Recurrent Glioblastoma Multiforme: ADC Histogram Analysis Predicts Response to Bevacizumab Treatment[1]

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Purpose: To determine if apparent diffusion coefficient (ADC) histogram analysis can stratify progression-free survival in patients with recurrent glioblastoma multiforme (GBM) prior to bevacizumab treatment.

Materials and Methods: The study was approved by the institutional review board and was HIPAA compliant; informed consent was obtained. Bevacizumab-treated and control patients (41 per cohort) diagnosed with recurrent GBM were analyzed by using whole enhancing tumor ADC histograms with a two normal distribution mixture fitting curve on baseline (pretreatment) magnetic resonance (MR) images to generate ADC classifiers, including the overall mean ADC as well as the mean ADC from the lower curve (ADC_L). Overall and 6-month progression-free survival (as defined by the Macdonald criteria) was determined by using Cox proportional hazard ratios and the Kaplan-Meier method with log-rank test.

Results: For bevacizumab-treated patients, the hazard ratio for progression by 6 months in patients with less than versus greater than mean ADC_L was 4.1 (95% confidence interval: 1.6, 10.4), and there was a 2.75-fold reduction in the median time to progression. For the control patients, there was no significant difference in median time to progression for the patients with low versus high ADC_L (hazard ratio, 1.8; 95% confidence interval: 0.9, 3.7). For bevacizumab-treated patients, pretreatment ADC more accurately stratified 6-month progression-free survival than did change in enhancing tumor volume at first follow-up (73% vs 58% accuracy, $P = .034$).

Conclusion: Pretreatment ADC histogram analysis can stratify progression-free survival in bevacizumab-treated patients with recurrent GBM.

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Glioblastoma multiforme (GBM) is the most aggressive and lethal primary brain tumor. Vascular endothelial growth factor (VEGF) and its receptors are more highly expressed in GBM than in other brain tumors (1). VEGF is a potent mediator of cerebrovascular permeability and is thought to play a substantial role in tumor progression (2). Bevacizumab is a nonselective monoclonal antibody to VEGF (3), and when compared with historical controls it has been shown to improve response rate, 6 month progression-free survival, and overall survival in a phase II trial of recurrent GBM when used alone or in combination with the chemotherapeutic agent irinotecan (4–6). Not all patients respond to bevacizumab regimen therapy, however, and currently, to our knowledge, there is no way to predict which patients will have a good response.

One possible reason for the mixed response to bevacizumab is the variable expression level of VEGF in GBM. VEGF is upregulated in regions of high cell density (pseudopalisades) surrounding necrotic areas (7). Increased VEGF expression has been shown to correlate with radiographic response (8). Processes that degrade cellular integrity, such as necrosis caused by therapy or tumor growth, are thought to increase the apparent diffusion coefficient (ADC) of tissue (9,10). Conversely, since water molecules are more restricted in their movement within cells than in the extracellular space, high cell density is associated with a low ADC (11,12). ADC has been used to assess brain tumor response to therapy (12,13) and to predict survival in patients with newly diagnosed GBM (14–16). We hypothesized that bevacizumab treatment may be most effective for highly necrotic tumors, which would be reflected in higher ADCs, resulting from diminished cellular integrity and density. Therefore the purpose of our study is to determine if ADC histogram analysis can stratify progression-free survival in patients with recurrent GBM prior to treatment with bevacizumab.

Advance in Knowledge

- In this preliminary study, pretreatment apparent diffusion coefficient (ADC) histogram analysis could be used to stratify 6-month progression-free survival in patients with recurrent glioblastoma multiforme (GBM) treated with salvage chemotherapy incorporating bevacizumab.

