Published in final edited form as:
Clin Cancer Res. 2010 May 15; 16(10): 2890–2898. doi:10.1158/1078-0432.CCR-09-3151.

Rationally designed pharmacogenomic treatment using concurrent capecitabine and radiotherapy for glioblastoma; gene expression profiles associated with outcome

Jessica M. Grunda[1], **John Fiveash**[2], **Cheryl A. Palmer**[3], **Alan Cantor**[4], **Hassan M. Fathallah-Shaykh**[5], **L. Burt Nabors**[5], and **Martin R. Johnson**[1]

[1]Departments of Pharmacology and Toxicology, Division of Clinical Pharmacology; University of Alabama at Birmingham, Alabama 35294

[2]Department of Radiation Oncology; University of Alabama at Birmingham, Alabama 35294

[3]Department of Pathology; Division of Neuropathology; University of Alabama at Birmingham, Alabama 35294

[4]Department of Biostatistics, Biostatistics and Bioinformatics Unit; University of Alabama at Birmingham, Alabama 35294

[5]Department of Neurology, Division of Neuro-oncology; University of Alabama at Birmingham, Alabama 35294

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/m^2 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 performed on tissues obtained prior to therapy. The relationship between gene expression with time-to-progression (TTP) and overall survival (OS) was investigated using univariate Cox proportional hazards regression, semi-supervised principle component analysis (SSPCA), and class prediction modeling.

Results—The maximum tolerated dose of capecitabine was 625 mg/m^2 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. SSPCA analysis identified two patient populations significantly different in both TTP ($p=0.005$) and OS ($p=0.015$). Class prediction modeling determined that 8 genes (RAD54B, MTOR, DCTD, APEX2, TK1, RRM2, SLC29A1, ERCC6) could collectively classify patients into outcome subgroups with 100% accuracy and precision.

CORRESPONDING AUTHORS: Martin R. Johnson, Ph.D, Department of Pharmacology & Toxicology, Comprehensive Cancer Center, University of Alabama at Birmingham, Birmingham, AL 35294, L. Burt Nabors, M.D., Department of Neurology, Comprehensive Cancer Center, University of Alabama at Birmingham, Birmingham, AL 35294.
The trial was completed at the University of Alabama at Birmingham, Comprehensive Cancer Center, Birmingham, AL.

Conclusions—Capecitabine and concurrent radiation for newly diagnosed GBM appears 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.

Introduction

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 (RT) was recently adopted as the standard of care for GBM following a clinical study reporting a 4 month increase in patient survival compared to RT alone (4). Unfortunately, a significant portion of patients (approximately 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).

Capecitabine is an oral fluoropyrimidine prodrug which has not been previously examined for use in GBM therapy. Following administration, capecitabine is sequentially converted to 5'-deoxy-5-fluorocytidine (5’-DFCR) and 5'-deoxy-5-fluorouridine (5’-DFUR) by carboxylesterase (CE) and cytidine deaminase (CD) respectively (10). The final and rate limiting step in the activation of capecitabine is the intratumor hydrolysis of 5’-DFUR into 5-fluorouracil (5-FU) by thymidine phosphorylase (TP) (11,12). The metabolic activation of capecitabine exploits the elevated TP levels reported in some solid tumors (compared to normal tissue) to achieve selective intratumor activation and, ultimately, minimizes systemic exposure to 5-FU (11,12). Preclinical pharmacogenomic studies demonstrated 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 both TP and DPD, it has been demonstrated that elevated TP expression results in higher intratumor levels of 5-FU while (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 antitumor 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 to an 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 2 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), while 5-FU clearance from the tumor and normal tissues should be similar (equivalent DPD expression in GBM and uninvolved tissue) (8). Further, *in vivo* studies demonstrated 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. which described the successful treatment of brain metastasis with capecitabine (9). This study was particularly noteworthy since previous whole brain radiotherapy and systemic chemotherapy,

including treatment with 5-FU, proved ineffective suggesting that 1.) 5-FU resistance does not predict response to capecitabine and; 2.) capecitabine may achieve therapeutic concentrations in brain tissues (a significant consideration for developing chemotherapy options for the treatment of CNS malignancies). These results also supported earlier pharmacokinetic studies which demonstrated that both 5-FU (18,19) and 5'-DFUR (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 RT, 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

Material and Methods

Patients

Nineteen patients with newly diagnosed GBM were consented and enrolled contingent on meeting the following criteria: ≥18 years of age, histological established GBM (according to the World Health Organization guidelines) (23), maintained on a stable dose of corticosteroids for ≥5 days, a Karnofsky performance status of ≥60%, adequate hematologic, renal, and hepatic function, and were capable of providing informed consent. Tumor tissue was obtained via debulking or biopsy prior to initiation of chemotherapy and radiotherapy and was immediately formalin-fixed and subsequently paraffin embedded. All studies using human tissues were approved by and conducted in accordance with the policies of the Institutional Review Board at UAB.

