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*Clinical Study*

# The prognostic value of tumor markers in patients with glioblastoma multiforme: analysis of 32 patients and review of the literature

John F. Reavey-Cantwell[1], Raymond I. Haroun[1], Marianna Zahurak[2], Richard E. Clatterbuck[1], Ricardo J. Parker[3], Rita Mehta[3], John P. Fruehauf[3] and Henry Brem[1]
[1]*Hunterian Neurosurgical Laboratory, Department of Neurosurgery,* [2]*Department of Biostatistics, The Johns Hopkins University School of Medicine, Baltimore, MD, USA;* [3]*Oncotech Corporation, Irvine, CA, USA*

*Key words:* DNA index, glioblastoma multiforme, glutathione-S-transferase pi, p53, Ki-67, multidrug resistance gene 1, ploidy, S-phase fraction

**Summary**

Although several studies have examined brain tumor markers for prognostic value, few investigations have stratified analysis based on specific histologic grade. The objective of this study was to evaluate a single histologic grade of glioma, the grade IV glioma or glioblastoma (World Health Organization Classification), with a comprehensive panel of tumor markers in an attempt to identify those with prognostic significance. Tumor samples from a cohort of patients with glioblastoma multiforme ($n = 32$) were examined for tumor markers, DNA analysis, and clinical variables in an attempt to determine a 'profile' for this tumor. We used univariate and multivariate statistical analysis to determine the prognostic value of tumor cell ploidy, percent S-phase, DNA index, p53, and Ki-67 labeling index, as well as the variables of gender, race, age, location of tumor, history of chemotherapy, and primary versus recurrent tumor. Two additional tumor markers, multidrug resistance gene 1 and glutathione-S-transferase subtype pi, were included in the sample testing, but were not analyzed statistically. Univariate analysis indicated that increasing age had a strong association with decreased survival. Female gender, increasing Ki-67, no chemotherapy before sample collection, and primary glioblastoma showed some association with decreased survival in the univariate model. The univariate results indicated that race, side of tumor, ploidy, S-phase, DNA index, and p53 had no prognostic value. Multivariate modeling demonstrated that age, gender, and Ki-67 were the strongest factors associated with survival. The relevant literature is reviewed.

## Introduction

Gliomas are the most common primary intracerebral tumors among the approximately 35,000 new brain tumors diagnosed every year in the United States [1]. The World Health Organization grade IV glioma, or glioblastoma, is the most common malignant glioma [2]. The prognostic significance of histology in astrocytic tumors is well established. A diagnosis of glioblastoma carries a dismal prognosis, with a 2-year survival rate of 5% [2]. There are, however, long-term survivors, suggesting that a wide variation in the biological characteristics of glioblastomas may exist. The identification of tumor markers with prognostic significance may help tailor treatment of the individual, resulting in increased long-term survival.

Our aim was the evaluation of a single histologic grade of glioma with a comprehensive panel of tumor markers in an attempt to identify those with prognostic significance. Many studies have analyzed the prognostic significance of markers in gliomas; however, very few studies [3–6] have analyzed a well-defined subtype or grade of tumor (i.e. only glioblastoma). In the present study, a cohort of patients with glioblastoma was chosen for analysis based upon the relatively high frequency of this neoplasm, its malignant nature, and a well-defined endpoint (all patients but one had died). Samples of each tumor were tested for ploidy, percent S-phase, DNA index, p53, Ki-67

labeling index, glutathione-S-transferase subtype pi (GSTpi), and multidrug resistance gene 1 (MDR-1). The clinical variables of age, race, gender, location of tumor, whether the collected sample was of a primary or recurrent glioblastoma, and history of chemotherapy before sample analysis, were collected for each patient. We performed univariate and multivariate analyses to determine whether any significant correlation exists between tumor markers and clinical variables with survival time.