Materials and Methods

Patients

All patients participating in this study signed institutional review board–approved informed consent. Data acquisition was performed in compliance with all applicable Health Insurance Portability and Accountability Act regulations. The study spanned from August 1, 2006, to January 29, 2008. Images were acquired at five participating sites. We analyzed two patient groups, with 41 subjects each. The first group of patients was retrospectively selected from our institution's neuro-oncology database. All patients (n = 41) who met the following criteria were selected: *(a)* pathologically confirmed GBM with recurrence based on magnetic resonance (MR) imaging and clinical data, *(b)* patients regularly treated every 2 weeks per cycle with bevacizumab (Avastin, Genentech, South San Francisco, Calif; 5 or 10 mg per kilogram of body weight), alone or in combination with chemotherapy (carboplatin, irinotecan, etoposide, lomustine), and *(c)* baseline (ie, before bevacizumab treatment) and at least one follow-up MR study that included diffusion-weighted images. Additional exclusion criteria for subgroups analyses are given below. Follow-up images were obtained at approximately 4–6-week intervals. At the time of last assessment (June 2008), 37 of 41 patients had disease progression. For bevacizumab-treated patients, 24 patients were being treated with steroids at the time of initial imaging (dose range, 0.125–18 mg dexamethasone), and 17 patients were not being treated with steroids. Of the 41 bevacizumab-treated patients, 28 were treated at first recurrence, seven at second recurrence, and six at third or later recurrence.

All patients were treated with radiation therapy (6000 cGy) and subtotal or total resection at the time of initial tumor presentation. We considered the possibility that early radiation necrosis ("pseudoprogression") or late radiation necrosis could be a confounding factor. Therefore we also analyzed 6-month progression-free survival for the following bevacizumab-treated patient subgroups: *(a)* patients who underwent radiation therapy more than 3 months before the baseline MR study (n = 36), *(b)* patients who were more than 1 year out from radiation therapy (n = 14), *(c)* patients with ADC from the lower curve (ADC_L) greater than 1201 (see below), with progressive nonenhancing or solidly enhancing tumor that would not be compatible with radiation necrosis, and/or positive positron emission tomography (PET) scan and/or pathologic evidence of tumor recurrence at autopsy (n = 10), and *(d)* patients who were more than 3 months out from radiation therapy (to avoid pseudoprogression) and had recurrent tumor confirmed by means of positive PET scans (n = 7). Six-month progression-

Implication for Patient Care

- ADC histogram analysis may help determine prognosis in patients with recurrent GBM treated with bevacizumab.

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Abbreviations:
ADC = apparent diffusion coefficient
ADC_L = ADC from the lower curve
GBM = glioblastoma multiforme
LCP = lower curve proportion
VEGF = vascular endothelial growth factor

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Guarantor of integrity of entire study, W.B.P.; study concepts/study design or data acquisition or data analysis/interpretation, all authors; manuscript drafting or manuscript revision for important intellectual content, all authors; approval of final version of submitted manuscript, all authors; literature research, W.B.P., T.C., S.R.; clinical studies, W.B.P., J.A., V.S., J.R.Y., L.T., T.C., A.L., P.N., S.R., J.G.; experimental studies, J.H., M.S.B., J.R.Y., L.T., T.C., P.S.M., S.R., J.G.; statistical analysis, W.B.P., H.J.K., J.A., D.G., J.G.; and manuscript editing, W.B.P., J.A., M.S.B., D.G., T.C., P.S.M., A.L., P.N., S.R., J.G.

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free survival was used for analysis because it has been shown to be a strong predictor of survival (17).

The second (control) group of patients ($n = 41$) with recurrent GBM were not treated with bevacizumab. Control patients were treated for recurrent GBM at multiple centers with a non–VEGF targeting investigational agent. Steroid doses for this cohort were not available.

