

Cancer Therapy: Clinical

Rationally Designed Pharmacogenomic Treatment Using Concurrent Capecitabine and Radiotherapy for Glioblastoma; Gene Expression Profiles Associated with Outcome

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Abstract

Purpose: Previous preclinical studies suggested that concurrent capecitabine and radiation could be an effective new treatment modality for glioblastoma (GBM). In the current study, we investigate toxicity and response to this regimen and explore associations between gene expression and patient outcome.

Experimental Design: Eighteen newly diagnosed GBM patients received concurrent capecitabine at 625 mg/m² BID (25% escalation) and irradiation (60 Gy total) for 6 weeks followed by 4 weeks of capecitabine only. Maintenance capecitabine was administered for 14 days every 3 weeks until progression or unacceptable toxicity. Expression analysis of 94 genes involved in capecitabine metabolism and radiation response was done on tissues obtained before therapy. The relationship of gene expression with time-to-progression (TTP) and overall survival (OS) was investigated using univariate Cox proportional hazards regression, semi-supervised principle component analysis, and class prediction modeling.

Results: The maximum tolerated dose of capecitabine was 625 mg/m² BID. Median patient TTP and OS were 247 and 367 days, respectively. Cox regression identified 24 genes significantly ($P < 0.025$) associated with patient outcome. Semi-supervised principle component analysis identified two patient populations significantly different in both TTP ($P = 0.005$) and OS ($P = 0.015$). Class prediction modeling determined that eight genes (*RAD54B*, *MTOR*, *DCTD*, *APEX2*, *TK1*, *RRM2*, *SLC29A1*, and *ERCC6*) could collectively classify patients into outcome subgroups with 100% accuracy and precision.

Conclusions: Capecitabine and concurrent radiation for newly diagnosed GBM seems to be well tolerated and comparable to temozolomide and radiation. A gene expression profile predictive of patient outcome that may be useful in patient stratification for therapy was also elucidated. *Clin Cancer Res*; 16(10); 2890–8. ©2010 AACR.

Glioblastoma (GBM) remains the most common and malignant form of primary brain neoplasm (1). Average survival for patients diagnosed with GBM is 9 to 12 months, with fewer than 2% surviving over 5 years (2). GBM is characterized by a diffuse infiltrative nature, making complete resection difficult and, in most cases, necessitating adjuvant chemoradiotherapy (3). Concurrent temozolomide and radiation therapy was recently adopted as the standard of care for GBM following a clinical study reporting a 4-month increase in patient sur-

vival compared with radiation therapy alone (4). Unfortunately, a significant portion of patients (~55%) fail to respond to this chemoradiotherapy regimen (5, 6). The slow incremental progress made in the development of effective treatment paradigms for GBM emphasizes the limitations of empirically designed treatment regimens for this particularly lethal cancer. Interestingly, several recent preclinical and clinical studies suggested that capecitabine may be an effective new treatment paradigm for GBM (7–9).

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Translational Relevance

In this study, a novel pharmacogenomic approach was used to rationally design a phase I clinical study using concurrent administration of capecitabine and irradiation for patients newly diagnosed with glioblastoma. Results suggest that treatment with capecitabine (an antimetabolite) may be as effective as temozolomide (an alkylating agent which is the current standard of care). Important clinical and research implications are that (a) capecitabine may provide an alternative treatment for patients who do not respond to temozolomide (50-70%) or for patients who become refractory to temozolomide; and (b) given that capecitabine and temozolomide have different mechanisms of action and toxicity profiles, it may be possible to combine these treatment regimens with concurrent administration of both drugs. Lastly, molecular analysis of tumor biopsies obtained before treatment identified an eight-gene expression profile that may prove useful in the future stratification of GBM patients to help guide therapy.

Capecitabine is an oral fluoropyrimidine prodrug that has not been previously examined for use in GBM therapy. Following administration, capecitabine is sequentially converted to 5'-deoxy-5-fluorocytidine and 5'-deoxy-5-fluorouridine by carboxylesterase and cytidine deaminase, respectively (10). The final and rate-limiting step in the activation of capecitabine is the intratumor hydrolysis of 5'-deoxy-5-fluorouridine into 5-fluorouracil (5-FU) by thymidine phosphorylase (TP; refs. 11, 12). The metabolic activation of capecitabine exploits the elevated TP levels reported in some solid tumors (compared with normal tissue) to achieve selective intratumor activation and, ultimately, minimizes systemic exposure to 5-FU (11, 12). Preclinical pharmacogenomic studies showed that intratumor expression of TP and dihydropyrimidine dehydrogenase (DPD) are associated with response to capecitabine (11-13). DPD is the initial and rate-limiting enzyme responsible for the catabolic elimination of 5-FU (12). By comparing response independently to TP and DPD, it has been shown that elevated TP expression results in higher intratumor levels of 5-FU whereas (11, 12), conversely, high DPD expression results in increased 5-FU degradation and decreased response (12). Of particular importance to combination therapy, ionizing radiation has been shown to significantly increase the anti-tumor efficacy of capecitabine through a tumor-associated induction of TP expression (14).