## Materials and methods

### *Cohort description, clinical data, and tissue specimen collection*

Between 1994 and 1998, tumor samples were collected from a subset of patients admitted to the Johns Hopkins Hospital for resection of a brain tumor. The tumors were reviewed by members of the division of neuropathology and classified according to the revised World Health Organization classification [7]. Surgical specimens were all placed aseptically into Oncotech transport vials containing sterile transport medium (RPMI containing 15% fetal calf serum, 2 mM L-glutamine, and Pen/Strep {200 IU/ml Pen + 200 μg/ml Strep} plus 1.25 ng/ml fungizone) and sent to Oncotech in Irvine, CA for testing. Specimens larger than 2 g were divided into separate vials. Specimens arrived at Oncotech via FEDEX within 24–48 h after resection, assigned a tracking number, and then processed. The tumor specimens chosen for this study were all classified as grade IV gliomas/glioblastoma. Initially, 35 samples were collected from 32 patients. One patient had three samples collected from three different resections and a second patient had two samples collected from two different resections. For the study, each patient was paired with only one set of data. For the two patients with multiple samples, the sample with the most comprehensive analysis was chosen. Otherwise, the sample from the first surgical procedure was chosen. One of the 32 patients was excluded from the statistical analysis because that individual initially presented with grade II astrocytoma before progression to glioblastoma multiforme.

Clinical history was obtained retrospectively from the patients' charts. This information included gender, race, age, primary versus recurrent glioblastoma, history of chemotherapy at any time prior to sample collection, and side of the tumor (left or dominant versus right or nondominant). The statistical endpoint of the study was survival, defined as the time from definitive diagnosis of glioblastoma at first surgical procedure until death. Thirty-one of 32 patients had died by the time of the final data analysis. The final patient was alive at last follow-up with a survival time of 206 weeks.

### *Percent S-phase, ploidy, DNA index*

DNA ploidy and S-phase fraction are common DNA flow cytometry markers used in the diagnosis and management of many solid tumor types. A diploid (normal ploidy) tumor has the normal amount of DNA, whereas aneuploid tumors have either more or less DNA than expected. The S-phase fraction is the proportion of tumor cells that are in the DNA synthesis-phase of the cell cycle and is thus a measure of proliferation. In this study, multiparameter flow cytometry was used to analyze cell proliferation based on cell phase distribution, which discriminates noncycling ($G_0$) from cycling ($G_1$) cell populations, and $G_2$-phase cells from cells in mitosis. The DNA index is a commonly reported ratio measuring the DNA content of a cell relative to reference cells with a known DNA content, such as normal diploid human cells. It is the ratio of the mean position of the $G_0/G_1$ peak (in a DNA histogram) of the experimental cells to the mean position of the $G_0/G_1$ peak of the reference cells.

An aliquot of cells from suspensions of brain tumor cells was harvested and prepared for flow cytometric analysis by using the propidium iodide fluorochrome (peak emission at 620 nm) as an intercalating DNA dye. Briefly, $1 \times 10^6$ tumor cells were washed once in phosphate-buffered saline (PBS) containing 1% fetal calf serum, and resuspended in 1 ml of Krishan buffer to release the nuclei, after which propidium iodide was added. This material was then stored at 4°C until flow analysis. Data were acquired on a FACScan flow cytometer (Becton Dickinson Immunocytometry Systems, San Jose, CA) equipped with a 15 mW, air-cooled argon ion laser. FACScan (488 nm excitation) fluorescence emission was obtained after passing through band pass filters. Multiparameter histograms, forward angle light scatter, and side scatter were obtained for 10,000 events. Data were collected and analyzed on a FACScan interface model 340 Hewlett Packard computer by using LYSYS II software (Becton Dickinson Immunocytometry), and CellFIT software (BDI) was used to analyze DNA histogram data and determine the percent S-phase, ploidy, and DNA index.

*Immunohistochemistry*

Immunohistochemical detection of the biomarkers p53, Ki-67, MDR-1, and GSTpi was performed according to previously published methods [8–10]. Briefly, 5 μm sections of fixed, paraffin-embedded specimens were cut, mounted on poly-L-lysine slides (VWR Superfrost Plus) and then de-paraffinized in Histoclear (National Diagnostics, Atlanta, GA) for 15 min. Specimens were then rehydrated by sequential washing in ethanol solutions. Antigen retrieval utilized pronase digestion for Ki-67 or microwave boiling for 15 min in citrate buffer for p53, MDR-1, and GSTpi. Endogenous peroxidase activity was blocked by a 10 min incubation in 3% hydrogen peroxide (Peroxide Block, Biogenex, San Ramon, CA), and slides were then washed in phosphate buffered saline. Protein blocking was performed by treatment of slides with normal goat serum (Protein Block, Biogenex) for 10 min to abolish non-specific binding, followed by application of biotinylated goat anti-mouse primary antibody. All primary antibody reagents were commercially available from Signet Laboratories, Dedham, MA (MDR-1), Santa Cruz Biotech, Santa Cruz, CA (p53 antibody DO1), Immunotech, Inc., Westbrook, ME (Ki-67), Dako, Carpinteria, CA (GSTpi). The StrAviGen Super Sensitive Immunodetection Kit (Biogenex), using the horseradish-peroxidase method, was used as the second antibody reagent. The slides were then rinsed in PBS for 5 min, counterstained with hematoxylin for 1 min, rinsed for 10 min in tap water, dehydrated in ascending ethanol series, cleared in xylene, cover slipped in Permount and viewed under 40× magnification. Tissue sections were scored by a pathologist for percentage of cells staining positively and staining intensity. Estimations were based on entire sections rather than selected fields. Staining was considered negative when less than 5% of cells were stained. A histoscore was calculated for each tissue section by multiplying the percentage of positively stained cells by the intensity of staining plus 1. Intensity was scored as 1+ (mild), 2+ (moderate), 3+ (equal to positive controls), and 4+ (greater than positive controls). Proliferating endothelial cells were excluded from the analysis of Ki-67. For each specimen examined, a section tested against a non-specific antibody served as the negative control.