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 utilizing cytochrome p450 enzyme-inducing anticonvulsant drugs (EIADs) (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 utilized for capecitabine administration. The first dose level of capecitabine was at 625 mg/m^2 BID (1250 mg/m^2/day). Doses were escalated by 25% increments in consecutive cohorts of 3 patients until the maximum tolerated dose (MTD) was achieved. After a one week hiatus the patients entered the maintenance phase with capecitabine at a dose of 1250 mg/m2 BID (2500 mg/m^2/day) on a schedule of 14 days on and 7 days off. Cycles were discontinued upon 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 (DLTs) in more than 2 of 3 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-μm tissue sections using the Roche High Pure RNA Paraffin Kit (Roche Diagnostics, Mannheim, Germany) as per the manufacturer's instructions. Concentration was determined through S9 housekeeping gene expression analysis on an ABI

7900HT Sequence Detection System as previously described (Applied Biosystems, Foster City, CA) (25). Reverse transcription was performed using the High Capacity cDNA Archive Kit (Applied Biosystems, Foster City, CA) 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, Foster City CA). The precision, accuracy, 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 metabolism of capecitabine as well as genes associated with response to RT. TLDA analysis was performed using the Applied Biosystems Prism 7900HT sequence detection system (Applied Biosystems, Foster City, CA). Gene expression values were calculated using the comparative C_t method with normal brain cDNA used as the 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, Cary, NC) (28).

Univariate Cox proportional hazards regression modeling was performed to assess the association of each gene's expression with TTP and OS (29). Genes with a mean standard deviation 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, 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 utilized the genes significantly correlated with patient outcome from the Cox regression analysis in a semi-supervised principle component analysis (SSPCA) (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 (LOOCV) 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[1] (32–33). Analyses were conducted and graphed using GenePattern software[2] (31).

[1] http://tcga-data.nci.nih.gov
[2] http://www.broad.mit.edu/cancer/software/genepattern

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 Figure 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 9 patients were taking non-EIADs or were not taking anticonvulsants. No statistical difference in age, sex, patient TTP or OS was demonstrated between the EIAD and 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 (HFS; 50%), and fatigue (33%) were also reported. A DLT was observed in one patient for each EIAD arm at the lowest dose level of To 625mg/m^2/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 750mg/m^2/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 HFS in the EIAD arm. On the basis of DLTs occurring in two of the three patients enrolled at the 750mg/m^2/BID (1500mg/m^2/day) dose level for both the EIAD and non-EIAD arms, the MTD was determined to be 625mg/m^2/BID (1250mg/m^2/BID) for capecitabine concurrent with RT 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 a standard error of 40.2 days. The median OS was 366.5 days with a standard error of 54.9 days.

Gene Expression-Based Predictor Model Development

Prior to administration of chemoradiotherapy, all GBM patients underwent surgery for tumor resection and/or biopsy. Tissues were formalin fixed and paraffin embedded for histological examination. A total of 13/19 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 pathway responsible for capecitabine metabolism, as well as genes previously associated with radiation response (see Supplemental Table 1). Univariate Cox proportional hazards regression identified a significant association between 19 genes and patient TTP (Table 2) and 12 genes with OS (Table 3). Due to multiplicity of tests, a more stringent significance cut-off 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 SSPCA. Since unsupervised principle component analysis may identify cancer subtypes that are unrelated to patient survival, we utilized 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 both 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 both patient TTP (Figure 2A; p=0.005) and OS (Figure 2B; p=0.015).

Genes identified through Cox regression analysis were utilized to examine predictor models of patient outcome to capecitabine radiotherapy. Weighted-voting algorithm assessment of test predictor models identified an 8 gene expression-based model that accurately (100%) segregated all patients into either poor or good outcome subgroups (Figure 3A). These analysis also demonstrated that the expression of all 8 genes was significantly higher in the poor outcome (patients 1, 18, 19, 3, 6, and 15) compared to the good outcome (patients 16, 13, 11, 4, 17, 7, and 12) subgroup (Figure 3B). Subsequent examination of a separate dataset containing 228 GBM patients who were not treated with capecitabine (TCGA database) demonstrated 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 utilized a novel pharmacogenomic approach to design a new treatment paradigm for patients diagnosed with GBM. Earlier studies demonstrated: 1.) A favorable molecular profile of drug metabolizing enzymes in GBM and uninvolved brain tissue for treatment with capecitabine (8); 2.) Exposure of glioma xenografts to ionizing radiation results in a significant, tumor associated induction of TP and subsequent increased anti-tumor efficacy to capecitabine (8); 3.) 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 prior to 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 neurological or GI toxicities. As illustrated in Figure 1, we selected an aggressive chemoradiation schedule of capecitabine daily during the six weeks of radiation therapy and continued for four additional weeks. This schedule for our induction phase was chosen based on pre-clinical studies that concluded increased TP expression persisted post-radiation in our animal models of malignant glioma (8). This 10 week period was the study observation time during which grade 3 or 4 non-hematological toxicities or grade 4 hematological 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 utilizing EIADs (24). The DLTs that defined the MTD in this study were similar to those seen in other cancers such as GI when capecitabine was administered concurrently with radiation therapy (34). In our study, hand-foot syndrome and diarrhea were equally responsible for dose-limiting toxicities in our study occurring in 16.7% of patients at 625 mg/m2 BID and 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 capecitabine metabolism and clearance is not significantly