Early clinical investigations of systemic 5-FU as a single agent or in combination with radiation for the treatment of malignant glioma did not significantly improve survival (15-17). However, recent studies suggest that minimal response may have resulted from the limited availability of

5-FU in the tumor and not from the intrinsic resistance of gliomas to fluoropyrimidines. In clinical studies, direct implantation of 5-FU-loaded microspheres in the wall of the surgical bed following surgical resection in GBM patients resulted in an overall median survival time of almost 2 years, with two patients achieving disease remission at 139 and 153 weeks (7). In other studies, molecular analyses of clinical GBM biopsies reported a distribution of TP and DPD expression, which should result in selective intratumor activation of capecitabine (increased TP expression in GBM), whereas 5-FU clearance from the tumor and normal tissues should be similar (equivalent DPD expression in GBM and uninvolved tissue; ref. 8). Further, *in vivo* studies showed that exposure of glioma xenografts to irradiation results in a significant, tumor-associated induction of TP and subsequent increased anti-tumor efficacy to capecitabine (8, 14). Publication of these results coincided with a report by Wang et. al. describing the successful treatment of brain metastasis with capecitabine (9). This study was particularly noteworthy because previous whole-brain radiotherapy and systemic chemotherapy, including treatment with 5-FU, proved ineffective, suggesting that (a) 5-FU resistance does not predict response to capecitabine and (b) capecitabine may achieve therapeutic concentrations in brain tissues (a significant consideration for developing chemotherapy options for the treatment of central nervous system malignancies). These results also supported earlier pharmacokinetic studies which showed that both 5-FU (18, 19) and 5'-deoxy-5-fluorouridine (20-22) cross the blood brain barrier. Taken collectively, these studies provided the rationale for the examination of capecitabine for the treatment of primary GBM.

In the current study, we examine response to treatment of newly diagnosed primary GBM patients to concurrent administration of capecitabine and radiation. In addition, all known genes involved in capecitabine metabolism as well as genes previously associated with response to fluoropyrimidine drugs and radiation therapy were examined to develop a gene expression model predictive of outcome. This represents the first study to examine genetic signatures corresponding to capecitabine response in GBM patients, which may ultimately be used to rationally stratify patients for future clinical studies examining capecitabine and to develop new treatment paradigms.

Materials and Methods

Patients. Nineteen patients with newly diagnosed GBM consented and were enrolled contingent on meeting the following criteria: ≥ 18 years of age; histologically established GBM (according to the WHO guidelines; ref. 23); maintained on a stable dose of corticosteroids for ≥ 5 days; a Karnofsky performance status of $\geq 60\%$; adequate hematologic, renal, and hepatic functions; and capable of providing informed consent. Tumor tissue was obtained via debulking or biopsy before initiation of chemotherapy and radiotherapy and was immediately formalin fixed and

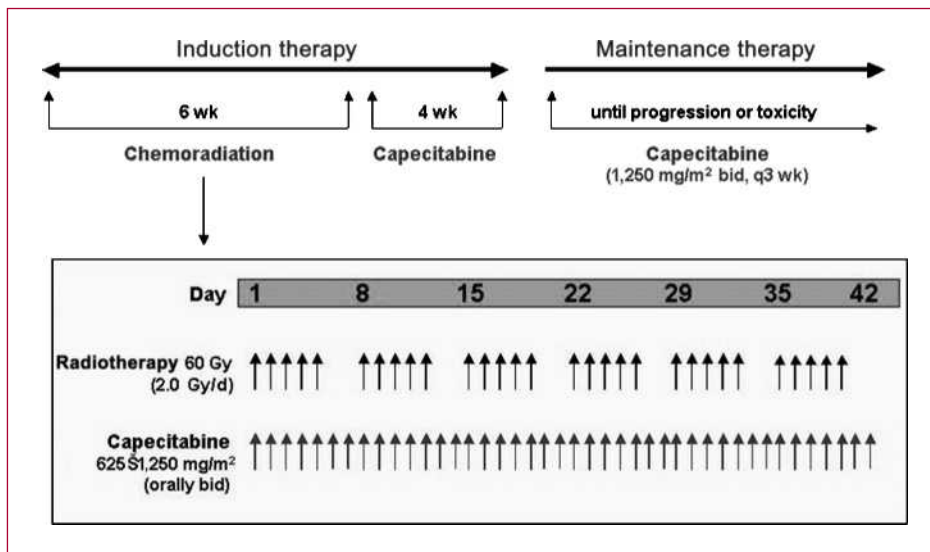


Fig. 1. Patient treatment schematic with capecitabine and radiotherapy.