MR Imaging

MR sequences were performed by using a 1.5-T imager and typically included axial T1-weighted (repetition time msec/echo time msec, 400/15; section thickness, 5 mm), T2-weighted fast spin-echo (4000/126–130; section thickness, 5 mm), and diffusion-weighted and contrast material–enhanced (gadopentetate dimeglumine, Magnevist; Berlex, Wayne, NJ; 0.1 mmol/kg) axial and coronal T1-weighted (400/15; section thickness, 3 mm) imaging, with a field of view of 24 cm and a matrix size of 256 × 256. Postcontrast images were acquired immediately after contrast agent injection. The retrospective nature of this study resulted in some variability in diffusion-weighted acquisition protocols. For the majority of patients, section thickness of 3–5 mm, field of view of 24 cm, and matrix size of 256 × 256 were used, and ADCs were calculated from the trace images. Most of the data from group 1 were collected with a 1.5-T Signa unit (GE Medical Systems, Waukesha, Wis) by using the standard diffusion-weighted imaging pulse sequence supplied by the manufacturer. This pulse sequence performs one image acquisition at $b = 0$ sec/mm^2 and three diffusion-weighted acquisitions at $b = 1000$ sec/mm^2. The data from group 2 were collected as part of a multiple-center clinic trial. For these studies, data were obtained by using GE Medical Systems and Siemens (Erlangen, Germany) MR units with methods equivalent to those described above. How-