*Statistical methods*

The primary outcome in this study was survival. Event time distributions for the survival endpoint were estimated using the method of Kaplan and Meier [11] and compared by the log-rank statistic [12] or the proportional hazards regression model [13]. The simultaneous effect of two or more factors was studied using the multivariate proportional hazards model. Factors tested for prognostic value included diagnosis (primary or recurrent glioblastoma) at time of sample collection, gender, race, age, location of tumor (left versus right), prior history of chemotherapy before sample analysis, ploidy, percent S-phase, DNA index, p53, and Ki-67 labeling index. All $p$ values are two-sided. Computations were performed using the Statistical Analysis System (SAS Institute, 1985, Cary, NC) or EGRET (Statistics, 1988, Seattle, WA).

## Results

The median age at the time of diagnosis was 52.5 years (range, 34–91 years); there were 10 women (31%) and 22 men (69%). The tumor was located on the left side 56% of the time, with a right side incidence of 44%. Overall median survival for the 32 patients in this study was 59 weeks, [range, 2 weeks to 206+ weeks (one patient still living)]. Radiation was the predominant adjuvant treatment; 84% of patients received this modality. In addition, 78% of the patients received some type of chemotherapy or experimental protocol; 41% of patients received chemotherapy before sample collection and analysis. Recurrent glioblastomas accounted for 37.5% of the samples. One of the 32 patients initially presented with a grade II astrocytoma. For statistical analysis, we excluded this patient so that only primary glioblastomas or recurrences of primary glioblastomas would be included in the analysis. Table 1 summarizes the patient data.

Factors that demonstrated some association with decreased survival in univariate Cox Proportional Hazard models included increasing age, female gender, and Ki-67 labeling index. Age was the strongest prognostic factor for survival, with patients age 60 or older having almost three times the risk of death compared to patients less than 60, Cox proportional hazard ratio (HR) = 2.9 (95% Confidence Interval (CI): 1.3, 6.4; $p = 0.007$) (Figure 1). Women appeared to have a higher risk of death, HR = 2.1 (95% CI: 0.9, 4.8; $p = 0.08$) (Figure 2). Increasing Ki-67 labeling index was also marginally associated with increasing risk of death, HR = 1.03 (95% CI: 0.99, 1.05; $p = 0.09$). By using the median value as a cutoff, we grouped patients

*Table 1*. Clinical history and demographics

| Patient number | Sex | Race | Age at diagnosis | History of radiation | History of chemo | Other treatment |
|---|---|---|---|---|---|---|
| 240 | M | ME | 73 | − | − | — |
| 427 | M | ME | 54 | + | + | — |
| 300 | M | C | 58 | − | − | BoronNC |
| 742 | F | C | 48 | + | + | — |
| 385 | F | C | 91 | − | − | — |
| 591 | M | C | 44 | + | + | IDT, Mino |
| 773 | M | C | 34 | + | + | — |
| 269 | M | C | 46 | + | − | — |
| 442 | M | AA | 72 | + | − | — |
| 865 | M | C | 48 | + | − | RSR-13 |
| 231 | M | C | 40 | + | + | — |
| 509 | F | C | 36 | + | + | — |
| 144 | M | C | 43 | + | + | — |
| 730 | M | C | 49 | + | + | — |
| 767 | M | C | 48 | + | + | — |
| 098 | M | C | 73 | + | + | — |
| 301 | M | C | 55 | + | + | — |
| 016 | M | C | 57 | + | + | — |
| 665 | M | C | 66 | + | + | — |
| 712 | M | C | 45 | + | + | — |
| 348 | F | C | 62 | + | − | — |
| 763 | F | C | 53 | + | + | — |
| 029 | F | C | 72 | + | + | — |
| 099 | M | C | 67 | − | − | — |
| 389 | F | C | 77 | + | − | — |
| 774 | M | C | 86 | + | − | — |
| 057 | M | C | 34 | + | + | — |
| 360 | F | C | 35 | + | + | — |
| 417 | M | C | 52 | + | + | — |
| 423 | F | C | 48 | + | + | — |
| 622 | M | C | 46 | + | + | — |
| 292 | F | C | 66 | + | + | — |