subsequently paraffin embedded. All studies using human tissues were approved by and conducted in accordance with the policies of the Institutional Review Board at the University of Alabama at Birmingham.

Treatment plan. In the current study, patients were first stratified by anticonvulsant use based on previous reports of altered clearance of chemotherapy agents in patients using cytochrome P450 enzyme-inducing anticonvulsant drugs (EIAD; ref. 24). During the induction phase, patients received capecitabine on a continuous daily basis during the 6 weeks of radiotherapy as well as the 4 weeks following radiotherapy for a total of 10 weeks. Patients received radiotherapy for a total dose of 60 Gy given in 30 fractions over the 6 weeks. A standard chemotherapy dose escalation design was used for capecitabine administration. The first dose level of capecitabine was at 625 mg/m² BID (1,250 mg/m²/d). Doses were escalated by 25% increments in consecutive cohorts of three patients until the maximum tolerated dose (MTD) was achieved. After a 1-week hiatus, the patients entered the maintenance phase with capecitabine at a dose of 1,250 mg/m² BID (2,500 mg/m²/d) on a schedule of 14 days on and 7 days off. Cycles were discontinued on radiographic evidence of tumor progression (assessed monthly), clinical deterioration, or voluntary withdrawal. The MTD was defined as the dose level below the dose that induced dose-limiting toxicities (DLT) in more than two of three patients. A DLT was defined as any grade 3 or 4 toxicity attributable to the study drug, evaluated according to the National Cancer Institute Common Toxicity Criteria version 3.

Gene expression analysis. RNA was extracted from 20- μ m tissue sections using the Roche High Pure RNA Paraffin Kit (Roche Diagnostics) as per manufacturer's instructions. Concentration was determined through S9 housekeeping gene expression analysis on an ABI 7900HT Sequence

Detection System as previously described (Applied Biosystems; ref. 25). Reverse transcription was done using the High Capacity cDNA Archive Kit (Applied Biosystems) as per manufacturer's instructions and stored at -80°C until analysis.

Individual RTQ assays were formatted into a TaqMan low-density array (TLDA; Applied Biosystems). The precision, accuracy, and intra- and inter-assay variability have been previously described in detail by our laboratory (26, 27). Samples were normalized to the S9 housekeeping gene, which has been validated for use in irradiated tissues (25). The 94 genes selected for inclusion on the TLDA included all known genes in the anabolic and catabolic metabolisms of capecitabine as well as genes associated with response to radiation therapy. TLDA analysis was done using the Applied Biosystems Prism 7900HT sequence detection system. Gene expression values were calculated using the comparative C_t method with normal brain cDNA used as calibrator (26).

Statistical analysis. Patient characteristics and toxicities were summarized using appropriate descriptive statistics. Time-to-progression (TTP) and overall survival (OS) were calculated from date of diagnosis until disease progression or death, respectively, or date of last follow-up. The method of Kaplan and Meier was used to estimate TTP and OS using SAS version 9.1.3 (SAS Institute; ref. 28).

Univariate Cox proportional hazards regression modeling was done to assess the association of expression of each gene with TTP and OS (29). Genes with a mean SD below 0.1 were filtered to eliminate genes with low variation across patient samples. All analyses were adjusted for patient age and capecitabine dose. Hazard ratios were computed to reflect the relationship between each gene and patient outcome (TTP and OS). To partially account for the multiplicity of tests, a more stringent $P < 0.025$ was used to establish statistically significant associations,

Table 1. Demographics of the 18 enrolled evaluable patients

| Characteristic | |
|---------------------------------|----------|
| Age, y | |
| Median | 49 |
| Range | 18-78 |
| Sex, <i>n</i> | |
| Male | 15 |
| Female | 3 |
| Karnofsky performance status, % | |
| Median | 80 |
| Range | 60-100 |
| Race, <i>n</i> (%) | |
| White | 18 (100) |
| Black | |
| Asian | |
| Histology, <i>n</i> (%) | |
| Glioblastoma multiforme | 18 (100) |

rather than $P < 0.05$, which is typically used. Genes that were found to be significantly associated with patient TTP and OS in the univariate analysis were considered for future statistical analyses.

