills, weight loss), death, dermatology/skin (alopecia, ecchymosis, pruritus, rash), gastrointestinal (colonic obstruction, dysphagia, esophagitis, flatulence, gastritis, heartburn, hemorrhage/bleeding (epistaxis, hemoptysis, melena, petechiae, subdural hematoma), infection (febrile neutropenia, infection), lymphatics (limb edema, swelling face), metabolic/laboratory (alkaline phosphatase, hyperbilirubinemia, hypercalcemia, hyperkalemia, hypermagnesemia, hypernatremia, hyperuricemia, hypoalbuminemia, hypomagnesemia, hyponatremia, low serum bicarbonate), musculoskeletal/soft tissue (gait/walking, rib fracture), neurology (ataxia, confusion, depression, dizziness, sensory neuropathy, speech impairment, tremor), ocular/visual (eyelid ptosis), pain (back pain, chest pain, face pain, flank pain, gingival pain, groin pain, headache, joint pain, limb pain, muscle pain, neck pain, pharyngolaryngeal pain), pulmonary/upper respiratory (dyspnea, hiccoughs, pleural effusion, pneumonitis/pulmonary infiltrates, sinus congestion, wheezing), renal/genitourinary (urinary frequency, urinary incontinence, urinary retention), vascular (deep vein thrombosis).

Dose limiting toxicities are anorexia, dehydration, diarrhea, and fatigue. Prothrombin time and INR prolongations have been reported in patients taking vorinostat concomitantly with coumarin derivative anticoagulants. Thus, monitor these patients more frequently for alterations in their coagulation parameters may be necessary.

To prevent dehydration, patients should consume at least 2 liters of fluid orally, daily. If patients are experiencing dysgeusia, popsicles or Gatorade may be recommended. If diarrhea occurs, it should be managed with loperamide according to institutional guidelines. Nausea should be managed according to standard practice; 5HT-3 antagonists have proven effective.

Vorinostat was evaluated in a panel of genetic toxicity assays; *in vivo* and *in vitro* assays were found to be positive. Therefore, vorinostat should not be taken by pregnant women. Both women and men of reproductive potential participating in vorinostat studies must be completely informed of the unknown risks of pregnancy and agree not to become pregnant or father a child during the time they are participating in the study until at least 30 days after the final dose. Further, appropriate contraception must be used.

Vorinostat-related Clinical or Laboratory Adverse Experiences by Preferred Term as experienced by ≥ 10% of all Patients

	Total Patients (N=305)	
	Related Experiences Only	
	All Grades	Grade 3-5
	n %	n %
Fatigue	195 (63.9)	42 (13.8)
Nausea	170 (55.7)	17 (5.6)
Diarrhoea	151 (49.5)	14 (4.6)
Anorexia	141 (46.2)	15 (4.9)
Vomiting	100 (32.8)	5 (1.6)
Hyperglycaemia	79 (25.9)	10 (3.3)
Blood Creatinine Increased	75 (24.6)	2 (0.7)
Weight Decreased	75 (24.6)	4 (1.3)
Thrombocytopenia	69 (22.6)	34 (11.1)
Constipation	54 (17.7)	3 (1.0)
Dysgeusia	54 (17.7)	0 (0.0)
Haemoglobin Decreased	53 (17.4)	10 (3.3)
Dehydration	45 (14.8)	25 (8.2)
Hypocalcaemia	42 (13.8)	3 (1.0)
Platelet Count Decreased	41 (13.4)	13 (4.3)
Dyspnoea	40 (13.1)	1 (0.3)
Anaemia	36 (11.8)	7 (2.3)
Hyponatraemia	36 (11.8)	5 (1.6)
Alopecia	35 (11.5)	0 (0.0)
Dry Mouth	35 (11.5)	0 (0.0)
Aspartate Aminotransferase Increased	34 (11.1)	2 (0.7)
Alanine Aminotransferase Increased	33 (10.8)	1 (0.3)
Hypokalaemia	33 (10.8)	6 (2.0)
Blood Alkaline Phosphatase Increased	31 (10.2)	1 (0.3)
Hypoalbuminaemia	31 (10.2)	1 (0.3)
White Blood Cell Count Decreased	31 (10.2)	4 (1.3)

A patient is counted only once within a specific preferred term, even if more than one adverse experience with specific preferred term occurred. Adverse experience terms are from MedDRA Version 8.1

3.3 Temozolomide

3.3.1 Therapeutic Classification

Alkylator agent, antineoplastic.

3.3.2 Pharmacologic Data

Temozolomide (TMZ, Temodar®) is an orally administered alkylating agent with activity against malignant gliomas. It is a prodrug that spontaneously converts at physiologic pH to the active alkylating agent 5-(3- methyltriazen-1-yl)imidazole-4-carboximide (MTIC) under physiologic conditions. The cytotoxicity of temozolomide is principally mediated through methylation of DNA at the O6 position of guanine Temozolomide is rapidly and completely absorbed after oral administration; peak plasma concentrations occur in 1 hour. Food reduces the rate and extent of

temozolomide absorption. Mean peak plasma concentration and AUC decreased by 32% and 9%, respectively, and Tmax increased 2-fold (from 1.1 to 2.25 hours) when temozolomide was administered after a modified high-fat breakfast.

Temozolomide is rapidly eliminated with a mean elimination half-life of 1.8 hrs and exhibits linear kinetics over the therapeutic dose range. Temozolomide has a mean apparent volume of distribution of 0.4 L/kg (%CV=13%). It is weakly bound to plasma proteins; the mean percent bound of drug-related total radioactivity is 15%.

Temozolomide is spontaneously hydrolyzed at physiologic pH to the active species MTIC and to temozolomide acid metabolite. MTIC is further hydrolyzed to 5-amino-imidazole-4-carboxamide (AIC), which is known to be an intermediate in purine and nucleic acid biosynthesis and to methylhydrazine, which is believed to be the active alkylating species. Cytochrome P450 enzymes play only a minor role in the metabolism of temozolomide and MTIC. Relative to the AUC of temozolomide, the exposure to MTIC and AIC is 2.4% and 23%, respectively. Approximately 38% of the. administered temozolomide total radioactive dose is recovered over 7 days: 37% in urine and 0.8% in feces. The majority of the recovery of radioactivity in urine is as unchanged temozolomide (5.6%) AIC (12%), temozolomide acid metabolite (2.3%), and unidentified polar metabolite(s) (17%). Overall clearance of temozolomide is ~5.5 L/hr/m.

3.3.3 Pharmaceutical Data

Temozolomide is not directly active but undergoes rapid non-enzymatic conversion at physiologic pH to the reactive compound MTIC. The cytotoxicity of MTIC is thought to be primarily due to alkylation of DNA. Alkylation (methylation) occurs mainly at the O6 and N7 positions at guanine. Temozolomide should be stored at room temperature. The capsules are packaged in 30 cc 28 mm-48-Type I amber glass bottles (30 capsules/bottle) and should be stored between 2 and 30 degrees Centigrade. Capsules are stable for at least 30 months when stored in amber glass bottles at this temperature.

3.3.4 Usual Dosage Range

The typical dose regimen for patients with recurrent malignant glioma is 150 -200 mg/m2/day orally days 1-5 every 28 days [12]. According to clinical circumstances the initial dosage may require reduction by 20 to 25% in patients with risk factors such as prior myelosuppressive therapy and poor performance status. Determining haematologic nadir by frequent blood counts during initial courses is recommended for future dosage adjustment and scheduling of temozolomide.

3.3.5 Route of Administration

Oral.

3.3.6 Formulation

Temozolomide (Temodar®) is supplied in white, opaque, preservative free, 2-piece, hard gelatin capsules in the following p.o. dosage strengths: 5 mg, 20 mg, 100 mg, 140 mg, 180 mg and 250 mg. Capsules should not be opened or chewed. If capsules are accidentally opened or damaged, inhalation or contact with the skin should be avoided. Each capsule contains drug substance in combination with lactose, anhydrous NF, colloidal silicon dioxide NF, sodium starch glycolate NF, tartaric acid NF, and stearic acid NF. The capsule shells contain gelatin NF, titanium dioxide USP, and sodium lautyl sulfate NF.