impacted 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 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 CNS 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 Figure 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 which forms of *06*-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) (35,36). Alternatively this regimen provides a potential avenue for the use of capecitabine as second line therapy for the approximate 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 7 genes (DPD, RRM1, CTPS, UCK2, RAD51, RAD54B and XRCC1) significantly associated with both TTP and OS (Table 3, bold).

Previous molecular studies demonstrated that TP expression in GBM is approximately 13-fold higher compared to uninvolved brain while 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), while 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). While 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 prior to treatment did not correlate with survival (34,37). Subsequent studies examining pancreatic biopsies prior to and during treatment with capecitabine and concurrent radiation showed a significant induction of TP. Taken collectively, these studies suggest a dual role for TP: 1.) as reported in several solid tumors, elevated TP expression prior to treatment indicates a more aggressive phenotype characterized by increased vascularization and a poor prognosis (38,39) and, 2.) clinical studies in pancreatic cancer and preclinical models in colorectal, mammary, and GBM suggest that TP is induced following radiation which results in increased anti-tumor 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 both gene expression and clinical data to identify molecular subtypes associated with patient outcome (30,31). As shown in Figure 2, SSPCA (utilizing the 24 unique genes identified by Cox regression analysis) identified two

patient subgroups that were significantly different in both TTP (Figure 2A) and OS (Figure 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 8-gene model that accurately (100%) segregated all patients into either poor or good outcome subgroups (Figure 3). 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 & APEX2), drug metabolizing (RRM1, TK DCTD), transport (SLC29A1) and cell proliferation (mTOR) genes in this model suggest that response to this treatment regimen is multifactoral 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 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 appears 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 include: 1.) capecitabine may provide an alternative treatment for the 50–70% of GBM patients who do not respond to temozolomide or for patients who become refractory to temozolomide; 2.) since capecitabine and temozolomide have different mechanisms of action and toxicity profiles, it may be possible to combine these treatment regiments with concurrent or sequential administration of both drugs; 3.) gene expression profiles may prove useful in the future stratification of GBM patients to help guide therapy.

Statement of 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 include: 1.) capecitabine may provide an alternative treatment for patients who do not respond to temozolomide (50–70%), or for patients who become refractory to temozolomide; 2.) since capecitabine and temozolomide have different mechanisms of action and toxicity profiles, it may be possible to combine these treatment regiments with concurrent administration of both drugs. Lastly, molecular analysis of tumor biopsies obtained prior to treatment identified an 8 gene expression profile which may prove useful in the future stratification of GBM patients to help guide therapy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The results published here are in part based upon data generated by The Cancer Genome Atlas Pilot Project established by the NCI and NHGRI. Information about TCGA and the investigators and institutions who constitute the TCGA research network can be found at http://cancergenome.nih.gov. This research was supported by grants from the National Cancer Institute P50 CA97247 and ACS grant RSG-04-030-01-CCE.