To identify GBM patient outcome risk groups, we used the genes significantly correlated with patient outcome from the Cox regression analysis in a semi-supervised principle component analysis (SSPCA; refs. 30, 31). In this study, the first three principle components were used to cluster patient samples into two groups based on shrunken centroids. The Kaplan-Meier method in conjunction with log-rank tests were used to examine significant differences ($P < 0.025$) in patient TTP and OS between the two patient outcome risk subgroups (28).

Genes significantly associated with patient TTP and OS were used to build gene expression-based predictor models of GBM patient outcome. Model assumptions were examined using the weighted-voting algorithm with leave-one-out cross-validation to test performance and accuracy (30). To determine if the resulting prognostic model was exclusive to GBM patients treated with capecitabine, associations between gene expression and clinical outcome were examined in an unrelated data set of 228 GBM patients who were not treated with capecitabine [obtained through The Cancer Genome Atlas (TCGA) database, <http://tcga-data.nci.nih.gov>; refs. 32, 33]. Analyses were conducted and graphed using the GenePattern software (<http://www.broad.mit.edu/cancer/software/genepattern>; ref. 31).

Table 2. Genes associated with GBM patient TTP

| Accession no. | Gene | Description | Hazard ratio* | <i>P</i> |
|-------------------------------|----------------|------------------------------------|---------------|----------|
| Capecitabine metabolism genes | | | | |
| NM_001033 | <i>RRM1</i> | Ribonucleotide reductase M1 | 2.30 | 0.002 |
| NM_000110 | <i>DPYD</i> | Dihydropyrimidine dehydrogenase | 1.23 | 0.002 |
| NM_012474 | <i>UCK2</i> | Uridine-cytidine kinase 2 | 2.14 | 0.004 |
| NM_001905 | <i>CTPS</i> | CTP synthase | 19.63 | 0.009 |
| NM_004955 | <i>SLC29A1</i> | Solute carrier family 29, member 1 | 1.06 | 0.010 |
| NM_001953 | <i>TP</i> | Thymidine phosphorylase (ECGF1) | 1.11 | 0.010 |
| NM_001785 | <i>CDA</i> | Cytidine deaminase | 1.66 | 0.020 |
| Radiation response genes | | | | |
| NM_002875 | <i>RAD51</i> | RAD51 homolog | 1.06 | 0.007 |
| NM_002524 | <i>NRAS</i> | Neuroblastoma RAS | 1.49 | 0.007 |
| NM_005432 | <i>XRCC3</i> | XRCC3 | 6.05 | 0.008 |
| NM_012415 | <i>RAD54B</i> | RAD54 homolog B | 1.21 | 0.009 |
| NM_005732 | <i>RAD50</i> | RAD50 homolog | 1.62 | 0.009 |
| NM_003401 | <i>XRCC4</i> | XRCC4 | 1.28 | 0.010 |
| NM_001641 | <i>APEX1</i> | APEX nuclease 1 | 2.20 | 0.012 |
| NM_000124 | <i>ERCC6</i> | ERCC6 | 1.90 | 0.013 |
| NM_014481 | <i>APEX2</i> | APEX nuclease 2 | 1.74 | 0.018 |
| NM_021141 | <i>XRCC5</i> | XRCC5 | 2.48 | 0.019 |
| NM_000251 | <i>MSH2</i> | mutS homolog 2 | 37.50 | 0.022 |
| NM_006297 | <i>XRCC1</i> | XRCC1 | 1.35 | 0.023 |

NOTE: Cox regression analysis was used to assess the association between TTP and each individual gene expression value, adjusted for age and dose.

*Hazard ratio > 1: higher gene expression, shorter time-to-progression; hazard ratio < 1: higher gene expression, longer time-to-progression; hazard ratio = 1: no association.