Figure 1

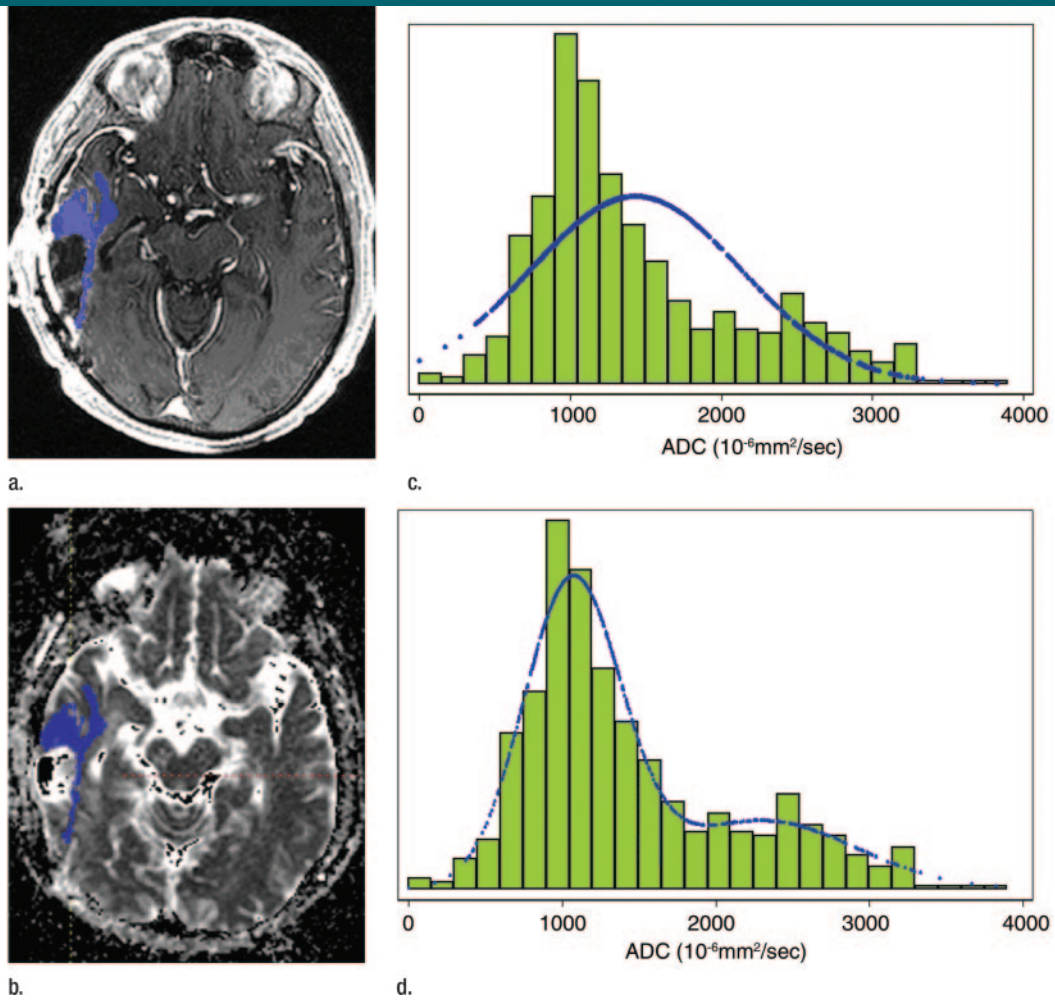

Figure 1: Generation of ADC histograms. **(a)** Total enhancing tumor volume was segmented on axial postcontrast T1-weighted images in a 52-year-old woman with recurrent GBM and transferred to **(b)** the corresponding ADC map image for generation of ADC histogram (**c, d**). **(c)** A single distribution fitting curve provided a poor fit of the data, which could be substantially improved by using **(d)** a two-component normal mixture model.

ever, several centers provided six- and 12-direction diffusion tensor imaging by studies using $b = 0$ and $b = 1000$ from which ADCs were calculated.

Volume Acquisition and ADC Histograms

Enhancing tumor volumes were segmented on postcontrast T1-weighted images at baseline and first follow-up by using a semiautomated adaptive thresholding technique so that all pixels above the threshold value were selected (18). Therefore, regions of macroscopic necrosis that were not enhancing as well as cystic areas were excluded. The resulting regions of interest encompassing the entire enhancing tumor volume were verified by a board-certified neuroradiologist (W.B.P.; 5 years of experience), blinded to clinical outcome, and mapped to the ADC images. ADCs calculated on a pixel-by-pixel basis for the entire enhancing volume were used for histogram analysis and expressed in units of 10^{-6} mm^2/sec (J.H.; blinded to clinical outcome). Since ADC histograms were often bimodal or skewed, we used a two-mixture normal distribution to provide optimal fitting (19). As judged by visual inspection, this model yielded better fitting curves than either data transformation or higher-level mixtures. We then generated means for the upper and lower peak (ADC_L, lower curve mean, and ADC_H, upper-curve mean), in addition to the overall mean ADC (Fig 1). We also considered the proportion of total ADCs that were attributable to the lower peak, and termed this the lower curve proportion (LCP). For several analyses, we dichotomized the ADC classifiers at the mean (ADC_L = 1201; overall mean ADC = 1371; LCP mean = 0.68). We also dichotomized change in enhancing tumor volume between baseline and first follow-up at the mean (59% reduction).

Statistical Analysis

The Fisher exact test was used to compare the interval between radiation and baseline imaging in the bevacizumab and control groups. Progression-free survival from the time of bevacizumab treatment was determined based on the Macdonald criteria (20). The paired *t* test was used to determine significance levels for changes between the first follow-up and pretreatment imaging studies. A test of the proportional hazards assumption was used after fitting univariate Cox models, and 95% confidence intervals were generated (21). To test the effect of patient age and size of tumor at recurrence on 6-month progression-free survival in conjunction with the ADC classifiers, the covariates of age at recurrence, contrast-enhanced volume at recurrence, percent change in the tumor volume at first follow-up, and the change in mean ADC at first follow-up were standardized, and a multivariate Cox model, which also included site of image acquisition, was used. The Kaplan-Meier method with log-rank test was used to estimate progression-free survival in bevacizumab-treated and control patients (22). For the Kaplan-Meier plots, we dichotomized the data on the basis of mean values of the ADC histogram analysis from the baseline bevacizumab-treated patients. Sensitivity and specificity for the ADC classifiers and change in tumor volume at first follow-up for stratification of 6-month progression-free survival were calculated. For all analyses, $P < .05$ was accepted as indicating a significant difference (statistical analysis performed by D.G. and H.J.K.). Statistical analysis was performed with statistical software (Stata 10, 2008; Stata, College Station, Tex).