3.3.7 Supply: Provided by Merck, Sharp and Dohme.

3.3.8 Side Effects and Toxicities

3.3.8.1 Known Potential Adverse Events
Hematologic: Thrombocytopenia, leukopenia, myelodysplastic syndrome
Gastrointestinal: Nausea, vomiting, anorexia
Hepatic: Elevated liver enzymes (reversible)
Skin: Rash
Neurologic: Convulsions, weakness (one side of the body), incoordination, paralysis
Other: Constipation, diarrhea, stomatitis, fatigue, decreased performance status, headache

A. Likely (occurring in more than 20% of patients)

- fatigue
- headache
- seizures
- alopecia
- nausea
- vomiting
- constipation
- anorexia
- Lymphopenia

B. Common (occurring in 3-20% of patients)

- dizziness
- abnormal muscle movements/ coordination
- abnormal gait
- hemiplegia or partial paralysis
- upper and/or lower extremity edema
- weakness (such as weakness on one side of the body)
- tickling/tingling sensation
- pain (such as in the abdomen, joints, back, and/or muscles)
- breast pain in females
- memory problems
- depression
- difficulty sleeping
- drowsiness
- skin rash
- pruritus
- dry skin
- mucositis
- dysphagia
- dysgeusia or taste changes
- diarrhea
- excess steroid in the body (possible bruising and/or increase in size of the face and/or neck)
- viral infection
- anxiety
- leukopenia
- weight gain
- loss of urinary control
- urinary tract infection
- frequent urination
- abnormal vision
- blurry vision
- diplopia or double vision
- fever
- cough
- sore throat
- sinusitis
- dyspnea
- allergic reaction
- confusion
- neutropenia thrombocytopenia

C. Rare but serious (occurring in fewer than 3% of patients)

- bone marrow disease where not enough blood cells are made
- hallucinations
- nervous system disease (possible pain and/or weakness)
- neuropathies (nerve damage- possible numbness, tingling, and pain)
- hyperglycemia (possible diabetes)
- hypokalemia (possible weakness)
- severe allergic reaction
- new occurrence of cancer (including myeloid leukemia)
- allergic skin reaction
- severe skin damage with loss of a large portion of skin
- weight loss
- fever due to low white blood cell counts
- bruising
- hemorrhage
- Steven-Johnson Syndrome
- damage from radiation (such as skin damage)
- pneumonitis (lung inflammation)
- flu-like symptoms
- injection site reactions (skin redness, irritation, pain, itching, swelling, and/or warmth)
- opportunistic infection
- herpes infection causing painful skin rash (shingles)

3.3.9 Special Populations:

3.3.9.1 Creatinine Clearance: Population pharmacokinetic analysis indicates that creatinine clearance over the range of 36-130 mL/min/m^2 has no effect on the

clearance of temozolomide after oral administration. The pharmacokinetics of temozolomide have not been studied in patients with severely impaired renal function (CLcr < 36 mL/min/m^2). Caution should be exercised when temozolomide is administered to patients with severe renal impairment. Temozolomide has not been studied in patients on dialysis.

3.3.9.2 Hepatically Impaired Patients: In a pharmacokinetic study, the pharmacokinetics of temozolomide in patients with mild to moderate hepatic impairment (Child's-Pugh Class I-II) were similar to those observed in patients with normal hepatic function. Caution should be exercised when temozolomide is administered to patients with severe hepatic impairment.

3.3.9.3 Gender: Population pharmacokinetic analysis indicates that women have an approximately 5% lower clearance (adjusted for body surface area) for temozolomide than men. Women have higher incidences of grade 4 neutropenia and thrombocytopenia in the first cycle of therapy than men.

3.3.9.4 Age: Population pharmacokinetic analysis indicates that age (range 19-78 years) has no influence on the pharmacokinetics of temozolomide. In the anaplastic astrocytoma study population, patients 70 years of age or older had a higher incidence of grade 4 neutropenia and grade 4 thrombocytopenia in the first cycle of therapy than patients <70 years of age. In the entire safety database, however, there did not appear to be a higher incidence in patients ≥70 years of age.

3.3.9.5 Drug-Drug Interactions: In a multiple dose study, administration of temozolomide with ranitidine did not change the C_{max} or AUC values for temozolomide or MTIC. Population analysis failed to demonstrate any influence of co-administered dexamethasone, prochlorperazine, phenytoin, carbamazepine, ondansetron, H_2-receptor antagonists, or phenobarbital on the clearance of orally administered temozolomide.

The following side effects have been reported in research studies with temozolomide. However, their causality related to temozolomide is uncertain:

- BUN blood level increase
- creatinine blood elevated
- lymphopenia
- thrombocytopenia
- neutropenia
- death
- hypoxia
- weight loss
- epistaxis
- sepsis
- cholecystitis
- pancreatitis
- GI perforation
- dehydration

3.3.9.6 Temozolomide is potentially mutagenic and should be handled with appropriate precautions by both staff and patients. Capsules should not be opened. If capsules are accidentally opened or damaged, rigorous precautions should be taken with the capsule contents to avoid inhalation or contact with the skin or mucous membranes. Procedures for proper handling and disposal of anticancer drugs should be considered.

3.3.10 Contraindications: Temozolomide is contraindicated in patients who have a history of a hypersensitivity reaction to any of its components or to DTIC.

3.3.11 Pregnancy Category D

Temozolomide may cause fetal harm when administered to a pregnant woman. There are no adequate and well-controlled studies in pregnant women. If this drug is used during pregnancy, or if the patient becomes pregnant while taking this drug, the patient should be apprised of the potential hazard to the fetus. **Women of childbearing potential should be advised to avoid becoming pregnant during therapy with temozolomide.**

Treatment of a man with temozolomide may increase the risk of birth defects if he causes a woman to become pregnant while he is taking temozolomide. Men treated with temozolomide may have difficulty causing a woman to become pregnant after their treatment is completed. Men receiving temozolomide should be directed to use effective contraception while they are being treated. There is insufficient data to know what the risk of subsequent problems with fertility will be. Similarly, women who are treated with temozolomide may have difficulty becoming pregnant in the future and may at be at increased risk of having children with birth defects. There is insufficient evidence to determine what the risk of these complications will be.

4.0 ELIGIBILITY CRITERIA

4.1 Patients with histologically proven supratentorial glioblastoma multiforme, gliosarcoma or anaplastic glioma will be eligible for the Phase I component of this protocol. Anaplastic gliomas include anaplastic astrocytoma (AA), anaplastic oligodendroglioma (AO), anaplastic mixed oligoastrocytoma (AMO), or malignant glioma NOS (not otherwise specified). Patients will be eligible if the original histology was low-grade glioma and a subsequent histological diagnosis of a malignant glioma is made. Only patients with histologically proven supratentorial glioblastoma multiforme or gliosarcoma will be eligible for the Phase II component.

4.2 Patients must have shown unequivocal evidence for tumor recurrence or progression by MRI scan and should have failed radiation therapy. The scan done prior to study entry documenting progression will be reviewed by the treating physician to document changes in tumor dimension to confirm recurrence. Patients with prior therapy that included interstitial brachytherapy or stereotactic radiosurgery must have confirmation of true progressive disease rather than radiation necrosis.

4.3 Patients may have had up to 2 prior relapses provided the functional status and other eligibility criteria for enrollment are met.

4.4 All patients must sign an informed consent indicating their awareness of the investigational nature of this study in keeping with the policies of this hospital.

4.5 The baseline on-study MRI should be performed within 14 days (+ 3 working days) prior to registration and on a steroid dosage that has been stable or decreasing for at least 5 days. If the steroid dose is increased between the date of imaging and the initiation of therapy (or at that time), a new baseline MRI is required. The same type of scan, i.e., MRI, must be used throughout the period of protocol treatment for tumor measurement.

4.6 Patients having undergone recent resection of recurrent or progressive tumor will be eligible as long as all of the following conditions apply:
a) They have recovered from the effects of surgery.
b) Evaluable or measurable disease following resection of recurrent tumor is not mandated for eligibility into the study.
c) To best assess the extent of residual measurable disease post-operatively, a MRI should be done no later than 96 hours in the immediate post-operative period or 4-6 weeks post-operatively.

4.7 Patients must be 18 years old or older.

4.8 Patients must have a Karnofsky performance status (KPS) equal or greater than 60 (Appendix G).

4.9 Patients must have recovered from the toxic effects of prior therapy to < grade 2 non hematological or grade 2 or lesser hematological toxicity per CTC ver 3 (except deep vein thrombosis – see section 5.3): 4 weeks from prior cytotoxic therapy and/or at least two weeks from vincristine, 6 weeks from nitrosoureas, 3 weeks from procarbazine administration, and 1 week for non-cytotoxic agents, e.g., interferon, tamoxifen, cis-retinoic acid, etc. (radiosensitizer does not count). Patients who receive anticancer agents for non-therapeutic purposes unrelated to this study (such as presurgically for obtaining pharmacology data for the agent) will be eligible to enter the study provided they have recovered from the toxic effects of the agent if any. Because the trial is based on the hypothesis that the combination of agents used will be synergistic in their effects, and that HDAC inhibition will potentially overcome resistance to retinoids, prior treatment with cRA is allowed. Any questions related to the definition of non-cytotoxic agents should be directed to the Study Chair.

4.10 Patients must have adequate bone marrow function (ANC$\geq$ 1,500/mm^3 and platelet count of $\geq$ 100,000/mm^3), adequate liver function (SGPT $\leq$ 3 times normal and alkaline phosphatase $\leq$ 2 times normal, bilirubin $\leq$1.5 mg/dl), adequate renal function (BUN and creatinine $\leq$1.5 times institutional normal) and normal serum amylase and lipase prior to starting therapy. Elevated cholesterol and triglycerides are not a contraindication to study enrollment, but should be managed as clinically appropriate by the treating physician.

4.11 Patients with a history of any other cancer (except non-melanoma skin cancer or carcinoma in-situ of the cervix or bladder), unless in complete remission and off of all therapy for that disease for a minimum of 3 years are ineligible.

4.12 Patients must not have:
a) active infection
b) disease that will obscure toxicity or dangerously alter drug metabolism, especially liver disease including cirrhosis or hepatic dysfunction
c) serious intercurrent medical illness
d) prior recurrence with other HDAC inhibitors. However, patients who have received anticancer agents for non-therapeutic purposes (for eg., as part of a pharmacology study without therapeutic intent) will remain eligible for enrollment into the study

4.13 Patients receiving valproic acid (VPA), an anticonvulsant drug with HDAC inhibitor properties, will be excluded, unless they are switched to an alternative agent prior to treatment initiation. No wash out period is required.