References

1. CBTRUS. CBTRUS Statistical Report: Primary Brain and Central Nervous System Tumors Diagnosed in the United States in 2004–2005: Central Brain Tumor Registry of the United States. 2009
2. McLendon RE, Halperin EC. Is the long-term survival of patients with intracranial glioblastoma multiforme overstated? Cancer 2003;98(8):1745–1748. [PubMed: 14534892]
3. Castro MG, Cowen R, Williamson IK, et al. Current and future strategies for the treatment of malignant brain tumors. Pharmacol Ther 2003;98(1):71–108. [PubMed: 12667889]
4. Stupp R, Mason WP, van den Bent MJ, et al. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. N Engl J Med 2005;352(10):987–996. [PubMed: 15758009]
5. Brada M, Hoang-Xuan K, Rampling R, et al. Multicenter phase II trial of temozolomide in patients with glioblastoma multiforme at first relapse. Ann Oncol 2001;12(2):259–266. [PubMed: 11300335]
6. Yung WK, Prados MD, Yaya-Tur R, et al. Temodal Brain Tumor Group. Multicenter phase II trial of temozolomide in patients with anaplastic astrocytoma or anaplastic oligoastrocytoma at first relapse. J Clin Oncol 1999;17(9):2762–2771. [PubMed: 10561351]
7. Menei P, Capelle L, Guyotat J, et al. Local and sustained delivery of 5-fluorouracil from biodegradable microspheres for the radiosensitization of malignant glioma: a randomized phase II trial. Neurosurgery 2005;56(2):242–248. discussion 42-8. [PubMed: 15670372]
8. Blanquicett C, Gillespie GY, Nabors LB, et al. Induction of thymidine phosphorylase in both irradiated and shielded, contralateral human U87MG glioma xenografts: implications for a dual modality treatment using capecitabine and irradiation. Mol Cancer Ther 2002;1(12):1139–1145. [PubMed: 12481438]
9. Wang ML, Yung WK, Royce ME, Schomer DF, Theriault RL. Capecitabine for 5-fluorouracil-resistant brain metastases from breast cancer. Am J Clin Oncol 2001;24(4):421–424. [PubMed: 11474279]
10. Ishitsuka H. Capecitabine: preclinical pharmacology studies. Invest New Drugs 2000;18(4):343–354. [PubMed: 11081570]
11. Schuller J, Cassidy J, Dumont E, et al. Preferential activation of capecitabine in tumor following oral administration to colorectal cancer patients. Cancer Chemother Pharmacol 2000;45(4):291–297. [PubMed: 10755317]
12. Tsukamoto Y, Kato Y, Ura M, et al. A physiologically based pharmacokinetic analysis of capecitabine, a triple prodrug of 5-FU, in humans: the mechanism for tumor-selective accumulation of 5-FU. Pharm Res 2001;18(8):1190–1202. [PubMed: 11587492]
13. Ishikawa T, Sekiguchi F, Fukase Y, Sawada N, Ishitsuka H. Positive correlation between the efficacy of capecitabine and doxifluridine and the ratio of thymidine phosphorylase to dihydropyrimidine dehydrogenase activities in tumors in human cancer xenografts. Cancer Res 1998;58(4):685–690. [PubMed: 9485021]
14. Sawada N, Ishikawa T, Sekiguchi F, Tanaka Y, Ishitsuka H. X-ray irradiation induces thymidine phosphorylase and enhances the efficacy of capecitabine (Xeloda) in human cancer xenografts. Clin Cancer Res 1999;5(10):2948–2953. [PubMed: 10537364]
15. Cascino TL, Veeder MH, Buckner JC, et al. Phase II study of 5-fluorouracil and leucovorin in recurrent primary brain tumor. J Neurooncol 1996;30(3):243–246. [PubMed: 8943099]
16. Levin VA, Edwards MS, Wara WM, Allen J, Ortega J, Vestnys P. 5-Fluorouracil and 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU) followed by hydroxyurea, misonidazole, and irradiation for brain stem gliomas: a pilot study of the Brain Tumor Research Center and the Childrens Cancer Group. Neurosurgery 1984;14(6):679–681. [PubMed: 6087193]
17. Stewart DJ, Dahrouge S, Soltys K. A phase II study of 5-fluorouracil plus folinic acid in malignant gliomas in adults. J Neurooncol 1995;23(3):249–252. [PubMed: 7673988]
18. Bourke RS, West CR, Chheda G, Tower DB. Kinetics of entry and distribution of 5-fluorouracil in cerebrospinal fluid and brain following intravenous injection in a primate. Cancer Res 1973 Jul;33 (7):1735–1746. [PubMed: 4198466]
19. Levin VA, Chadwick M, Little AD. Distribution of 5-fluorouracil-2- 14 C and its metabolites in a murine glioma. J Natl Cancer Inst 1972 Dec;49(6):1577–1584. [PubMed: 4265113]