Results

Patient Characteristics

Table 1 shows the baseline characteristics of and responses in patients with recurrent GBM. There was no significant difference in baseline ADCs between patients treated with steroids versus the patients not treated with steroids ($P = .65$). All bevacizumab-treated patients received a total of 6000 cGy. Seventy-eight percent of control patients (32 of 41) received a radiation dose of 5900–6100

Table 1

Baseline Patient Demographics and Response

Parameter	Bevacizumab-treated Patients			Control Patients		
	Male ($n = 27$)	Female ($n = 14$)	All ($n = 41$)	Male ($n = 25$)	Female ($n = 16$)	All ($n = 41$)
Mean age (y)	52 ± 15	55 ± 16	53 ± 15	53 ± 10	52 ± 15	53 ± 12
Age range (y)	24–78	24–74	24–78	35–70	24–78	24–78
Baseline ADC_L (10^{-6} mm^2/sec)	1171 ± 199	1259 ± 178	1201 ± 195	1135 ± 376	1054 ± 237	1103 ± 328
Follow-up ADC_L (10^{-6} mm^2/sec)	964 ± 232	1066 ± 284	1000 ± 253	1138 ± 240	1110 ± 277	1127 ± 252
Baseline enhancing tumor volume (mL)	16.4 ± 12.4	13.8 ± 11.8	15.5 ± 12.1	24.0 ± 23.0	18.9 ± 10.0	22.0 ± 19.0
Follow-up enhancing tumor volume (mL)	6.4 ± 7.8	8.4 ± 11.5	7.1 ± 9.1	41.3 ± 43.4	33.4 ± 28.4	38.2 ± 38.1
Radiation dose (cGy)	6000 ± 0.0	6000 ± 0.0	6000 ± 0.0	5873 ± 1564*	5931 ± 452*	5894 ± 1262*
Radiation-free interval (mo)†						
0–3	4	1	5	1	2	3
3–6	9	5	14	15	7	22
6–12	6	2	8	3	2	5
>12	8	6	14	5	3	8

Note.—Except where indicated, data are mean ± standard deviation. For the bevacizumab-treated group, follow-up was in 40 patients, owing to complete tumor response in one patient.

* Data are from 24 male and 14 female patients (radiation dose was unavailable for three control patients).

† Indicates radiation-free interval before baseline imaging. Data are numbers of patients (data unavailable for three patients: one male, two female).

cGy. The interval between radiation and baseline imaging was also similar between the bevacizumab-treated and control patients ($P = .22$, Fisher exact test). There was no significant difference in baseline ADC between the bevacizumab-treated and control patients ($P = .10$).

Progression-free Survival

For bevacizumab-treated patients, the log-rank test showed that mean ADC, ADC_L, and LCP, but not the upper-curve mean ADC, were predictive of 6-month progression-free survival (respective P values of .016, .001, .001, and .972). In a univariate Cox model, ADC_L was the most accurate predictor of 6-month progression-free survival. Table 2 shows the sensitivity and specificity of all three significant ADC classifiers, as well as changes in enhancing volume in terms of ability to stratify 6-month progression-free survival. Pretreatment ADCs were more accurate at stratifying 6-month progression-free survival than was determining response at first follow-up as defined by the Macdonald criteria (73% vs 58% accuracy, $P = .034$).

Multivariate Cox Model

Change in enhancing tumor volume was a significant predictor of 6-month progression-free survival ($P = .004$, Table 3), whereas change in mean ADC, the size of tumor, and age at recurrence were not significant ($P = .787$, .203, and .155, respectively). Mean ADC also was not significant ($P = .144$). However, both ADC_L and LCP were predictive of 6-month progression-free survival (ADC_L hazard ratio of 5.45, $P = .004$; LCP hazard ratio of 3.70, $P = .014$). Thus it appeared that the histogram analysis was superior to the mean ADC alone in stratifying 6-month progression-free survival in the multivariate Cox model analysis.