4.14 Prior treatment with dose dense regimens of temozolomide is not allowed (e.g, 7 days on/7 days off, 21 day/28 day and daily low dose continuous dosing). However, standard day 1-5 dosing and low dose daily dosing as part of chemoradiation therapy are allowed .

4.15 Patients receiving treatment with other antiepileptic medications will not be excluded. Vorinostat is not metabolized by cytochrome P450 3A4 (CYP 3A4). However, vorinostat may potentially suppress CYP 3A4 activity. Therefore, patients should preferably be treated with non-enzyme inducing anti-epileptic medications to avoid any potential interactions with vorinostat. However, the use of non-enzyme inducing anti-epileptic medications is not mandatory. If enzyme-inducing antiepileptic drugs are used, monitoring of drug levels should be considered, as considered clinically appropriate by the treating physician.

4.16 Patients with a known allergy to any component of vorinostat, or a known allergy to temozolomide and/or isotretinoin will be excluded.

4.17 Patients must be willing and able to comply with the FDA mandated iPLEDGE program for treatment with isotretinoin (cRA). Patients must sign specific informed consents for treatment with cRA, as mandated by iPLEDGE guidelines. Women of childbearing potential must not be pregnant, must not be breast-feeding and must practice adequate contraception (please refer to 6.4 and Appendix I for details and definitions – woman of childbearing potential; adequate methods of contraception).

4.18 Male patients on treatment with vorinostat must agree to use an adequate method of contraception for the duration of the study, and for 30 days after the last dose of study medication (please refer to Appendix I for definition of adequate methods of contraception).

4.19 Patient must be able to tolerate the procedures required in this study including periodic blood sampling, study related assessments, and management at the

treating institution for the duration of the study. Inability to comply with protocol or study procedures (for example, an inability to swallow tablets) will be an exclusion criteria.

4.20 This study was designed to include women and minorities, but was not designed to measure differences of intervention effects. Males and females will be recruited with no preference to gender.

4.21 No exclusion to this study will be based on race. The malignant glioma patient population treated at MDACC over the past year is as follows:

American Indian or Alaskan Native - 0
Asian or Pacific Islander - <2%
Black, not of Hispanic Origin - 3%
Hispanic - 6%
White, not of Hispanic Origin - 88%
Other or Unknown - 2%
Total-100%

5.0 TREATMENT PLAN

5.1. Phase I Component

The Phase I component of the trial was designed with three treatment arms (see below). This component is a non randomized trial in which Arm 1 and Arm 2 were to be opened simultaneously with consecutive patients enrolled alternately to each arm, and sequentialy to each dose level. If the MTD was reached earlier in one arm, all subsequent patients were to be enrolled to the other arm. Once the MTD for Arm 1 and Arm 2 was known, Arm 3 would begin accrual. A maximum of 54 patients were to be enrolled in this component of the trial. The MTDs obtained in the Phase I component were planned to be used as the Phase II dose in the next component of the trial.

5.1.1. Arm 1 – Vorinostat plus isotretinoin

This arm assessed the MTD of vorinostat in combination with a fixed dosage of isotretinoin (cRA) as shown in the table below. A cycle of treatment was 28 days (+ 3 days); the treatment schedule began with cRA on days 1-21. The total daily dose was to be given orally, divided BID (twice a day). Vorinostat was administered on days 1-14. The total daily dose was given once daily orally. Days 22-28 was considered as a no-treatment "rest" period.

A conventional phase I design was used with 3 patients enrolled into the first dose level and monitored for 3 weeks. If no DLT was seen, the next 3 patients were to be enrolled at the next dosage level. A maximum of three dosage levels will be utilized as shown in the table below. If no DLT was noted after dose escalation to Level III,

these doses were to be utilized for the phase II components of the study. If 1/3 patients shows ≥ grade 3 toxicity, the cohort was to be expanded to 3 more patients, If 2/6 patients experience ≥ grade 3 toxicity, the DLT was considered reached, and previous (lower) dosage level was to be declared as the MTD of vorinostat in combination with cRA.

Level	Arm 1	
	Vorinostat (mg/day x 14 days)	cRA (mg/m2/day x 21 days)
0 (starting dose)	300 mg	100
I	400 mg	100
II	500 mg	100

This arm of the trial completed accrual as planned and an MTD was established for the combination of vorinostat (400 mg/day on days 1-14) and isotretinoin (100 mg/m2/day on days 1-21) on a 28 day schedule. We propose to use this dose in the phase II trial.

5.1.2. Arm 2 – Temozolomide plus isotretinoin

This arm was originally designed to assess the MTD of carboplatin (CBT) in combination with fixed doses of isotretinoin (cRA). The accrual to this arm of the trial was also completed and the MTD for this combination was established with carboplatin (AUC 5 on day 1) and isotretinoin (100 mg/m2/day on days 1-21). However, in the current proposal, we will not use carboplatin and will replace this with temozolomide. Thus, the combination of interest in Arm 2 will be dose-dense temozolomide with isotretinoin. A recent phase I trial of temozolomide in combination with several different agents has established the MTD of temozolomide adminstered in combination with isotretinoin at 150 mg/m2/day of temozolomide on days 1-7 and days 15-21 in patients with glioblastoma (Gilbert et al, Neuro-oncology 2010). Thus, we propose to use this regimen in the phase II portion of the current trial without the need for a phase I component.

5.1.3. Arm 3 – Vorinostat plus isotretinoin plus temozolomide

This arm will assess the MTD of vorinostat and temozolomide in combination with fixed doses of cRA. The enrollment will proceed as outlined for Arm 1 and 2 (cohorts of 3 patients enrolled to each dose level), declaring MTD for vorinostat in combination with temozolomide and cRA once DLT is noted. In this arm, cRA will be given on days 1-21 and vorinostat on days 1-7 and 15-21 . Temozolomide will be administered orally on days 1-7 and 15-21. Days 22-28 will be considered a no-treatment "rest" period. A maximum of three dose levels of vorinostat will be used as shown in the table below.

Level	Arm 3		
	Vorinostat (mg/day x days 1-7 & 15-21)	cRA (mg/m2/day x 21 days)	Temozolomide (mg/m2/day on days 1-7 & 15-21

-III	300	100	100
-II	300	100	125
-I	400	100	125
0 (starting dose)	400	100	150
I	500	100	150

5.1.3.1 Escalation of Dose Level

- If the initial cohort of patients at starting dose level 0 do not experience DLT, the next cohort of patients will be treated by dose escalation to level I (with increase in the dose of vorinostat). If no patients experience DLT in this cohort, 3 more patients will be enrolled to expand this cohort to a total of 6 patient to confirm that MTD has been achieved. If no DLT is noted or if only 1/6 patients experience DLT after this expansion of level I, this dose level will be declared the MTD and hence the phase II dose.
- If 2/6 patients have DLT after expansion of dose level I, dose level 0 will be declared as the MTD and hence the phase II dose.

5.1.3.2 De-escalation of Dose Level

- If Dose level 0 exceeds the MTD, lower doses will be studied as noted in the table above. If 1/3 patients have DLT at any dose level, the cohort will be expanded by 3 more patients. If ≥2/6 patients experience DLT, the MTD will be considered as being exceeded and the dose will be de-escalated to the next lower dose level and 3 new patients will be enrolled at that level. If none or only 1/6 patients experience DLT after dose de-escalation to the lower level, this dose level will be declared as the MTD, and will be utilized for the phase II component of the study.

5.2. Phase II Component

5.2.1. Non-surgical Arm

In this component, patients will be randomized between three competing treatment arms: vorinostat + cRA, versus TMZ + cRA, versus vorinostat + cRA + TMZ using an adaptive randomization design (see 11.0 Statistical Section). The dosage of agents in each arm will be derived from the MTD of the combination determined in the phase I component of the study. The treatment schedule will be identical to that described above in the phase I component, with each course comprising 28 days (+ 3 days). Patients will continue treatment for at least one year from registration provided they experience neither tumor progression nor unacceptable toxicity (see 8.9). Treatment beyond this period is at the discretion of the treating physician after discussion with the patient. Vorinostat will continue to be provided free of charge to patients continuing treatment beyond 1 year.

Optional procedures: if available, brain tumor tissue from prior surgical procedures (original surgery, or definitive surgery, or the surgery closest to initiation of this clinical trial) will be obtained for determination of acetylation status and p21 levels (see 6.7). Blood samples for acetylation status of histones and p21 levels in

peripheral mononuclear cells will be also collected at baseline prior to treatment (6.6). Patients enrolled in arms 1 (vorinostat + cRA) and 3 (vorinostat + cRA + TMZ) will be evaluated with blood samples before and after treatment with vorinostat in day 1 of cycles 1 and 2 (see 7.10).

5.2.2. Surgical Arm

Ten patients who require resection for recurrent glioblastoma multiforme will be included in this arm of the study. These patients will receive treatment with vorinostat at a dose of 400 mg once daily for 3 consecutive days prior to surgery. Blood samples for acetylation status of histones and p21 levels in peripheral mononuclear cells will be collected at baseline prior to treatment, and after the first dose of vorinostat. The last dose prior to surgery will be administered the morning of surgery within 6 hours of the tumor removal. Tumor tissue and intraoperative blood samples will be obtained for analysis of drug levels, histone acetylation and p21 levels. Approximately 2 weeks after surgery is performed, each patient will be randomized to one of the three treatment arms of the phase II component (arm 1: vorinostat + cRA; arm 2: TMZ + cRA; arm 3: vorinostat + cRA + TMZ). Patients enrolled in arms 1 and 3 will be evaluated with blood samples before and after treatment with vorinostat in cycles 1 and 2 as described for patients in the non-surgical arm (see 5.2.1, and 7.10). In surgical patients, cycle 1 will be the first cycle of treatment after surgery.