Abbreviations: M, male; F, female; C, Caucasian; AA, African American; ME, Middle Eastern; Chemo, chemotherapy; BoronNC, boron neutron capture; IDT, intratumor diphtheria toxin; Mino, minotoxin; RSR-13, 2-[4-[[(3,5-dimethylanilino)carbonyl]methyl]phenoxy]-2-methylproprionic acid.

with high values of Ki-67 labeling index (>20%) and compared them to those with values ≤20%. The hazard for patients with a Ki-67 labeling index >20% was 1.8 (95% CI: 0.8, 4.2; $p = 0.16$) times that in the group with values ≤20%. Median survival in the two groups, Ki-67 labeling index ≤20% and >20%, was 61 and 42 weeks, respectively (Figure 3). The variables of side of tumor, race, ploidy, S-phase, DNA index, and p53 markers were not associated with the survival endpoint. In our cohort, 50% of tumors were p53 negative and 50% were p53 positive. Furthermore, 47% of *de novo* tumors were p53 positive while only 54% of recurrent tumors were p53 positive. In addition, although screening for the tumor markers MDR-1 and GSTpi was performed, the data were not analyzed because only 1 of 16 and 3 of 29 patient samples tested positive, respectively.

Multivariate modeling indicated that the strongest factors associated with survival were age, gender, and Ki-67 labeling index, as shown in Table 2. Age, adjusted for Ki-67 labeling index and gender, was the strongest factor associated with survival, HR = 3.0 (95% CI: 1.33, 6.86; $p = 0.008$). Adjusting for gender and age, patients with elevated Ki-67 labeling index (>20%) had 2.2 times the risk of death (95% CI: 0.89, 5.17; $p = 0.089$) compared to the group with lower values for this marker. Gender also remained a marginal risk factor for death when adjusted for Ki-67

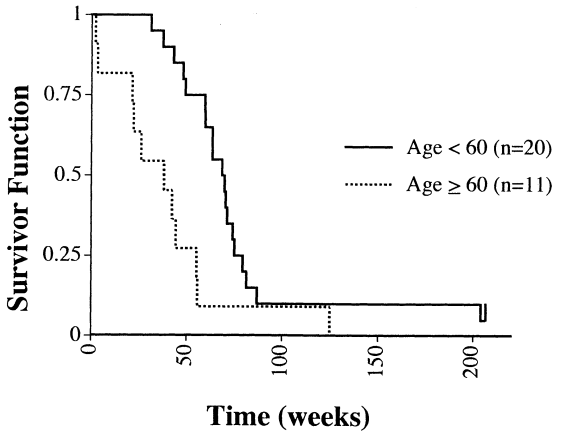


*Figure 1*. Kaplan Meier survival curve showing survival for patients greater than or equal to 60 years old (- - -) or patients less than 60 years old (—).

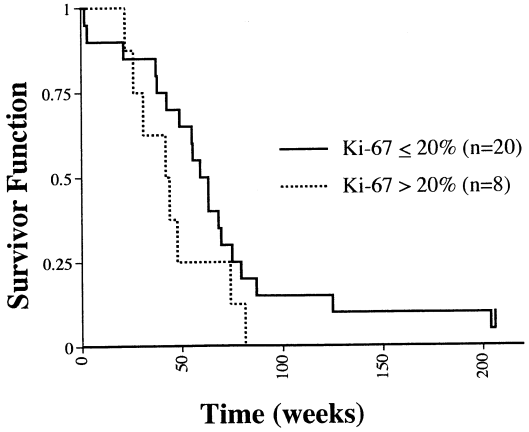


*Figure 3*. Kaplan Meier survival curve showing survival for patients with tumor cells that expressed Ki-67 at a level ≤20% (—) or >20% (- - -).