20. Phillips TW, Chandler WF, Kindt GW, et al. New implantable continuous administration and bolus dose intracarotid drug delivery system for the treatment of malignant gliomas. Neurosurgery 1982 Aug;11(2):213–218. [PubMed: 6289163]
21. Shibui S. Pyrimidine nucleoside phosphorylase in brain tumors and anti-tumor effect of 5'-DFUR. Neurol Med Chir (Tokyo) 1984 Feb;24(2):65–72. [PubMed: 6205290]
22. Yamashita M, Hashimoto T, Hirakawa T, et al. 5'-Deoxy-5-fluorouridine and 5-fluorouracil concentrations and thymidine phosphorylase activity in brain tumors following intravenous administration of 5'-deoxy-5-fluorouridine][Article in Japanese. Neurol Med Chir (Tokyo) 1985 Aug;25(8):613–619. [PubMed: 2415854]
23. Louis DN, Ohgaki H, Wiestler OD, et al. The 2007 WHO classification of tumours of the central nervous system. Acta Neuropathol 2007;114(2):97–109. [PubMed: 17618441]
24. Gilbert MR, Supko JG, Batchelor T, et al. Phase I clinical and pharmacokinetic study of irinotecan in adults with recurrent malignant glioma. Clin Cancer Res 2003;9(8):2940–2949. [PubMed: 12912940]
25. Blanquicett C, Johnson MR, Heslin M, Diasio RB. Housekeeping gene variability in normal and carcinomatous colorectal and liver tissues: applications in pharmacogenomic gene expression studies. Anal Biochem 2002;303(2):209–214. [PubMed: 11950223]
26. Grunda JM, Nabors LB, Palmer CA, et al. Increased expression of thymidylate synthetase (TS), ubiquitin specific protease 10 (USP10) and survivin is associated with poor survival in glioblastoma multiforme (GBM). J Neurooncol 2006;80(3):261–274. [PubMed: 16773218]
27. Steg A, Wang W, Blanquicett C, et al. Multiple gene expression analyses in paraffin-embedded tissues by TaqMan low-density array: Application to hedgehog and Wnt pathway analysis in ovarian endometrioid adenocarcinoma. J Mol Diagn 2006;8(1):76–83. [PubMed: 16436637]
28. Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. Journal of the American Statistical Association 1958;(53):457–481.
29. Cox D. Regression Models and Life Tables. J R Stat Soc 1972;B(34):187–220.
30. Bair E, Tibshirani R. Semi-supervised methods to predict patient survival from gene expression data. PLoS Biol 2004;2(4):E108. [PubMed: 15094809]
31. Mullighan CG, Su X, Zhang J, et al. Deletion of IKZF1and prognosis in acute lymphoblastic leukemia. N Engl J Med 2009;360(5):470–480. [PubMed: 19129520]
32. Cancer Genome Atlas Research Network. Comprehensive genomic characterization defines human glioblastoma genes and core pathways, Supplementary Information. Nature 2008 Oct 23;455(7216):1061–1068. [PubMed: 18772890]
33. Bredel M, Scholtens DM, Harsh GR, et al. A network model of a cooperative genetic landscape in brain tumors. JAMA 2009 Jul 15;302(3):261–275. [PubMed: 19602686]
34. Saif MW, Eloubeidi MA, Russo S, et al. Phase I study of capecitabine with concomitant radiotherapy for patients with locally advanced pancreatic cancer: expression analysis of genes related to outcome. J Clin Oncol 2005;23(34):8679–8687. [PubMed: 16314628]
35. Andre T, Louvet C, Raymond E, Tournigand C, de Gramont A. Bimonthly high-dose leucovorin, 5-fluorouracil infusion and oxaliplatin (FOLFOX3) for metastatic colorectal cancer resistant to the same leucovorin and 5-fluorouracil regimen. Ann Oncol 1998;9(11):1251–1253. [PubMed: 9862058]
36. Schmoll HJ, Arnold D. Update on capecitabine in colorectal cancer. Oncologist 2006;11(9):1003–1009. [PubMed: 17030642]
37. Saif MW, Black G, Roy S, et al. Phase II study of capecitabine with concomitant radiotherapy for patients with locally advanced pancreatic cancer: up-regulation of thymidine phosphorylase. Cancer J 2007;13(4):247–256. [PubMed: 17762760]
38. Akiyama S, Furukawa T, Sumizawa T, et al. The role of thymidine phosphorylase, an angiogenic enzyme, in tumor progression. Cancer Sci 2004;95(11):851–857. [PubMed: 15546501]
39. Liekens S, Bronckaers A, Perez-Perez MJ, Balzarini J. Targeting platelet-derived endothelial cell growth factor/thymidine phosphorylase for cancer therapy. Biochem Pharmacol 2007;74(11):1555–1567. [PubMed: 17572389]
40. Alizadeh AA, Eisen MB, Davis RE, et al. Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. Nature 2000;403(6769):503–511. [PubMed: 10676951]