Progression Free Survival (PFS) in patients enrolled in the surgical arm will be determined from the date of randomization into the chemotherapy arms (i.e. after surgery for resection of recurrent tumor), and not from the date of registration in the trial.

5.3. Definition of dose limiting toxicities (DLT)

Toxicities will be graded according to the Common Terminology Criteria for Adverse events (CTCAE) Version 3.0. (see Appendix B). If multiple toxicities are seen, the presence of DLT should be based on the most severe toxicity experienced. DLT will be defined as any of the following events occurring during treatment and attributable to the study drugs:

5.3.1 Any grade 4 hematological toxicity excluding lymphopenia . (if the toxicity is Grade 4 neutropenia without fever, it must NOT resolve/regress to ≤ Grade 1 within 7 days of onset to be considered a DLT).

5.3.2 Any non-hematologic grade 3 toxicity, weight gain (in patients on steroids), alopecia, and venous thromboembolic disease *. Grade 3 nausea, vomiting, or diarrhea that cannot be controlled by medical therapy will be considered dose-limiting. Isolated laboratory abnormalities not of clinical significance will be discussed with the IRB and the decision to declare these DLTs will be based on the IRB recommendations.

5.3.3 Failure to recover from toxicities to be eligible for re-treatment within 4 weeks of the last dose of the drugs.

***Non-Relevant Toxicities for Brain Tumor Protocols**

Lymphopenia is a common finding among patients with primary brain tumors and is directly attributable to concurrent use of corticosteroids. To date, even Grade III lymphopenia has not been associated with a worsening of clinical outcome. Therefore, we will not consider this parameter in the determination of optimal dosing of drugs.

Weight gain is a common finding among patients with primary brain tumors. This too is directly attributed to the concurrent use of corticosteroids. Doses of steroids or study agents will not be modified as a consequence of weight gain. Corticosteroid dosage will be based on maintenance of control of edema in the brain and the standard clinical practice to use the minimal effective dose of corticosteroids will be employed.

Alopecia is a common occurrence in patients with brain tumors as a consequence of cranial radiotherapy. Alopecia will not be recorded or graded as toxicity.

Venous thromboembolic disease is a common complication occurring in up to 30% of patients with malignant gliomas [32, 33]. In this study, patients who develop deep vein thrombosis may receive anticoagulation and resume therapy once they are stable on anticoagulation. Grade III deep vein thrombosis will not be considered a DLT.

5.4. Dose modifications

5.4.1 Subsequent Cycles

Treatment cycles will be repeated every 28 days (+ 2 days) from day 1 provided that the tumor has not progressed and the patient has recovered from treatment-related adverse events associated with a prior course. Recovery has occurred once all of the following conditions have been met:

a) ANC ≥1500/mL;
b) Platelet Count ≥100,000/mL;
c) All drug-associated non-hematological toxicities have recovered to either Grade 0 or 1(except for deep venous thrombosis as below).

If recovery has not occurred by **Day 28** (+ 3 days), the subsequent cycle will be delayed until these criteria have been met. If the patient has not recovered within 4 weeks of rest, he/she will be taken off study. However, if the patient develops a lower extremity deep venous thrombosis and requires anticoagulation or intervention (grade 3 toxicity), treatment may be restarted after a 2 week rest period if the patient

is stably anticoagulated and is judged medically stable to begin chemotherapy, in the opinion of the treating physician. (Please note: DVT is graded in the CTC version 3.0 as grade 1= not defined, grade 2= DVT without need for anticoagulation or intervention, grade 3= DVT needing anticoagulation or intervention).

The administration of vorinostat, isotretinoin and temozolomide in subsequent cycles will continue at the same doses without interruption as long as there is no tumor recurrence or progression and toxicity is acceptable.

5.4.2 Dosage Reduction

Patients experiencing toxicity will be dose reduced according to the agent causing the toxicity. Because of the different profile of side effects of agents combined in Arms 1 and 2 (vorinostat + cRA; TMZ + cRA), no significant overlapping toxicities are expected. In case of thrombocytopenia or anemia in Arm 3 (vorinostat + cRA + TMZ), temozolomide will be dose-reduced first, and then vorinostat on an alternating basis for each time a dose reduction becomes necessary.

5.4.2.1 Vorinostat

Vorinostat will be administered from day 1 -14 in arm 1 and on days 1-7 and 15-21 in arm 3. Treatment will be initiated at the MTD dose determined in the phase I component of the trial for each arm. This dose will be maintained as long as there are no toxicities ≥ grade 3. For grade 3 or greater toxicities, treatment will be withheld and the patients will be monitored until toxicities resolve to < grade 2 (except for deep venous thrombosis as noted in section 5.3). Subsequent treatment will be at one dose level lower than the one that caused the toxicity. If there is grade 3 or greater non-hematologic toxicity, a minimum of 2-week rest period will be required. Subsequently, patients will restart treatment at one dose level less than the one that resulted in the toxicity as indicated below.

Dose adjustments for the subsequent cycles: dose will be one level below the dose that produced toxicity of grade 3 or greater per dose schedule changes indicated below. If grade 3 or 4 toxicity recurs, patients will again discontinue the vorinostat until toxicity returns to grade 1 or less. The estimated dose levels below are shown for various MTD that may be determined in the phase I study.

In case of thrombocytopenia or anemia in Arm 3 (vorinostat + cRA + TMZ), TMZ will be dose-reduced first, and then vorinostat on an alternating basis for each time a dose reduction becomes necessary.

Dose Level:	**-2**	**-1**	**0**
Drug			**MTD Phase I**
Vorinostat	**N/A**	**200 mg once daily**	**300 mg once daily**
	200 mg once daily	**300 mg once daily**	**400 mg once daily**

	300 mg once daily	**400 mg once daily**	**500 mg once daily**

Only 2 dose reductions of vorinostat are permitted as above. If the patient experiences grade 3 or greater toxicity after 2 dose reductions, he/she will be taken off study. If starting dose is 300 mg once daily, only 1 dose reduction of vorinostat is allowed.

5.4.2.2 Isotretinoin

Isotretinoin will be administered from day 1 to day 21 in the 3 arms of the study at an initial dose of 100 mg/m2/day. Doses will be rounded down, depending on body surface area and available capsule sizes. Treatment will continue at the same dose as long as there are no toxicities ≥ grade 3. For grade 3 or greater toxicities, treatment will be withheld and the patients will be monitored until toxicities resolve to < grade 2 (except for deep venous thrombosis as noted in Section 5.3).

Dose adjustments for the subsequent cycles: Subsequent cycles will start (as long as the treatment is beneficial) after resolution of toxicities to grade 1 or less (except for deep venous thrombosis as noted in Section 5.3). A minimum of 2-week rest period will be required if there is grade 3 or greater non-hematologic toxicity.

Dosage of cRA for the subsequent cycle will be one dose level below the dose that produced toxicity of grade 3 or greater as shown below.

Dose level:	**-2**	**-1**	**0**
Isotretinoin	**50 mg/m^2**	**75 mg/m^2**	**100mg/m^2**

Only 2 dose reductions of cRA are permitted. If the patient experiences grade 3 or greater toxicity after two dose reductions, he/she will be taken off study.

5.4.2.3 Temozolomide

Dosing is based on adverse events (AEs) during the prior treatment cycle. If multiple AEs are seen, the dose administered should be based on the dose reduction required for the most severe grade of any single AE.

Dose Level	**Dose mg/m^2**	**Remarks**
- 2	75	Reduction if prior AE
-1	100	Reduction if prior AE
0	125	Starting dose for cycle 1; increase to 150 mg/m^2 for cycle 2 and beyond if non hematologic toxicity ≤ grade 2

		and hematologic toxicity ≤ grade 1
+1	150	Highest possible dose level

a) First Cycle: Temozolomide will be initiated at a dose of 125 mg/m^2/day.

b) Second Cycle: The dose of temozolomide will be determined according to: (1) treatment related non-hematologic AE during the preceding treatment cycle, as well as (2) the worst ANC and platelet counts. For patients at dose level 0, the dose may be increased to 150 mg/m2/dose for cycle 2 and beyond. If this dose is not tolerated, the dose may be reduced to the previous dose level.

c) Delay: On day 1 of each cycle (within the prior 72 hours), ANC ≥ 1.5 x 10^9/L, platelet count ≥ 100 x 10^9/L and all grade 2, 3 or 4 non-hematologic AEs (except for alopecia, nausea, vomiting, weight change or DVT/PE – see section 5.3.3 – nonrelevant toxicities) must have resolved (to grade ≤ 1). For DVT/PE, the patient must have had adequate management of the event and be medically stable as determined by the treating physician prior to resuming treatment – this will be considered on a case by case basis after discussion between the treating physician and the study chair.

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

Dose Escalations and Reductions: If, during the first cycle, all hematologic AEs observed were ≤ grade 1 and treatment related non-hematologic AEs observed were grade ≤ 2 (except alopecia, lymphopenia, nausea, vomiting, weight gain and DVT/PE) then the temozolomide dose can be raised to 150 mg/m2/dose for subsequent cycles.