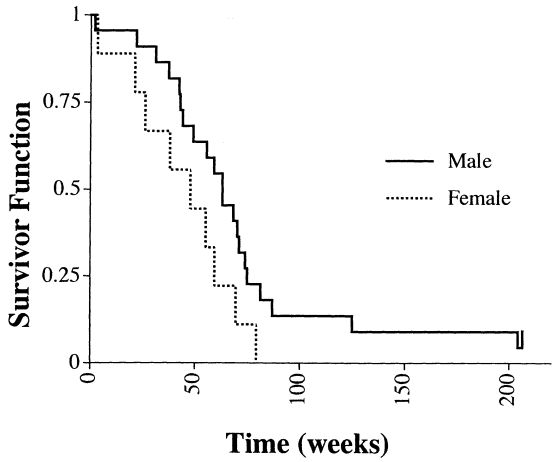


*Figure 2*. Kaplan Meier survival curve showing survival for female (- - -) or male (—) patients with glioblastoma multiforme.

*Table 2*. Estimated cox proportional hazards model for survival (multivariate analysis)

| Factor | Hazard ratio | 95% CI | *p* value |
|---|---|---|---|
| *Age* | | | |
| <60 | 1.00 | | |
| ≥60 | 3.03 | (1.33, 6.86) | 0.008 |
| *Gender* | | | |
| Male | 1.00 | | |
| Female | 2.34 | (0.99, 5.52) | 0.052 |
| *Ki-67* | | | |
| ≤20 | 1.00 | | |
| >20 | 2.15 | (0.89, 5.17) | 0.089 |

Abbreviations: CI, confidence interval.

labeling index and age, HR = 2.3 (95% CI: 0.99, 5.52; $p = 0.052$).

## Discussion

Although the median survival of patients with glioblastoma multiforme ranges from 7 to 12 months, long-term survivors have been documented, suggesting a disparity in the behavior of this tumor from patient to patient [2,4,14]. Identification of the differences in these tumors, which we term the tumor 'profile,' could enable treatments to be individually tailored for each patient. In this study, we explore the tumor profile of glioblastomas by evaluating the prognostic significance of a battery of tumor markers, DNA analyses, and clinical variables of the patients.

The demographic characteristics of our group are comparable to those in previous studies of patients with glioblastoma. The median age at the time of diagnosis was 52.5 years, which was similar to the 53-year median age found by Baxendine-Jones et al. and 56-year-old median age found by Bouvier-Labit et al. [3,4]. The gender distribution of 69% men and 31% women also was close to the 65% male and 35% female distribution of the Baxendine-Jones study [3]. The median survival of our cohort of patients was 59 weeks (413 days), consistent with the established range in the literature [2,4,14].

*Clinical variables*

In the univariate and multivariate Cox Proportional Hazard models, age was the strongest prognostic factor for survival. Patients 60 years and older had three times the risk of death compared to patients younger than 60, and this difference remained after adjusting for gender and Ki-67 labeling index. The inverse correlation between advancing age and survival is well documented in the literature, and our results support this conclusion [14–18]. Our univariate analysis suggests that females had a decreased length of survival, a factor which remained a marginal risk factor ($p = 0.052$) after adjusting for age and Ki-67 labeling index. This finding supports the results of Coons et al. [17], who found that female gender was a risk factor. In contrast, the studies by Baxendine-Jones et al. [3] and Dirks et al. [19] did not uncover any association between gender and survival in their respective cohorts of patients with glioblastoma.

The univariate analysis of primary versus recurrent glioblastoma samples was calculated with overall survival from time of diagnosis, not with survival from the moment of sample collection. If the analysis had been conducted with survival from collection date, we would likely have uncovered an association of recurrent tumor with shorter survival. This idea is supported by the work of Dirks et al. [19], who found that median survival after first operation was 57 weeks and median survival after reoperation was 19 weeks.

In our study, patients with primary tumors had a mildly increased risk of death compared to patients with recurrent tumors (relative risk = 1.69). We compared the mean values of the different variables for each group to find an explanation (data not shown). Since the samples of the primary tumors were taken from patients who were older (mean age of 60 versus mean age of 49 for patients with recurrence; $p = 0.04$), age may be responsible for the apparent trend. Alternatively, primary tumor samples may have been obtained from a subpopulation of patients with more aggressive tumors. Patients with more aggressive tumors would not be included in the recurrent tumor population because they did not survive long enough or had an unresectable recurrence.