Progression-free Survival: Bevacizumab versus Control Group

ADC_L values were predictive of overall progression-free survival in the bevacizumab-treated patients but not in the control patients (Fig 2). This was also the case for 6-month progression-free survival. ADC_L values were predictive in the bevacizumab-treated patients (low vs high ADC_L in bevacizumab-treated patients: hazard ratio, 4.1; 95% confidence interval: 1.6, 10.4; 2.75-fold reduction in median time to progression), but not in the control patients (low vs high ADC_L in control patients: hazard ratio, 1.8; 95% confidence interval: 0.9, 3.7; no difference in median time to progression). When we combined the bevacizumab-treated and control patients and used a multivariate analysis (Table 4), ADC_L and LCP values were predictive of overall progression-free survival.

For patients with ADC_L of 1201 or greater (bevacizumab-treated and control patients), not treating with bevacizumab resulted in a hazard ratio for progression by 6 months of 5.74 (95% confidence interval: 2.0, 16.2). For patients with ADC_L of less than 1201, the hazard ratio was 3.0 (95% confidence interval: 1.59, 5.69). Median survival increase with bevacizumab treatment was 6.6-fold for patients with ADC_L of 1201 or greater, compared with a 2.4-fold increase for patients with ADC_L of less than 1201. Thus, bevaci-

Table 2

Accuracy of ADC Classifiers for Stratifying Progression by 6 Months in Bevacizumab-treated Patients: Univariate Analysis

Predictive Classifier	Sensitivity (%)*	Specificity (%)*	Accuracy (%)*	Odds Ratio†	Log-Rank *P* Value	Cox *P* Value
Mean ADC_L	75.0 (18/24)	68.8 (11/16)	72.5 (29/40)	17.5 (2.0, 154)	.001	.003
Mean ADC	66.7 (16/24)	68.8 (11/16)	67.5 (27/40)	4.4 (1.1, 17.1)	.02	.02
Mean ADC_L and LCP‡	50.0 (12/24)	93.8 (15/16)	67.5 (27/40)	15.0 (1.7, 132)	<.0001	<.0001
Change in tumor volume§	75.0 (18/24)	37.5 (6/16)	60.0 (24/40)	1.8 (0.5, 7.1)	.79	.79
Macdonald criteria‖	79.2 (19/24)	25.0 (4/16)	57.5 (23/40)	1.3 (0.3, 5.7)	.67	.67

Note.—ADC_L, ADC, LCP, and tumor volume change are dichotomized based on means. Patients with values greater than the mean are compared with patients with values less than the mean. $P < .05$ indicates a significant difference.

* Data in parentheses are raw data used to calculate percentages.

† Data in parentheses are 95% confidence intervals.

‡ Indicates patients with both $ADC_L <$ mean and LCP > mean versus the remaining patients.

§ Indicates mean reduction in tumor volume between baseline and first follow-up (59%).

‖ Macdonald criteria for response at first follow-up.

Table 3

Multivariate Cox Model for Progression by 6 Months in Bevacizumab-treated Patients

Variable	Hazard Ratio	Standard Error	*P* Value	95% Confidence Interval
	Multivariate Cox Model with Mean ADC*			
Mean ADC < 1371	2.1	1.0	.14	0.8, 5.4
Age	0.7	0.2	.16	0.5, 1.1
Tumor volume	1.0	0.02	.20	1.0, 1.0
Percent change in tumor volume	2.2	0.6	.004	1.3, 3.7
Percent change in mean ADC	1.1	0.3	.79	0.6, 1.9
	Multivariate Cox Model with ADC_L and LCP†			
$ADC_L < 1201$	5.5	3.2	.004	1.7, 17.3
LCP > 0.68	3.7	2.0	.01	1.3, 10.5
Age	0.6	0.2	.08	0.4, 1.1
Tumor volume	1.0	0.02	.71	1.0, 1.0
Percent change in tumor volume	6.3	4.0	.004	1.8, 21.8
Percent change in mean ADC	1.0	0.02	.91	1.0, 1.0

Note.—Tumor volume indicates the initial enhancing tumor volume. $P < .05$ denotes a significant difference.

* Test of proportional hazards assumption ($P = .54$).