Dose reductions: If any treatment related non-hematologic AE observed was grade > 2 (except alopecia, lymphopenia, nausea, vomiting, weight gain and DVT/PE) 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. Patients who require more than three dose reductions will have treatment stopped.

If any treatment-related non-hematologic AE observed was grade 4 (except alopecia, lymphopenia, nausea, vomiting, weight gain and DVT/PE) then temozolomide treatment should be stopped.

Subsequent cycles: Any dose reductions of temozolomide 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 re-escalation in subsequent treatment cycles.

Summary of Dose Modifications or Discontinuation for Temozolomide-Related Adverse Events

Worst Treatment-Related Non-Hematologic AE (except for alopecia, nausea, and vomiting) During the Previous Cycles	
Grade	**Dose Modification**
0-2	No dose modifications for non-hematologic AEs. Dose reductions based on ANC and platelet counts are applicable.
3	Reduce by one dose level (except alopecia, lymphopenia, nausea, vomiting, weight gain and DVT/PE).
4	Stop (except alopecia, lymphopenia, nausea, vomiting, weight gain and DVT/PE). Dose modifications based on ANC and platelet counts are not applicable.

Worst Treatment-Related Hematologic AE During the Previous Cycle

Worst AE		**Platelets**		
		≥100 x 10^9/L	**50 – 99 x 10^9/L**	**< 50 x 10^9/L**
ANC	**≥ 1.5 x 10^9/L**	Dose unchanged	Dose unchanged	Reduce by 1 dose level
	≥1 & <1.5 x 10^9/L	Dose unchanged	Dose unchanged	Reduce by 1 dose level
	< 1 x 10^9/L	Reduce by 1 dose level	Reduce by 1 dose level	Reduce by 1 dose level

Note: A complete blood count must be performed on days 14 and 28 (± 72 hours) after the first daily dose of each treatment cycle.

Hematologic AE on Day 1 of Each Cycle (within the prior 72 hours before Day 1)	
AE	**Delay**
ANC< 1.5 x 10^9/L and/or Platelet count < 100 x 10^9/L	Delay up to 4 weeks until all resolved. If unresolved after 4 weeks then stop. If resolved, dose delay/reductions based on non-hematologic AEs are applicable. If treatment has to be delayed for AEs, then no escalation is possible.

Non-Hematologic AE (except alopecia, lymphopenia, nausea, vomiting, weight gain and DVT/PE) **On Day 1 of Each Cycle (within the prior 72 hours)**	
Grade	**Delay**
2-4	Delay up to 4 weeks until all resolved (to grade ≤ 1). If unresolved after 4 weeks, then stop. If resolved, dose delay/reductions based on ANC and platelets are applicable. If treatment has to be delayed for AE, then no escalation is possible.

In case of thrombocytopenia or anemia in Arm 3 (vorinostat + cRA + TMZ), TMZ will be dose-reduced first, and then vorinostat on an alternating basis for each time a dose reduction becomes necessary.

A minimum of 4 weeks of treatment shall be required for a patient to be considered as having received an adequate trial to evaluate efficacy. All patients will be considered evaluable for toxicity and will be followed for at least 30 days after completion of treatment.

5.5 Supportive care

1. G-CSF and erythropoetin administration: routine prophylactic use is not permitted. However, therapeutic use in patients with complications (severe neutropenia with fever or anemia), may be considered.

2. Steroids: should be used in the smallest dose to control symptoms of cerebral edema, and discontinued if possible.

3. Antiepileptic medications: should be used as indicated. Treatment with valproic acid is not allowed. Patients should preferably be treated with non-enzyme inducing antiepileptic medications to avoid any potential interactions with vorinostat. For patients on treatment with enzyme-inducing antiepileptic drugs, clinically relevant monitoring of drug levels is recommended, as considered appropriate by the treating physician.

4. Antiemetics: prophylactic and therapeutic use is allowed.

5. Other concomitant medications: therapies considered necessary for the well-being of the patient may be given at the discretion of the treating physician including prophylaxis for *pneumocystis carinii* pneumonia (PCP). All concomitant medications should be recorded.

6.0 PRE-STUDY EVALUATION

6.1 A complete history and neurological examination (including height, weight and Karnofsky Performance Status), as well as documentation of evaluable disease shall be performed on all patients within 14 days (+ 3 working days) of study entry.

6.2 The treating physician will review a Gd-DPTA MRI scan done prior to study entry documenting progression. For non-surgical arm patients, a baseline scan should be performed within 14 days (+ 3 working days) of registration, with day 0=registration date. The baseline on-study MRI should be performed on a steroid dosage that has been stable or decreasing for at least 5 days. If the steroid dose

is increased between the date of imaging and the initiation of therapy (or at that time), a new baseline MRI is required.

6.3 Pre-treatment laboratory tests will include CBC, differential, platelets, PT, PTT, INR, total protein, albumin, calcium, phosphorus, magnesium, glucose, BUN, creatinine, sodium, potassium, uric acid, total bilirubin, alkaline phosphatase, LDH, SGPT (ALT), SGOT (AST), amylase, lipase, lipid panel (total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides), and a pregnancy test for women of child bearing potential (see 6.4 and Appendices I and J). Anticonvulsant levels in patients on treatment with enzyme-inducing antiepileptic drugs (phenytoin, phenobarbital, carbamazepine) will also be obtained if considered appropriate by the treating physician. Blood tests must be performed within 14 days (+ 3 working days) of registration, with day 0 = registration date. Pregnancy test must be obtained 24 hours before treatment with isotretinoin starts. Patients whose clinical condition has significantly changed between the time of these tests and the initiation of treatment in the judgement of the treating physician, will have a repeat chemistry panel prior to the start of treatment.

6.4 Patients will be enrolled in the mandatory iPLEDGE Program to initiate Isotretinoin (cRA) therapy. This program dictates guidelines for pregnancy testing and contraception in women of childbearing potential who are treated with cRA (please refer to Appendix I for iPLEDGE definition of woman of childbearing potential). However, it is anticipated that for the vast majority of patients with recurrent malignant glioma, strict adherence to these guidelines would imply unacceptable delays in their treatment. For this reason, in women of childbearing potential who elect to participate in this protocol, an ”Exception Authorization for Oncology Patient” will be requested (Appendix J). This exception authorization will allow the patient to start treatment immediately after enrollment in iPLEDGE and obtention of a negative pregnancy test. The ”Exception Authorization for Oncology Patient” will allow patients to expedite the beginning of therapy by eliminating the need for a 1 month delay in the first prescription of cRA.

6.5 Patients will complete the baseline MD Anderson Symptom Inventory-Brain Tumor Module (MDASI-BT) within 14 days (+ 3 working days) of study entry (*).

6.6 Patients enrolled on the phase II component of the trial will have blood samples collected for determination of acetylation status of histones and p21 levels in peripheral mononuclear cells before starting treatment (*).

6.7 If available, unstained paraffin tissue from surgical samples will be obtained. A representative paraffin tissue block (at least 5 mm x 5 mm) or 20 unstained paraffin slides will be obtained from original surgery, or definitive surgery, or the surgery closest to initiation of this clinical trial. Paraffin slides will be used to evaluate histone acetylation levels and p21 levels by immunohistochemistry. These will be correlated with response. If available, frozen tissue ($\geq$ 1 g) from prior surgeries may also be sent for the same tests in frozen tissue (*).

* Note: **6.5**, **6.6** and **6.7** are optional procedures, and will be obtained if the patients gives his or her informed consent.

7.0 ON-STUDY EVALUATION

7.1 CBC, differential and platelets will be performed weekly (± 3 days) for the first cycle. If no significant toxicity is seen, monitoring will be changed to every two weeks (± 3 days) during treatment and prior to each new cycle. In addition, all patients will have the following tests every 2 weeks (± 3 days) for the first cycle and then every cycle as well as prior to each new cycle if no significant toxicities are noted: PT, PTT, INR, total protein, albumin, calcium, phosphorus, magnesium, glucose, BUN, creatinine, sodium, potassium, uric acid, total bilirubin, alkaline phosphatase, LDH, SGPT, SGOT. All patients will have serum amylase, lipase, and lipid panel drawn every 2 cycles of treatment. Patients may also be evaluated with labs at any time clinically indicated.

7.2 Patients on treatment with enzyme-inducing antiepileptic drugs (phenytoin, phenobarbital, carbamazepine) will be evaluated with anticonvulsant levels as considered appropriate by the treating physician.

7.3 A Gd-DPTA MRI scan shall be done after each **2 cycles** of treatment prior to initiating the next cycle. MRI scans may also be done at any time clinically indicated.

7.4 Physical and neurologic examinations will be performed after **cycle 1 and cycle 2**, and subsequently after each **2 cycles** of treatment prior to initiating the next cycle. Patients will also be evaluated anytime their clinical situation demands an assessment.

7.5 All patients receiving cRA must be enrolled in the iPLEDGE program (see Appendix I). Before starting treatment, an ”Exception Authorization for Oncology Patient” will be requested for women of childbearing potential (Appendix J). A negative serum pregnancy test will be obtained 24 hours before initiation of treatment with cRA. Once treatment has started, pregnancy testing and counseling should occur monthly. If pregnancy does occur during cRA treatment, the drug must be discontinued immediately and the treating physician notified. The treating physician will in turn notify the study chair immediately.