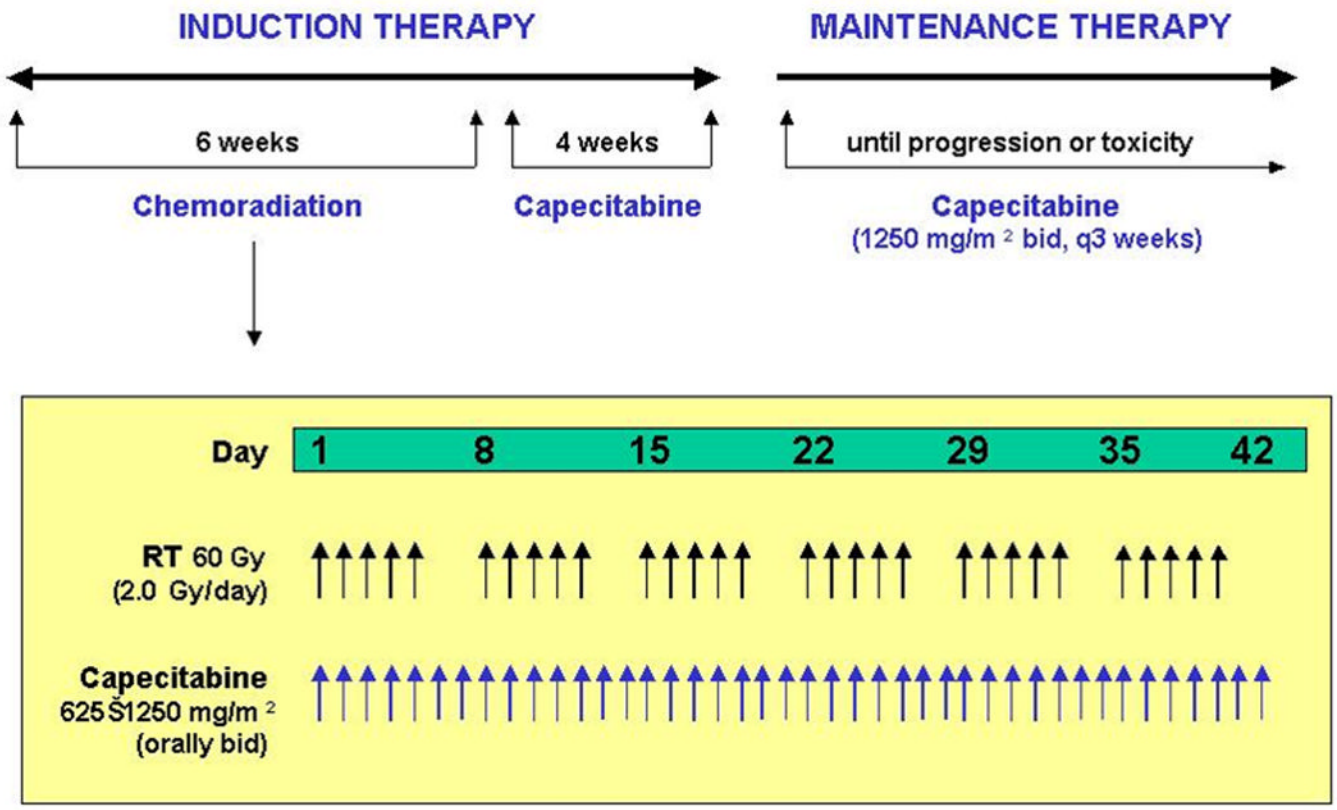

Fig 1.
Patient treatment schematic with capecitabine and radiotherapy.

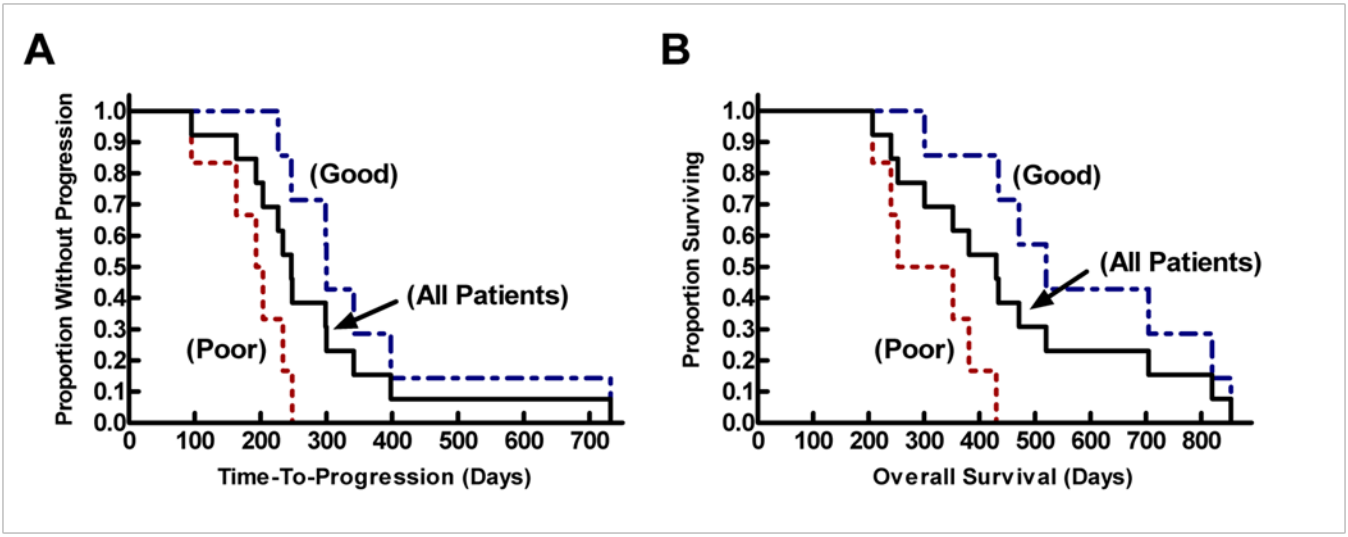

Fig 2.