† Test of proportional hazards assumption ($P = .45$).

zumab may extend progression-free survival by a greater amount in patients with the high compared with the low ADC_L. However, this difference in bevacizumab effect on progression-free survival between the two groups was not significant ($P = .33$), and thus this hypothesis would need to be confirmed in a larger cohort.

Radiation Necrosis

A significant difference between 6-month progression-free survival for the high versus low ADC_L in the bevacizumab-treated patient cohort was maintained when patients who underwent radiation therapy within 3 months of baseline imaging ($n = 5$) were excluded from analysis (median of 180 vs 88 days respectively, $P = .02$). Examination of the subset of patients who were more than 1 year out from radiation therapy ($n = 14$) also yielded a significant difference between the patients with high versus low ADC_L (median 6-month progression-free survival, 208 vs 72 days; $P = .04$). In addition, confining the high-ADC_L group to only those patients who had corroborating data showing viable tumor (including PET scans and autopsies, $n = 10$) and comparing these patients to the low-ADC_L patients showed a significant difference in median 6-month progression-free survival (197 vs 84 days, $P = .04$). Last, selecting the subset of patients that were more than 3 months out from radiation therapy (to avoid pseudoprogression [23]) and restricting patients in the $ADC_L > 1201$ group to those who had recurrent tumor confirmed on positive PET scans ($n = 7$), we found a trend toward longer 6-month progression-free survival with high versus low ADC_L (238 vs 84 days, $P = .057$).