7.6 The MDASI-BT will be completed at the time of physical and neurologic exam, i.e. after **cycle 1 and cycle 2**, and subsequently after every **2 cycles** of treatment prior to initiating the next cycle of treatment. (*)

7.7 All relevant information regarding drug doses, concomitant medications and doses, measurable lesions, tumor response, laboratory examinations, and

treatment-related toxicities shall be documented in the patient's medical record and flow sheets.

7.8 After treatment on protocol is completed**,** patients will be followed for overall survival every 3 months, when possible.

7.9 All patients enrolled in the surgical arm of the phase II component of the trial will have tumor tissue and intraoperative blood samples (8ml) collected during surgery. Vorinostat will be given at 400 mg once daily for 3 consecutive days prior to surgery, with the last dose given on the morning of surgery within 6 hours of the tumor removal. Blood samples for acetylation status of histones and p21 levels in peripheral mononuclear cells will be collected on day 1 before (pretreatment), and at 1, 2 and 6 hours after administration of vorinostat.

7.10 Blood samples for acetylation status of histones and p21 levels in peripheral mononuclear cells should be collected before (pretreatment) and then 1, 2 and 6 hours after administration of vorinostat on day 1 of cycle 1, and before and within 4 hours after administration of vorinostat on day 1 of cycle 2 in patients enrolled in arms 1 and 3 of the phase II component (surgical and non-surgical patients). Total volume is 144 mL (6 x 24 mL)*.

*Note: 7.6 and 7.10 are optional procedures, and will be obtained if the patients gives his or her informed consent.

8.0 RESPONSE CRITERIA

The primary endpoint is Progression Free Survival (PFS) for determining the "winner" of the three regimens tested. Secondary endpoints are a) radiological response to treatment, b) the progression free survival at 6 months compared with historical controls, and c) overall survival. MRI brain scans will be used to define overall response or progression.

8.1 Disease Evaluability

8.1.1 **Measurable Disease:** Bidimensionally measurable lesions with clearly defined margins by MRI scan.

8.1.2 **Evaluable Disease:** Unidimensionally measurable lesions, masses with margins not clearly defined. Patients with only this kind of imaging will not be allowed to enter this study unless they have recently undergone surgery and have histologically proven recurrent disease.

8.1.3 **Non-Evaluable Disease:** Not Applicable.

8.1.4 **Objective Status, To Be Recorded at Each Evaluation:** If there are too many measurable lesions to measure at each evaluation, choose the largest two to be followed before a patient is entered on study. The remaining

lesions will be considered evaluable for the purpose of objective status determination. Unless progression is observed, objective status can only be determined when ALL measurable and evaluable sites and lesions are assessed.

8.2 Response Criteria

8.2.1 **Complete Response (CR):** Complete disappearance of all measurable and evaluable disease. No new lesions. No evidence of non-evaluable disease. All measurable, evaluable and non-evaluable lesions and sites must be assessed using the same techniques as baseline. Responders must be on none or only maintenance doses of dexamethasone.

8.2.2 **Partial Response (PR):** Greater than or equal to 50% decrease under baseline in the sum of products of perpendicular diameters of all measurable lesions. No progression of evaluable disease. No new lesions. All measurable and evaluable lesions and sites must be assessed using the same techniques as baseline. Responders must be on the same or decreasing doses of dexamethasone and have stable or improved neurological exams.

8.2.3 **Partial Response, Non-Measurable (PRNM):** Not applicable.

8.2.4 **Stable/No Response:** Does not qualify for CR, PR, or progression. The designation of Stable/No Response requires a minimum of 8 weeks duration. All measurable and evaluable sites must be assessed using the same techniques as baseline. Responders must be on the same or decreasing doses of dexamethasone and have stable or improved neurological exams.

8.2.5 **Progression:** 25% increase in the sum of products of all measurable lesions over smallest sum observed (over baseline if no decrease) using the same techniques as baseline, OR clear worsening of any evaluable disease, OR appearance of any new lesion/site, OR failure to return for evaluation due to death or deteriorating condition (unless clearly unrelated to this cancer).

8.2.6 **Unknown:** Progression has not been documented and one or more measurable or evaluable sites have not been assessed.

8.3 Note:

8.3.1 For evaluable disease other than types specified in Section 8.1.2, the only objective statuses which apply are CR, stable/no response, progression and unknown.

8.3.2 Objective status must stay the same or improve over time until progression (unknown excepted).

8.4 **Neurological Exam:** Although not used for determining response, it is useful to evaluate improvement in the neurologic exam that should coincide with objective measurement of tumor size.

1 Normal exam
2 Improvement from on-study state (patient better)
3 Same as on-study state (patient same).
4 Worse than on-study state (patient worse).

8.5 **Performance Status:** Patients will be graded according to Karnofsky Performance Status (see Appendix G).

8.6 **Progression Free Survival (PFS):** From date of registration to the date of first observation of progressive disease, non-reversible neurologic progression or permanently increased steroid requirement (applies to stable disease only) or death due to any cause. Progression Free Survival (PFS) in patients enrolled in the surgical arm will be determined from the date of randomization (i.e. after surgery for resection of recurrent tumor), and not from the date of registration in the trial.

8.7 **Survival:** From date of registration to date of death due to any cause.

8.8 **Steroids**: Steroid dosage will be carefully monitored and recorded during each course of therapy and steroid dosage changes will be considered before response determinations are made.

8.9 **Unacceptable toxicity:** grade 3 or greater toxicity after 3 dose reductions of temozolomide or 2 dose reduction of any of the other drugs, or after 1 dose reduction if starting dose of vorinostat is 300 mg orally once daily.

9.0 DATA AND SAFETY MONITORING

9.1 Safety Assessments and Toxicity Monitoring:

All patients receiving Vorinostat, Isotretinoin and Temozolomide will be evaluated for safety. The safety parameters include all laboratory tests and hematological abnormalities, CNS observations, physical examination findings, and spontaneous reports of adverse events reported to the investigator by patients. All toxicities encountered during the study will be evaluated according to the NCI Common Terminology Criteria (CTCAE) version 3.0 and recorded prior to each course of therapy. Life-threatening toxicities should be reported immediately to the Study Chair, Institutional Review Board (IRB), and the FDA.

To provide formal safety monitoring in the Phase II portion of the trial, after enrollment of every 10 patients into each of the three arms, we will assess the group for toxicity. If dose limiting toxicity as defined in the phase I portion of the trial is reached =/> 1/3 of patients despite dose reduction, the arm will stop accrual. If dose reduction permits continuation of treatment in these patients, the

starting dose levels for the remainder of the patients on the trial will be modified to a lower starting dose (-1) level specified in the dose modification section

9.2 Adverse Event and Reporting Definitions

An adverse event or adverse experience is any untoward medical occurrence in a clinical investigation patient who is administered a medicinal product. An adverse event can be any unfavorable and unintended sign, symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product. Pre-existing conditions that increase in frequency or severity or change in nature during or as a consequence of use of a drug are also considered as adverse events. Adverse events may also include pre- or post-treatment complications that occur as a result of protocol-mandated procedures (eg, invasive procedures such as biopsies).

An AE does not include:

- Medical or surgical procedures (eg, surgery, endoscopy, tooth extraction, transfusion). The condition that leads to the procedure is the adverse event

- Situations where an untoward medical occurrence has not occurred (eg, hospitalization for elective surgery, social and/or convenience admissions)

- Overdose of either study drug or concomitant medication without any signs or symptoms unless the patient is hospitalized for observation.

The relationship to study drug therapy should be assessed using the following definitions:

Not Related: Evidence exists that the AE has an etiology other than the study drug (eg, pre-existing condition, underlying disease, intercurrent illness, or concomitant medication).

Possibly/Probably Related: A temporal relationship exists between the event onset and administration of the study drug. It cannot be readily explained by the patient's clinical state, intercurrent illness or concomitant therapies. In case of cessation or reduction of the dose, the event abates or resolves and reappears upon rechallenge. It should be emphasized that ineffective treatment should not be considered as causally related in the context of AE reporting.

A serious adverse event is defined as any adverse drug experience occurring at any dose that results in any of the following outcomes:

- Death

- Life-threatening situation (patient is at immediate risk of death)

-Inpatient hospitalization or prolongation of existing hospitalization (excluding those for study therapy, disease-related procedures, palliative or hospice care, or placement of an indwelling catheter, unless associated with other serious events)

- Persistent or significant disability/incapacity

- Congenital anomaly/birth defect in the offspring of a patient who received study drug

- Other: Important medical events that may not result in death, be immediately life-threatening, or require hospitalization, may be considered an SAE when, based upon appropriate medical judgment, they may jeopardize the patient or may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such events are:

  Intensive treatment in an emergency room or at home for allergic bronchospasm
  Blood dyscrasias or convulsions that do not result in hospitalization
  Development of drug dependency or drug abuse

9.3 Reporting of Serious Adverse Events

All Serious Adverse Events will be reported to the University of Texas M.D. Anderson Cancer Center IRB within five working days of knowledge of the event.