Results of SSPCA. Kaplan Meier plots representing all evaluable (n=13), poor outcome (n=6) and the good outcome (n=7) patient groups with median (A) time-to-progression of 247 (—), 199 (—), and 300 (—) days and (B) overall survival of 430 (—), 303 (—), and 520 (—) days respectively.

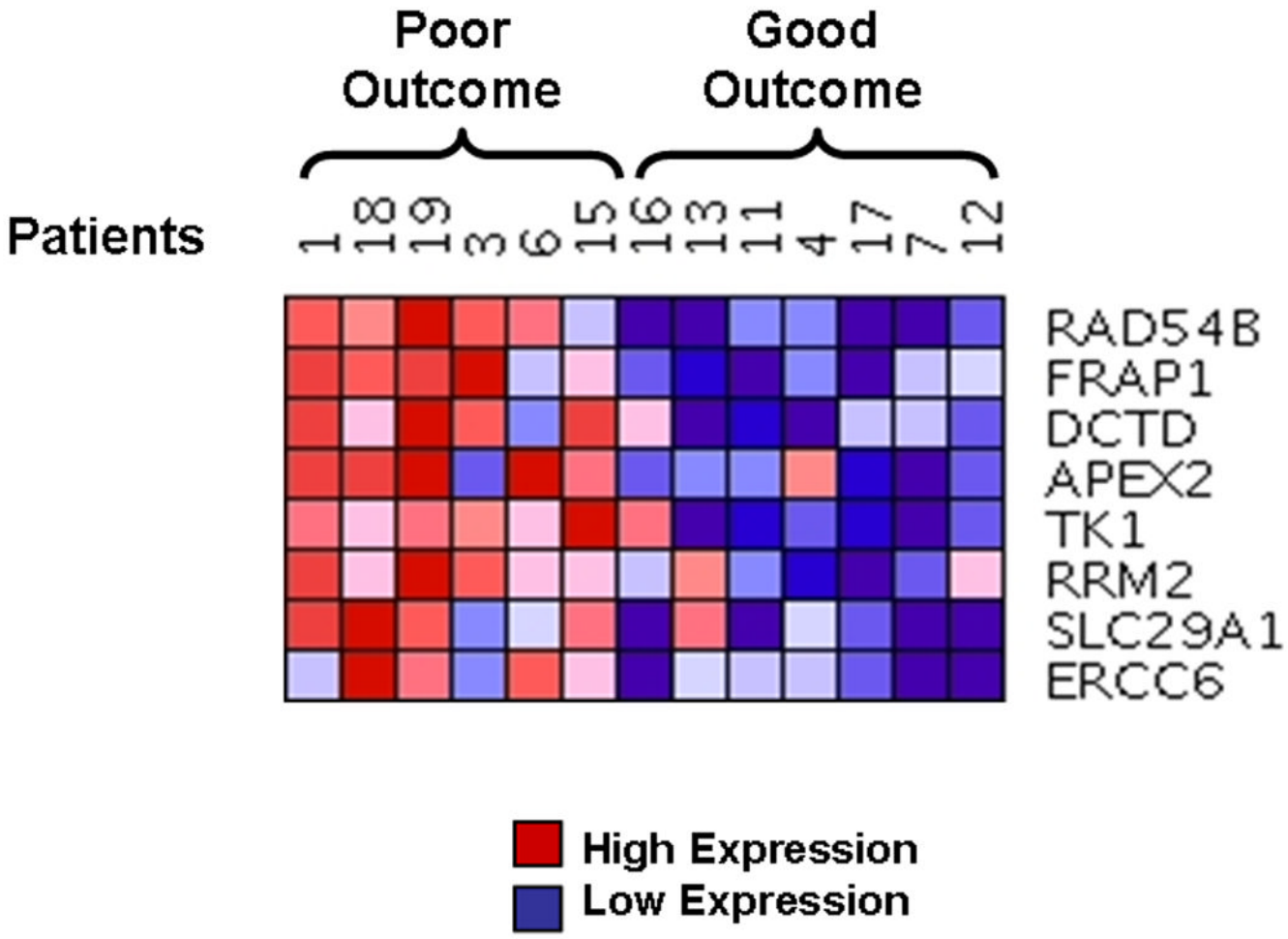

Fig 3.
Heat map of the 8 gene expression-based predictor model of patient outcome to capecitabine and radiotherapy treatment.