Table 3. Genes associated with GBM patient OS

| Accession no. | Gene | Description | Hazard ratio* | P |
|-------------------------------|----------------------|---|---------------|-------|
| Capecitabine metabolism genes | | | | |
| NM_001921 | <i>DCTD</i> | dCMP deaminase | 51.15 | 0.005 |
| NM_003258 | <i>TK1</i> | Thymidine kinase 1 | 156.73 | 0.006 |
| NM_000110 | <i>DPYD</i> | Dihydropyrimidine dehydrogenase | 1.21 | 0.006 |
| NM_001033 | <i>RRM1</i> | Ribonucleotide reductase M1 | 1.93 | 0.007 |
| NM_001034 | <i>RRM2</i> | Ribonucleotide reductase M2 | 1.04 | 0.008 |
| NM_001905 | <i>CTPS</i> | CTP synthase | 40.10 | 0.008 |
| NM_012474 | <i>UCK2</i> | Uridine-cytidine kinase 2 | 1.60 | 0.024 |
| Radiation response genes | | | | |
| NM_002875 | <i>RAD51</i> | RAD51 homolog | 1.08 | 0.003 |
| NM_012415 | <i>RAD54B</i> | RAD54 homolog B | 1.22 | 0.013 |
| NM_006297 | <i>XRCC1</i> | XRCC1 | 1.45 | 0.014 |
| NM_004958 | <i>mTOR</i> | Mechanistic target of rapamycin (FRAP1) | 6.55 | 0.020 |
| NM_001274 | <i>CHEK1</i> | CHK1 checkpoint homolog | 1.74 | 0.025 |

NOTE: Cox regression analysis was used to assess the association between OS and each individual gene expression value, adjusted for age and dose.

*Hazard ratio > 1: higher gene expression, shorter survival; hazard ratio < 1: higher gene expression, longer survival; hazard ratio = 1: no association.

Results

Patient characteristics. Between December 2002 and April 2004, a total of 19 patients with newly diagnosed GBM were enrolled into the study and treated according to the treatment schematic in Fig. 1. Demographic characteristics are listed in Table 1. One patient never received the study drug or any form of adjuvant therapy after surgical diagnosis and elected palliative care due to rapid tumor progression. Nine patients were taking EIADs and nine patients were taking non-EIADs or were not taking anticonvulsants. No statistical differences in age, sex, and patient TTP or OS were shown between the EIAD and the non-EIAD treatment groups. All patients had prior surgery.

Toxicity. The documentation of adverse events was in accordance with the National Cancer Institute Common Toxicity Criteria. The majority of events occurring in patients were gastrointestinal, consisting primarily of grade 1 or 2 nausea (67%), vomiting (67%), and diarrhea (67%). Grade 1/2 stomatitis (67%), hand-foot syndrome (50%), and fatigue (33%) were also reported. A DLT was observed in one patient for each EIAD arm at the lowest dose level of 625 mg/m² BID. In both cases, the DLT was a grade 3 diarrhea occurring during week 4 of the induction phase. Each arm was expanded to six patients with no further DLTs noted. The second dose level of 750 mg/m² BID enrolled an initial three patients in whom two patients developed a grade 3 diarrhea in the non-EIAD arm and two patients developed a grade 3 hand-foot syndrome in the EIAD arm. On the basis of DLTs occurring in two of the three patients enrolled at the 750 mg/m² BID (1,500

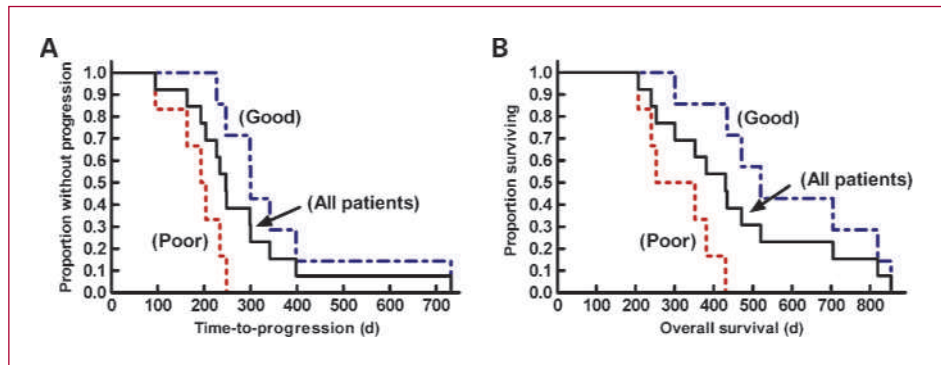
mg/m²/d) dose level for both the EIAD and the non-EIAD arms, the MTD was determined to be 625 mg/m² BID (1,250 mg/m² BID) for capecitabine concurrent with radiation therapy 7 days a week for 10 consecutive weeks in patients with newly diagnosed GBM.

Survival and progression. For 15 of the 18 patients with progression data, determined through measurable disease by monthly cranial magnetic resonance imaging, the median TTP was 247.0 days with an SE of 40.2 days. The median OS was 366.5 days with an SE of 54.9 days.