.Serious Adverse Events will also be reported to Merck, Sharp and Dohme, Corp.,. (Attn: Worldwide Product Safety; FAX 215-993-1220) with copies of all serious and/or unexpected adverse experiences, within two working days. Investigator will establish and maintain records and make reports to the IRB with copies, as described above, to Merck for the following Adverse Experiences: (1) all serious, unexpected, adverse events, (2) any significant increase in the frequency of serious expected adverse events, and (3) any significant increase in the frequency of therapeutic failures. Additionally, Investigator will report any pregnancy occurring in association with use of a Merck Product to Merck, Sharp and Dohme, Corp.,. (Attn: Worldwide Product Safety; FAX 215-993-1220). For any serious thromboembolic Adverse Experiences, investigator will fax, at the same time as the submission to Merck of the original report, the additional information set forth on the attached questionnaire (Appendix L) In addition, if any new information becomes available regarding any Adverse Experience, that new information will be submitted to Worldwide Product Safety within two working days.

10.0 CRITERIA FOR DISCONTINUATION OF TREATMENT

1. Progressive disease as defined above after one cycle of treatment. (Section 8.2.5).
2. The development of unacceptable toxicity.
3. Patient refusal.
4. Non-compliance or inability to comply with protocol requirements by patient
5. Treatment related adverse events not resolved within a 4 weeks rest period or requiring more than two dose de-escalations of any drug whichever is earlier.

Patients may remain on treatment as long as disease progression is not observed and the patient is not experiencing unacceptable toxicity (see 8.9). Although therapy is planned for one year, the patient may remain on treatment beyond this time if both the patient and physician agree that further therapy is in the patient's best interests.

11.0 STATISTICAL CONSIDERATIONS

11.1 Phase I component

The phase I component will be a non randomized trial in which Arm 1 and Arm 2 will be opened simultaneously with consecutive patients enrolled alternately to each arm, and sequentialy to each dose level. If the MTD is reached earlier in one arm, all subsequent patients will be enrolled to the other arm. Once the MTD for Arm 1 and Arm 2 is known, Arm 3 will begin accrual. A maximum of 54 patients will be enrolled in this component of the trial. The MTDs obtained in the Phase I component will be used as the Phase II dose in the next component of the trial.

11.1.1. Arm 1 of the phase I component was to assess the MTD of vorinostat in combination with a fixed dosage of cRA as shown in the table below. As noted above in section 5.1.1, the MTD of vorinostat in combination with cRA has been established in the current trial.

Level	Arm 1	
	Vorinostat (mg/day x 14 days)	cRA (mg/m2/day x 21 days)
0 (starting dose)	300	100
I	400	100
II	500	100

11.1.2. Arm 2 of the phase I component was aimed at assessing the MTD of Carboplatin (CBT) in combination with a fixed dosage of cRA. With the change in the combination to temozolomide with isotretinoin, the MTD is already known; hence, a phase I trial of this arm will not be needed.

11.1.3. Arm 3 of the phase I component will assess the MTD vorinostat in combination with fixed doses of temozolomide + cRA. For temozolomide, the Phase II dose determined in Arm 2 will be used. The enrollment will proceed as outlined for Arm 1 (cohorts of 3 patients enrolled to each dose level), declaring MTD for Temozolomide in combination with vorinostat and cRA once DLT is noted.

Level	Arm 3		
	Vorinostat (mg/day x days 1-7 and 15-21)	**cRA (mg/m2/day x 21 days)**	**Temozolomide (mg/m2/day on days 1-7 &15-21**
-III	300	100	100
-II	300	100	125
-I	400	100	125
0 (starting dose)	400	100	150
I	500	100	150

- If the initial cohort of patients at starting dose level 0 do not experience DLT, the next cohort of patients will be treated by dose escalation to level I (with increase in the dose of vorinostat). If no patients experience DLT in this cohort, 3 more patients will be enrolled to expand this cohort to a total of 6 patient to confirm that MTD has been achieved. If no DLT is noted or if only 1/6 patients experience DLT after this expansion of level I, this dose level will be declared the MTD and hence the phase II dose.
- If 2/6 patients have DLT after expansion of dose level I, dose level 0 will be declared as the MTD and hence the phase II dose.

- If Dose level 0 exceeds the MTD, lower doses will be studied as noted in the table above. If 1/3 patients have DLT at any dose level, the cohort will be expanded by 3 more patients. If ≥2/6 patients experience DLT, the MTD will be considered as being exceeded and the dose will be de-escalated to the next lower dose level and 3 new patients will be enrolled at that level. If none or only 1/6 patients experience DLT after dose de-escalation to the lower level, this dose level will be declared as the MTD, and will be utilized for the phase II component of the study.

11.2 Phase II component

This is an adaptive randomized phase II trial to compare three treatment arms: a) vorinostat + Isotretinoin, b) Temozolomide + Isotretinoin, and c) vorinostat + Isotretinoin + Temozolomide, in patients with recurrent GBM. The primary outcome is Progression Free Survival (PFS). Patients will be randomized among arms using a Bayesian adaptive algorithm. Technical details of this methodology are given in Appendix H. Patients will be randomized fairly among the three arms at the start of the trial (for the first 30 patients). Thereafter, as the trial progresses and data accrue, the randomization will become unbalanced in favor of the

treatment that, on average, has better results in terms of failure time. Therefore, each successive patient is more likely to receive the treatment with better results, on average. A minimum of 30 and a maximum of 135 patients will be accrued and there will be 6 months follow up after the last patient is accrued. Based on an anticipated accrual rate between 3 and 5 patients per month, the maximum trial accrual period will be between 45 and 27 months.

Patients enrolled in the surgical arm of the phase II component will be randomized to one of the three treatment arms after surgery is performed. In surgical patients, cycle 1 will be the first cycle of treatment after surgery. PFS in these patients will be determined from the date of randomization into the chemotherapy arms (i.e. after surgery for tumor resection), and not from the date of registration in the trial.

The tables given below summarize operating characteristics of the design. The historical median PFS is 3 months. The tables below assume an accrual rate of either 3 or 5 patients per month. The trial will be stopped early and a treatment selected as being “better” if the probability that one treatment’s median PFS is larger than the other’s PFS exceeds 0.975. If the trial does not stop early and the maximum 135 patients are accrued, a treatment is selected as being “better” if the probability that one treatment’s median PFS is larger than the other’s PFS exceeds 0.85. A treatment arm will be dropped at any point during the trial if the probability that the treatment’s median PFS is larger than 3 months is less than 0.01. The “# of patients treated” row is the average number of patients treated on a given arm under the given scenario. When the medians all equal to 3 months (scenario 1), the probability of selecting one of the three arms (i.e., a false positive result) is at most 6% for both accrual rates. The probability of selecting the best treatment (i.e., a true positive result) for scenario 4, when the medians PFS are 3, 3, and 7 months for the arms, respectively, is 99.5% for the accrual rate of 3 patients per month and 99.7% for the accrual rate of 5 patients per month.

At each evaluation when a new patient is coming in the study, the data for patients who have been followed till that time and not yet progressed are accounted in the analysis.

The trial will be conducted using a website developed in the Department of Biostatistics at MD Anderson Cancer Center. Through the web interface, the users have the ability to randomize patients, update last evaluation date and current patient status. When a patient is randomized the calculations are based on all available data entered into the website. The results of the randomization are displayed to the screen for the user to view. All data is stored in a secure SQL server database.

Run Date: Wed May-05-2010 03:46:39 PM
Trial Design

Primary endpoint	Time-To-Event
Number of arms	3

Minimum patient accrual	30
Maximum patient accrual	135
Number of patients to randomize fairly	30
Minimum randomization probability	0.1
Tuning parameter	1
Additional follow-up	6
Goal of study	Maximize θ

Prior Parameters

Arm Name	Description	Prior Parameters (a, b)
Arm1	V+A	(2.009, 3.027)
Arm2	T+A	(2.009, 3.027)
Arm3	V+A+T	(2.009, 3.027)

Stopping Rules
Suspend accrual to an arm if $Pr(\theta_i > \max(\theta_{j \neq i}) \mid Data) < P_L$
Stop the trial and select an arm as superior if $Pr(\theta_i > \max(\theta_{j \neq i}) \mid Data) > P_U$
At the final analysis select an arm as superior if $Pr(\theta_i > \max(\theta_{j \neq i}) \mid Data) > P_U^*$
Suspend accrual to an arm if $Pr(\theta_i > \theta_{min}) \mid Data) < P_L^*$

Stopping Rule Details

Arm	Group	P_L	P_U	P_U^*	θ_{min}	P_L^*
Arm1	No groups	0.01	0.975	0.85	3	0.01
Arm2	No groups	0.01	0.975	0.85	3	0.01
Arm3	No groups	0.01	0.975	0.85	3	0.01

SIMULATION PARAMETERS WITH ACCRUAL OF 3 PATIENTS/MONTH

Number of patients accrued each month	3
Number of repetitions	1000
Random number generator seed	123456789

Scenario 1

Average Trial Length 49.5 months

Arm	True Median Survival	Pr(Selected)	Pr(Selected Early)	Pr(Stopped Early)	# Patients (2.5%,97.5%)
V+A	3	0.054	0.015	0.082	43.7 (11, 88)

T+A	3	0.051	0.013	0.081	43.8 (11, 90)
V+A+T	3	0.061	0.015	0.083	43.8 (11, 89)

Scenario 2

Average Trial Length 43.3 months

Arm	True Median Survival	Pr(Selected)	Pr(Selected Early)	Pr(Stopped Early)	# Patients (2.5%,97.5%)
V+A	3	0.002	0.002	0.566	20.8 (10, 49)
T+A	4	0.038	0.018	0.301	36.7 (10, 89)
V+A+T	5	0.54	0.261	0.026	59.4 (10, 98)