*Ki-67 labeling index*

The Ki-67 antibody was developed in an attempt to establish a practical technique that could evaluate the proliferative potential of tumors. This antibody detects a non-histone nuclear antigen present in the G1-, S-, G2-, and M-phases of the cell cycle, primarily in the nucleolus. Early studies were designed to evaluate the ranges of Ki-67 expression in tumor samples [20] and then to correlate the level of expression with the histological classification [21–23]. The wide variation in the mean Ki-67 labeling index between studies, and even between different samples in the same tumor [24], however, remains a problem. This may represent a lack of uniformity of methods between laboratories, despite efforts by some authors to propose standardization for Ki-67 protocols in order to establish clinical utility [25]. Our multivariate analysis indicated that patients with a Ki-67 labeling index $>20\%$ had 2.2 times the risk of death compared with patients with a labeling index $\leq 20\%$, ($p = 0.09$). Our trend for the Ki-67 labeling index supports the conclusion of Sallinen et al. and Wakimoto et al. that the labeling index may be used for prognostication [26–29].

*Flow cytometry (ploidy, DNA index, percent S-phase)*

Attempts at DNA analysis in brain tumors have been equivocal. According to our univariate analysis, the tumor cell ploidy, DNA index, and percent S-phase did not have any prognostic value. Although, in general, well-differentiated tumors are diploid and malignant tumors are aneuploid [30], one previous study detected a survival benefit for patients with aneuploid glioblastoma [14]. In fact, their results are striking. Patients younger than 66.5 years old with an aneuploid tumor attained a median survival of 1316 days, while patients younger than 66.5 years old with a diploid tumor had a median survival of 402 days. Only two patients in our entire cohort exceeded the median survival of their first group, and they both had diploid glioblastomas. Other studies have suggested a survival benefit of patients with diploid tumors [30], and at least one other concurs with our data [31], finding no association with survival.

DNA index (DNA content of G1-phase tumor cells divided by the DNA content of G1-phase reference cells) also has equivocal value based on the literature. Some studies found that increasing DNA index correlates with decreasing survival [30,32], while other studies disagree and propose that no association exists [31,33]. However, most studies did not stratify gliomas based on grade or included too few glioblastomas to make any conclusive statements [30,32,33].

Struikmans et al. [31] in 1998 examined gliomas, stratified according to grade, and found no correlation using univariate and multivariate analyses. Our analysis of DNA index confirms this result.

Flow cytometry has been utilized to determine the percent of S-phase activity in cells, therefore gauging the amount of proliferative activity. Struikmans et al. [30] in 1997 found a correlation between high S-phase fraction and malignancy, but no significant S-phase differences between high and low grade gliomas. This same group in 1998 found tumors with low S-phase fraction trending to longer survival, but this was not significant in multivariate analysis. Evaluation of another cohort of astrocytomas, including 24 glioblastomas, found percent S-phase to be a strong prognostic indicator in univariate, but not multivariate, analysis [28]. In sharp contrast to this pattern is a study by Coons et al. [17], in which multivariate analysis confirmed the independent prognostic significance of the S-phase fraction. They stratified S-phase fraction into three groups (less than 3%, 3–5.9%, and greater than or equal to 6%), and despite modest risk ratios (1.31 for S-phase fraction), their study had statistically significant $p$ values [17]. We originally stratified our cohort into three tiers (less than 5%, 5–10%, and $>10\%$) based upon the median value (5.15) and the range (1.0–22.4) (data not shown). However, any statistical significance found with our three-tiered model was lost when S-phase fraction was analyzed as a continuous variable. It is interesting to note that, by differing mechanisms, S-phase fraction and Ki-67 labeling index both measure proliferative potentials. However, our results indicate that only the Ki-67 labeling index tended to predict prognosis. We propose that the relative sensitivity of each test could explain this disparity.