Gene expression-based predictor model development. Before administration of chemoradiotherapy, all GBM patients underwent surgery for tumor resection and/or biopsy. Tissues were formalin fixed and paraffin embedded for histologic examination. A total of 13 of 19 patient tissues were available for molecular analysis. A custom TLDA was designed to selectively examine the expression of 94 genes, which included all known genes in the anabolic and catabolic pathways responsible for capecitabine metabolism, as well as genes previously associated with radiation response (see Supplementary Table S1). Univariate Cox proportional hazards regression identified a significant association between 19 genes and patient TTP (Table 2) and between 12 genes and OS (Table 3). Due to multiplicity of tests, a more stringent significance cutoff of $P < 0.025$ was used. Comparison of Tables 2 and 3 reveals a total of 24 unique genes associated with patient outcome, 7 of which correlated with both patient TTP and OS (*DPD*, *RRM1*, *CTPS*, *UCK2*, *RAD51*, *RAD54B*, and *XRCC1*; Table 3, bold).

To examine if molecular subgroups existed in this patient population, we evaluated the expression data using

Fig. 2. Results of SSPCA. Kaplan-Meier plots representing all evaluable ($n = 13$), poor outcome ($n = 6$), and good outcome ($n = 7$) patient groups with median (A) TTP of 247 d (—), 199 d (---), and 300 d (---) and (B) OS of 430 d (—), 303 d (---), and 520 d (---), respectively.



SSPCA. Because unsupervised principle component analysis may identify cancer subtypes that are unrelated to patient survival, we used SSPCA, which uses the subset of genes identified by the Cox regression analysis, to identify patient outcome subgroups. This technique has been shown to be advantageous in that it combines gene expression and clinical data to identify molecular subtypes. SSPCA analysis of the 24 genes identified two patient subgroups ($n = 6$ and $n = 7$) that, when analyzed using Kaplan-Meier plots, were shown to be significantly different (log-rank tests) in patient TTP (Fig. 2A; $P = 0.005$) and OS (Fig. 2B; $P = 0.015$).

Genes identified through Cox regression analysis were used to examine predictor models of patient outcome to capecitabine radiotherapy. Weighted-voting algorithm assessment of test predictor models identified an eight-gene expression-based model that accurately (100%) segregated all patients into either poor or good outcome subgroups (Fig. 3). These analysis also showed that the expression of all eight genes was significantly higher in the poor outcome (patients 1, 18, 19, 3, 6, and 15) compared with the good outcome (patients 16, 13, 11, 4, 17, 7, and 12) subgroup (Table 4). Subsequent examination of a separate data set containing 228 GBM patients who

were not treated with capecitabine (TCGA database) showed no association with clinical outcome (TTP or OS), suggesting that this prognostic model is specific for GBM patients treated with capecitabine.

Discussion

The slow, incremental progress made in the development of effective treatment paradigms for GBM emphasizes the limitations of empirically designed treatment regimens for this particularly lethal cancer. This study used a novel pharmacogenomic approach to design a new treatment paradigm for patients diagnosed with GBM. Earlier studies showed (a) a favorable molecular profile of drug metabolizing enzymes in GBM and uninvolved brain tissue for treatment with capecitabine (8); (b) that exposure of glioma xenografts to ionizing radiation results in a significant, tumor-associated induction of TP and subsequent increased antitumor efficacy to capecitabine (8); and (c) the successful treatment of brain metastasis secondary to primary breast cancer with capecitabine, suggesting that therapeutic antitumor efficacy can be achieved in brain tissues (9). Collectively, these initial studies provided the rationale to design and implement this clinical trial examining concurrent administration of capecitabine and radiation in treatment-naïve patients diagnosed with primary GBM (to our knowledge, one of the few rationally designed clinical trials for GBM). This trial also incorporated a unique molecular component in that all resected GBM specimens (obtained before treatment) were evaluated for the expression of all known anabolic and catabolic genes involved in capecitabine metabolism as well as genes associated with response to radiation (primarily DNA repair enzymes).