Scenario 3

Average Trial Length 37.5 months

Arm	True Median Survival	Pr(Selected)	Pr(Selected Early)	Pr(Stopped Early)	# Patients (2.5%,97.5%)
V+A	3	0	0	0.918	14.3 (10, 28)
T+A	5	0.019	0.015	0.528	31.9 (10, 86)
V+A+T	7	0.758	0.486	0.015	57.6 (10, 101)

Scenario 4

Average Trial Length 21.9 months

Arm	True Median Survival	Pr(Selected)	Pr(Selected Early)	Pr(Stopped Early)	# Patients (2.5%,97.5%)
V+A	3	0	0	0.98	14.4 (10, 32)
T+A	3	0	0	0.979	14.3 (10, 32)
V+A+T	7	0.995	0.949	0	36 (10, 90)

Scenario 5

Average Trial Length 28.6 months

Arm	True Median Survival	Pr(Selected)	Pr(Selected Early)	Pr(Stopped Early)	# Patients (2.5%,97.5%)
V+A	3	0	0	0.935	14.3 (10, 28)
T+A	4	0.005	0.005	0.833	20.8 (10, 56)
V+A+T	7	0.96	0.803	0.005	47.5 (10, 97)

Scenario 6

Average Trial Length 10.3 months

Arm	True	Pr(Selected)	Pr(Selected	Pr(Stopped	# Patients

	Median Survival		Early)	Early)	(2.5%,97.5%)
V+A	1	0.025	0.001	0.819	10.2 (10, 13)
T+A	1	0.015	0	0.802	10.3 (10, 13)
V+A+T	1	0.012	0	0.806	10.3 (10, 13)

Run Date: Wed May-05-2010 03:57:33 PM
Trial Design

Primary endpoint	Time-To-Event
Number of arms	3
Minimum patient accrual	30
Maximum patient accrual	135
Number of patients to randomize fairly	30
Minimum randomization probability	0.1
Tuning parameter	1
Additional follow-up	6
Goal of study	Maximize θ

Prior Parameters

Arm Name	Description	Prior Parameters (a, b)
Arm1	V+A	(2.009, 3.027)
Arm2	T+A	(2.009, 3.027)
Arm3	V+A+T	(2.009, 3.027)

Stopping Rules
Suspend accrual to an arm if $Pr(\theta_i > \max(\theta_{j \neq i}) \mid Data) < P_L$
Stop the trial and select an arm as superior if $Pr(\theta_i > \max(\theta_{j \neq i}) \mid Data) > P_U$
At the final analysis select an arm as superior if $Pr(\theta_i > \max(\theta_{j \neq i}) \mid Data) > P_U^*$
Suspend accrual to an arm if $Pr(\theta_i > \theta_{min}) \mid Data) < P_L^*$

Stopping Rule Details

Arm	Group	P_L	P_U	P_U^*	θ_{min}	P_L^*
Arm1	No groups	0.01	0.975	0.85	3	0.01
Arm2	No groups	0.01	0.975	0.85	3	0.01
Arm3	No groups	0.01	0.975	0.85	3	0.01

SIMULATION PARAMETERS WITH ACCRUAL OF 5 PATIENTS/MONTH

Number of patients accrued each month	5
Number of repetitions	1000

Random number generator seed	123456789

Scenario 1

Average Trial Length 31.8 months

Arm	True Median Survival	Pr(Selected)	Pr(Selected Early)	Pr(Stopped Early)	# Patients (2.5%,97.5%)
V+A	3	0.04	0.014	0.076	43.8 (11, 90)
T+A	3	0.046	0.02	0.08	43.7 (11, 86)
V+A+T	3	0.055	0.018	0.073	43.5 (10, 88)

Scenario 2

Average Trial Length 28.9 months

Arm	True Median Survival	Pr(Selected)	Pr(Selected Early)	Pr(Stopped Early)	# Patients (2.5%,97.5%)
V+A	3	0.001	0.001	0.564	21.8 (10, 49)
T+A	4	0.042	0.021	0.253	38.8 (10, 87)
V+A+T	5	0.492	0.207	0.023	61.1 (14, 96)

Scenario 3

Average Trial Length 25.1 months

Arm	True Median Survival	Pr(Selected)	Pr(Selected Early)	Pr(Stopped Early)	# Patients (2.5%,97.5%)
V+A	3	0	0	0.915	15.5 (10, 33)
T+A	5	0.014	0.012	0.493	33.5 (10, 83)
V+A+T	7	0.745	0.444	0.012	60.4 (13, 99)

Scenario 4

Average Trial Length 15.3 months

Arm	True Median Survival	Pr(Selected)	Pr(Selected Early)	Pr(Stopped Early)	# Patients (2.5%,97.5%)
V+A	3	0.001	0.001	0.968	16 (10, 33)
T+A	3	0	0	0.973	15.8 (10, 33)
V+A+T	7	0.997	0.931	0.001	42.4 (10, 91)

Scenario 5

Average Trial Length 20.2 months

Arm	True	Pr(Selected)	Pr(Selected	Pr(Stopped	# Patients

	Median Survival		Early)	Early)	(2.5%,97.5%)
V+A	3	0	0	0.944	15.8 (10, 32)
T+A	4	0.003	0.003	0.803	23.7 (10, 64)
V+A+T	7	0.949	0.727	0.003	53.2 (11, 98)

Scenario 6

Average Trial Length 6.28 months

Arm	True Median Survival	Pr(Selected)	Pr(Selected Early)	Pr(Stopped Early)	# Patients (2.5%,97.5%)
V+A	1	0.02	0	0.768	10.4 (10, 14)
T+A	1	0.022	0	0.762	10.5 (10, 14)
V+A+T	1	0.024	0	0.757	10.5 (10, 15)

(Abbreviations: PFS: Progression free survival; V, vorinostat; A, accutane (isotretinoin); T, temozolomide)

Patients' demographic information at baseline will be analyzed, with data summarized in tables listing the number of subjects per treatment group in order to assess comparability. The Student t-test or the Wilcoxon rank sum test will be used to compare continuous variables between two different patient groups. The chi-squared test or the Fisher exact test will be applied to assess the association between two categorical variables.

Time-to-event outcomes, including progression-free survival and overall survival, will be estimated using Kaplan-Meier method. The log-rank test will be performed to test the difference in time-to-event distributions between patient groups. Cox proportional hazards model will be utilized to include multiple covariates in the time-to-event analysis.

Toxicity data will be summarized by frequency tables. The association between the types and severity of toxicity and the treatment groups will be evaluated. No formal statistical testing will be performed on these summary data.

To provide formal safety monitoring in the Phase II portion of the trial, after enrollment of every 10 patients into each of the three arms, we will assess the group for toxicity. If dose limiting toxicity as defined in the phase I portion of the trial is reached =/> 1/3 of patients despite dose reduction, the arm will stop accrual. If dose reduction permits continuation of treatment in these patients, the starting dose levels for the remainder of the patients on the trial will be modified to a lower starting dose (-1) level specified in the dose modification section.

For the efficacy endpoint, intent-to-treat analysis will be applied to the randomized and eligible patients. For the toxicity endpoint, as-treated analysis

will be used to include any patient who received the treatment – regardless of the eligibility nor the duration or dose of the treatment received.

11.3 Evaluation of symptom data (MDASI-BT)

The sample size for this trial was based on the primary end point of the study.

Received MDASI-BT forms will be checked versus the timing schedule and considered as valid if they fall within one week of the scheduled assessment. Compliance rates will be calculated as the number of received valid forms over the number of expected forms. Differences between groups in compliance will be tested by use of Fisher's exact test at every time point.

Estimates of differences in the mean symptom severity and mean symptom interference between groups will be estimated in the intent to treat population. All patients with at least one valid MDASI-BT will be included in the analyses. MDASI-BT completed at study registration will be considered baseline. All MDASI-BT data received after randomization will be used in the primary analyses.

For the MDASI-BT, differences of at least 2 points will be classified as the minimum clinically meaningful change in the symptom severity and symptom interference measures. For example, an increase of 2 points or more would mean a moderate improvement, whereas a decrease of 2 points or more would be interpreted as moderate worsening. For individual symptoms, a rise in a symptom score means deterioration, whereas a reduced score means improvement of the specific symptom.

12.0 COMPLIANCE AND DATA MANAGEMENT

12.1 Protocol Compliance:

The attending physician and oncology research nurse must see each patient after the first cycle, and subsequently after each 2 cycles of treatment prior to initiating the next cycle. All required interim and pretreatment data should be available and the physician must have made a designation as to tumor response and toxicity grade.

12.2 Data Entry:

All data will be entered on the computerized Protocol Data Management System (PDMS) of the Division of Medicine at MDACC. Patients must be registered in PDMS before a course of therapy can be given. A brief explanation for required but missing data should be recorded as a comment.

12.3 Accuracy of Data Collection:

A group consisting of the study chairman, the department chairman, the deputy chairman and an faculty member from the neuroradiology department will be the final arbiter of response or toxicity should a difference of opinion exist. The study chair will have the deciding vote in case of a tie.

Any life-threatening and/or unexpected and serious (grade 3 or 4) toxicity will be reported within 5 working days to the Study Chairman who, in turn, must notify the Surveillance Committee and the sponsoring agencies (See Appendix A).

13.0 REFERENCES

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