Table 1

Demographics of the 18 Enrolled Evaluable Patients

	Patients	
Characteristic	**N**	**%**
Age, years		
Median	49	
Range	18–78	
Sex		
Male	15	
Female	3	
Karnofsky performance status		
Median	80	
Range	60–100	
Race		
White	18	100
Black		
Asian		
Histology		
Glioblastoma multiforme	18	100

Table 2

Genes associated with GBM Patient Time-to-Progression

Accession No.	Gene	Description	Hazard Ratio[†]	P value
Capecitabine Metabolism Genes				
NM_001033	RRM1	ribonucleotide reductase M1	2.30	0.002
NM_000110	DPYD	dihydropyrimidine dehydrogenase	1.23	0.002
NM_012474	UCK2	uridine-cytidine kinase 2	2.14	0.004
NM_001905	CTPS	CTP synthase	19.63	0.009
NM_004955	SLC29A1	solute carrier family 29, member 1	1.06	0.010
NM_001953	TP	thymidine phosphorylase (ECGF1)	1.11	0.010
NM_001785	CDA	cytidine deaminase	1.66	0.020
Radiation Response Genes				
NM_002875	RAD51	RAD51 homolog	1.06	0.007
NM_002524	NRAS	neuroblastoma RAS	1.49	0.007
NM_005432	XRCC3	XRCC3	6.05	0.008
NM_012415	RAD54B	RAD54 homolog B	1.21	0.009
NM_005732	RAD50	RAD50 homolog	1.62	0.009
NM_003401	XRCC4	XRCC4	1.28	0.010
NM_001641	APEX1	APEX nuclease 1	2.20	0.012
NM_000124	ERCC6	ERCC6	1.90	0.013
NM_014481	APEX2	APEX nuclease 2	1.74	0.018
NM_021141	XRCC5	XRCC5	2.48	0.019
NM_000251	MSH2	mutS homolog 2	37.50	0.022
NM_006297	XRCC1	XRCC1	1.35	0.023

Note: Cox regression analysis was utilized to assess the association between time-to-progression and each individual gene expression value, adjusted for age and dose.

[†] Hazard Ratio >1: higher gene expression, shorter time-to-progression, <1: higher gene expression, longer time-to-progression, = 1: no association

Table 3

Genes associated with GBM Patient Overall Survival

Accession No.	Gene	Description	Hazard Ratio[†]	P value
Capecitabine Metabolism Genes				
NM_001921	DCTD	dCMP deaminase	51.15	0.005
NM_003258	TK1	thymidine kinase 1	156.73	0.006
NM_000110	**DPYD**	dihydropyrimidine dehydrogenase	1.21	0.006
NM_001033	**RRM1**	ribonucleotide reductase M1	1.93	0.007
NM_001034	RRM2	ribonucleotide reductase M2	1.04	0.008
NM_001905	**CTPS**	CTP synthase	40.10	0.008
NM_012474	**UCK2**	uridine-cytidine kinase 2	1.60	0.024
Radiation Response Genes				
NM_002875	**RAD51**	RAD51 homolog	1.08	0.003
NM_012415	**RAD54B**	RAD54 homolog B	1.22	0.013
NM_006297	**XRCC1**	XRCC1	1.45	0.014
NM_004958	mTOR	mechanistic target of rapamycin (FRAP1)	6.55	0.020
NM_001274	CHEK1	CHK1 checkpoint homolog	1.74	0.025

Note: Cox regression analysis was utilized to assess the association between overall survival and each individual gene expression value, adjusted for age and dose.

[†] Hazard Ratio >1: higher gene expression, shorter survival, <1: higher gene expression, longer survival, = 1: no association

Table 4

Gene expression statistics for the poor and good outcome patient subgroups

	Poor Outcome			Good Outcome				
Gene	**Ave.**	**Min**	**Max**	**Ave.**	**Min**	**Max**	**Fold Change‡**	**P-value**
RAD54B	14.60	7.47	20.86	4.60	2.91	7.06	3.17	0.002
mTOR	1.95	1.24	2.51	1.00	0.64	1.37	1.95	0.003
DCTD	1.39	0.71	1.80	0.61	0.31	0.99	2.28	0.004
APEX2	5.98	2.83	7.29	3.16	1.79	5.15	1.89	0.006
TK1	0.70	0.46	1.17	0.24	0.06	0.72	2.96	0.006
RRM2	98.04	73.82	132.73	49.50	12.71	88.05	1.98	0.007
SLC29A1	58.12	31.15	83.04	27.99	16.52	56.60	2.08	0.015
ERCC6	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.