Results indicate that capecitabine and concurrent radiotherapy for newly diagnosed GBM is well tolerated without unexpected neurologic or gastrointestinal toxicities. As illustrated in Fig. 1, we selected an aggressive chemoradiation schedule of capecitabine daily during the 6 weeks of radiation therapy and continued for 4 additional weeks. This schedule for our induction phase was chosen based on preclinical studies which concluded that increased TP expression persisted postirradiation in our animal models

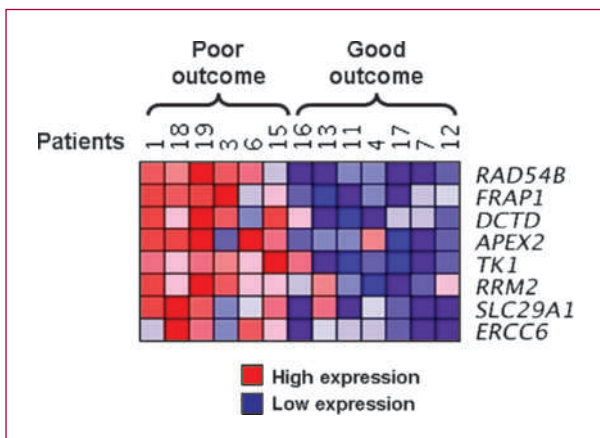


Fig. 3. Heat map of the eight-gene expression-based predictor model of patient outcome to capecitabine and radiotherapy treatment.

Table 4. Gene expression statistics for the poor outcome and good outcome patient subgroups

| Gene | Poor outcome | | | Good outcome | | | Fold change* | P |
|----------------|--------------|-------|--------|--------------|-------|-------|--------------|-------|
| | Ave. | Min | Max | Ave. | Min | Max | | |
| <i>RAD54B</i> | 14.60 | 7.47 | 20.86 | 4.60 | 2.91 | 7.06 | 3.17 | 0.002 |
| <i>mTOR</i> | 1.95 | 1.24 | 2.51 | 1.00 | 0.64 | 1.37 | 1.95 | 0.003 |
| <i>DCTD</i> | 1.39 | 0.71 | 1.80 | 0.61 | 0.31 | 0.99 | 2.28 | 0.004 |
| <i>APEX2</i> | 5.98 | 2.83 | 7.29 | 3.16 | 1.79 | 5.15 | 1.89 | 0.006 |
| <i>TK1</i> | 0.70 | 0.46 | 1.17 | 0.24 | 0.06 | 0.72 | 2.96 | 0.006 |
| <i>RRM2</i> | 98.04 | 73.82 | 132.73 | 49.50 | 12.71 | 88.05 | 1.98 | 0.007 |
| <i>SLC29A1</i> | 58.12 | 31.15 | 83.04 | 27.99 | 16.52 | 56.60 | 2.08 | 0.015 |
| <i>ERCC6</i> | 4.90 | 2.49 | 8.54 | 2.07 | 1.08 | 3.11 | 2.37 | 0.029 |

*Expression fold change relative to the poor outcome patient subgroup.

of malignant glioma (8). This 10-week period was the study observation time during which grade 3 or 4 non-hematologic toxicities or grade 4 hematologic toxicities would define DLTs. The starting dose of 625 mg/m² BID was selected based on previous experience with chemoradiation using capecitabine in pancreatic cancer (34). The study was stratified on patient anticonvulsant use based on previous reports of altered clearance of chemotherapy agents in patients using EIADs (24). The DLTs that defined the MTD in this study were similar to those seen in other cancers such as gastrointestinal cancer when capecitabine was administered concurrently with radiation therapy (34). In our study, hand-foot syndrome and diarrhea were equally responsible for DLTs, occurring in 16.7% of patients at 625 mg/m² BID and in 66.7% of patients at 750 mg/m² BID, thus defining the MTD. There was no difference in the incidence of DLTs based on anticonvulsant drugs, suggesting that capecitabine metabolism and clearance is not significantly affected by hepatic enzyme-inducing anticonvulsants. The majority of patients that experienced a DLT did so in the later weeks of the induction phase, suggesting that a continuous 70-day schedule may be too aggressive and that patients would tolerate a shorter induction period such as the 42-day concomitant course standard with temozolomide therapy. The incidences of adverse events or serious adverse events related to potential central nervous system toxicities were not increased and consisted primarily of fatigue and decreased energy. Based on these clinical results, the dose of oral capecitabine concurrent with radiation therapy recommended for future evaluation is 625 mg/m² BID with the 70-day schedule.

As shown in Fig. 2, the mean TTP and OS were 273 and 397 days, respectively. Collectively, clinical outcome suggests that this treatment paradigm may be as effective as temozolomide (an alkylating agent that forms of O⁶-alkylguanine DNA adducts). These studies suggest the exciting possibility that we could combine these two independent treatment modalities to obtain additive or potentially synergistic antitumor efficacy. The benefit of combining antimetabolite and alkylating agents has been established in

other models for the successful treatment of several cancers (i.e., 5-FU with oxaliplatin; refs. 35, 36). Alternatively, this regimen provides a potential avenue for the use of capecitabine as second-line therapy for approximately 55% to 70% of GBM patients who do not initially respond to temozolomide or for those patients who become refractory following treatment with temozolomide.