*MDR-1 and GSTpi*

MDR-1 codes for a cell membrane protein known as P-glycoprotein, which is an energy-dependent drug efflux pump [34]. P-glycoprotein is implicated in tumor resistance to chemotherapeutic agents and localizes to the brain tumor capillaries and, in some studies, brain tumor cells [34]. A study performed by von Bossanyi on samples of 53 astrocytomas (including 25 glioblastomas) found a correlation of expression with increasing grade of tumor, with 96% of all glioblastomas found to contain at least some MDR-1 expression [34]. In contrast, the tumor marker MDR-1 was only positive in 1 of 16 samples tested in our cohort. Billson et al. [35] examined 5 glioblastomas specimens and found no staining. Although many of the P-glycoprotein antibodies are thought to be of poor specificity [36], taken at face value, our results imply that the *MDR-1* gene does not play an active role in glioblastomas. This suggests that there are other mechanisms or molecules responsible for the tumor's ability to resist chemotherapy. Alternatively, the delay from sample acquisition to fixation and processing might account for the lower percentage of MDR-1 expression seen here.

Intracellular drug inactivation or transformation is a second method utilized by tumor cells to evade chemotherapy. One detoxifying enzyme, glutathione S-transferase, can catalyze the conjugation of glutathione to toxic compounds preventing these compounds from potentially damaging cellular DNA or critical proteins [34]. Four subtypes of GST exist in humans, however, the level of GSTpi expression correlates with tumor grade and is inversely related to patient survival [37, 38]. In contrast, von Bossanyi et al. [34] found that, although 100% of glioblastomas demonstrated GSTpi expression, there was no correlation of expression with grade of tumor. In our cohort of patients, only 3 of 29 patient samples tested positive for GSTpi. However, like MDR-1, the low percentage of GSTpi expressions seen here may be related to technical issues.

*p53*

The p53 protein is endowed with a large range of functions, including roles in cell cycle regulation, apoptosis, angiogenesis, cell differentiation, tumor suppression, and normal brain development [39]. Alterations of the *p53* gene are found in approximately 50% of all human cancers and some research suggests that certain mutations are associated with early onset of glioblastoma [40]. In our study, the p53 marker data failed to show any association with survival and does not support the inverse correlation between p53 and survival found by others [41,42]. A few studies suggest that p53 mutations occur preferentially in only some subpopulations of glioblastoma [39]. These studies propose that *de novo* glioblastomas are typically p53 negative and that secondary glioblastomas are p53 positive. In our cohort, 50% of tumors were p53 negative and 50% were p53 positive. Furthermore, 47% of *de novo* tumors were p53 positive while only 54% of recurrent tumors were p53 positive. Our results do not support the p53 differences between primary and recurrent glioblastomas seen in other studies. We stratified

our analysis of p53 in an attempt to differentiate subpopulations, but any correlation with survival was lost when p53 was analyzed as a continuous variable (data not shown). Other studies have also failed to find an association between p53 and survival [3–5,43]. Perhaps combining p53 status with other biomarkers associated with malignant tumor progression may provide a successful approach to predicting clinical outcome. It is also important to recognize that immunocytochemical methods only demonstrate the presence (or absence) of the p53 protein. Specific mutations in p53 that are likely more biologically relevant to patient survival are not assessed by this method.

Investigators have reported a significant relationship between mutant p53 and thrombospondin-1, a negative regulator of angiogenesis in breast cancer [44,45] and melanoma [8]. The relationship between these combined markers is also associated with survival in prostate and colorectal cancer (unpublished data). Together, p53, thrombospondin-1, and angiogenesis appear to be significantly associated with disease progression and prognosis. Evaluating this relationship in patients with glioblastoma is the next logical step.

Many studies have examined tumor markers for prognostic value; however, few investigations have stratified analysis based on histologic grade. The advantage of analysis limited by strict grading is the elimination of confounding factors introduced by the biological variability seen in astrocytomas of different grades. In our patients with glioblastoma, potential prognostic factors included female gender, increasing Ki-67, no chemotherapy before sample collection, and primary glioblastoma at time of sample analysis. The only definitive prognostic factor found in our cohort after multivariate analysis was age. Factors that did not appear to have any association with survival included race, location of tumor (left versus right), tumor cell ploidy, percent S-phase, DNA index, and p53. We believe that tumor profiling will eventually lead to tailored therapeutic regimens that will increase survival for patients with glioblastoma. However, much work remains to identify markers that carry unequivocal prognostic significance.

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*Address for offprints*: Henry Brem, Department of Neurosurgery, Johns Hopkins School of Medicine, Hunterian 817, 725 North Wolfe Street, Baltimore, MD 21205, USA; Tel.: (410)614-0477; Fax: (410)614-0478; E-mail: hbrem@welchlink.welch.jhu.edu