A unique component of this study was the inclusion of a molecular analysis that examined the expression of all known genes involved in capecitabine metabolism as well as genes associated with response to radiation. As shown in Tables 2 and 3, a total of 24 unique genes were individually associated with either TTP or OS, with seven genes (*DPD*, *RRM1*, *CTPS*, *UCK2*, *RAD51*, *RAD54B*, and *XRCC1*) significantly associated with both TTP and OS (Table 3, bold).

Previous molecular studies showed that TP expression in GBM is ~13-fold higher compared with uninvolved brain, whereas there was no significant difference in DPD levels in the same tissues (8). This distribution of TP and DPD should result in selective intratumor activation of capecitabine (elevated TP resulting in higher intratumoral 5-FU levels), whereas clearance from tumor and normal tissues should be similar (equivalent DPD expression). As shown in Tables 2 and 3, elevated DPD expression is significantly associated with poor patient outcome (both short TTP and OS). However, higher TP expression is significantly associated with shorter TTP (with no significant association with OS). Although this could be interpreted as contradictory to the hypothesis that elevated TP expression in GBM should result in increased activation of capecitabine, these results agree with previous studies in pancreatic cancer which reported that TP expression in biopsies obtained before treatment did not correlate with survival (34, 37). Subsequent studies examining pancreatic biopsies before and during treatment with capecitabine and concurrent radiation showed a significant induction of TP. Taken collectively, these studies suggest a dual role for TP: (a) As reported in several solid tumors, elevated TP expression before treatment indicates a more aggressive

phenotype characterized by increased vascularization and a poor prognosis (38, 39). (b) Clinical studies in pancreatic cancer and preclinical models in colorectal cancer, mammary cancer, and GBM suggest that TP is induced following radiation, which results in increased antitumor efficacy when administered concurrently with capecitabine (14). TP/DPD ratios (which have been reported as the best indicator of response to treatment with this regimen) did not correlate with either TTP or OS.

To examine if molecular subgroups existed in this patient population that could potentially be used to identify patients who would respond to this treatment regimen, we evaluated the expression data using SSPCA. This technique combines gene expression and clinical data to identify molecular subtypes associated with patient outcome (30, 31). As shown in Fig. 2, SSPCA (using the 24 unique genes identified by Cox regression analysis) identified two patient subgroups that were significantly different in both TTP (Fig. 2A) and OS (Fig. 2B). Segregation of patients into poor ($n = 6$) and good ($n = 7$) outcome subgroups based on their molecular profile allowed us to evaluate these data for predictors of patient outcome. Weighted-voting algorithm assessment of genetic profiles in each subgroup identified an eight-gene model that accurately (100%) segregated all patients into either poor or good outcome subgroup (Fig. 3; Table 4). Furthermore, analysis of gene expression and clinical data obtained from GBM patients who were not treated with capecitabine (TCGA database) suggested that this prognostic model may be specific for GBM patients treated with capecitabine. The combination of DNA repair (*RAD54B*, *ERCC6*, and *APEX2*), drug-metabolizing (*RRM1*, *TK1*, and *DCTD*), transport (*SLC29A1*), and cell proliferation (*mTOR*) genes in this model suggest that response to this treatment regimen is multifactorial and agree with other studies suggesting that analysis of multiple genes provides more accurate predictive or diagnostic potential. Although these

results are exploratory in nature due to the small sample size, the identification of a predictive model suggests that it may be possible to stratify patients toward more effective therapy.

This rationally designed treatment regimen seems to be well tolerated without unexpected toxicities. Tumor response and survival were comparable to standard treatment with temozolomide, although a larger trial comparing each arm independently would have to be conducted to confirm these results. These findings support the current consensus that clinical outcome of individuals with cancer can be predicted using gene expression profiles of primary tumors at diagnosis (40). Important clinical and research implications are that (a) capecitabine may provide an alternative treatment for the 50% to 70% of GBM patients who do not respond to temozolomide or for patients who become refractory to temozolomide; (b) because capecitabine and temozolomide have different mechanisms of action and toxicity profiles, it may be possible to combine these treatment regimens with concurrent or sequential administration of both drugs; and (c) gene expression profiles may prove useful in the future stratification of GBM patients to help guide therapy.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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