Figure 2

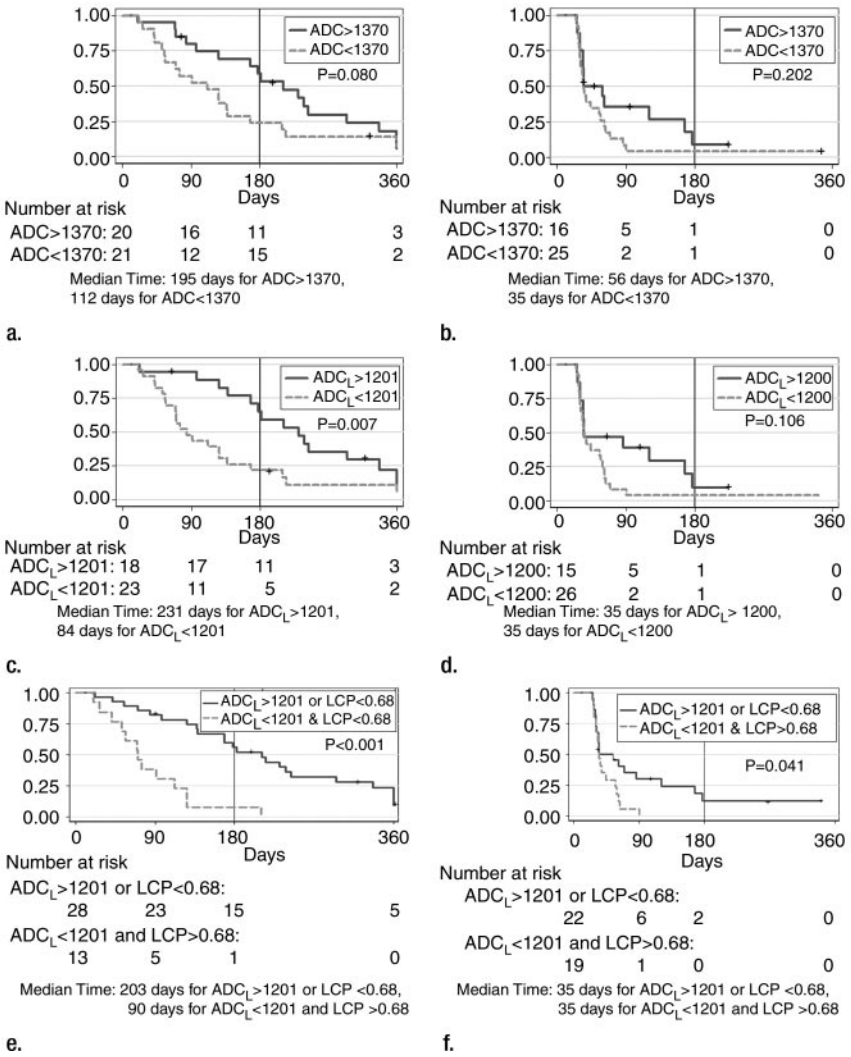

Figure 2: Kaplan-Meier curves for overall progression-free survival. The y-axis represents percentage not progressed. The x-axis is days after treatment, with the vertical line noting progression-free survival at 6 months. The left column (**a, c, e**) represents bevacizumab-treated patients and the right column (**b, d, f**) represents the control patients. Tick marks indicate time of patient data censoring.

Discussion

In patients with recurrent GBM, bevacizumab treatment has been shown to improve response rate, progression-free survival, and overall survival in comparison to historical controls (4–6). Not all patients respond to bevacizumab therapy, however. As targeted molecular therapies such as bevacizumab have been developed, the search for biomarkers that predict outcomes has intensified. We investigated the utility of ADC histogram analysis in stratifying response of recurrent GBM to bevacizumab therapy.

Since ADC histograms were asymmetrical, generally broad, and occasionally dual peaked, a two-component mixture normal distribution was selected to model their shape. As judged by means of visual inspection, this model yielded better-fitting curves than either data transformation or higher-level mixtures. One potential explanation for this observation is that some areas of tumor may have densely packed cells and little edema, resulting in low ADCs, whereas other areas of tumor are composed of a mixture of viable and necrotic cells with superimposed edema, which would tend to generate higher ADCs. Regardless of the underlying physiology, we found that ADC histogram analysis of MR images prior to bevacizumab treatment was highly accurate in stratifying 6-month progression-free survival. The accuracy of this approach was greater than that of assessing either change in tumor size or change in ADC at first follow-up examination. ADC

histogram analysis was a better predictor of median progression-free survival in bevacizumab-treated patients compared with control patients; this is particularly important as bevacizumab treatment is becoming the standard of care for recurrent GBM (24). Our hypothesis is that bevacizumab treatment is more effective for necrotic versus non-necrotic tumor (25,26). We found a greater increase in median time to survival in bevacizumab-treated patients with high ADCs in comparison with those patients with low ADCs (in comparison to control patients), potentially supporting this hypothesis. This difference was not significant, however, and will need to be confirmed with a larger study. Although change in enhancing tumor size is typically the reference standard by which response to treatment is determined, our findings suggest that ADC histogram analysis may be a superior and quicker biomarker of bevacizumab response than current methods.

Our results are compatible with other work showing a relationship between progression/survival and ADC in patients with newly diagnosed, rather than recurrent, GBM. For example, Oh et al (15) found that shorter median survival time was associated with a low mean normalized ADC within the peritumoral region of T2-weighted signal intensity abnormality. Higano et al (27) and Murakami et al (16) demonstrated that minimal pretreatment ADCs are prognostic of 2-year survival rates in patients with newly diagnosed GBM. One important difference between our study and that of Murakami et al (16) is that they used both enhancing and nonenhancing tumor, whereas our study was restricted to enhancing tumor to avoid confusion with gliosis and other causes of increased T2-weighted signal intensity. Additionally, Murakami et al used hand-selected regions of interest. We used the entire contrast-enhanced tumor volume to generate ADCs—an approach that has the advantage of reducing region of interest selection bias.

Another approach to assessing antiangiogenic therapies in GBM is with perfusion MR imaging. Perfusion MR imaging has been shown to be predictive of glioma grade (28) and time to tumor progression (29). Responders to antiangiogenic therapies demonstrate a "normalization" of tumor vasculature reflected in MR perfusion parameters with decreased cerebral blood volume and permeability. These changes have been correlated with a clinical response (30,31). Perfusion data were not acquired for our patients. Recurrence with bevacizumab treatment tends to be less enhancing and necrotic, and more infiltrative, than recurrence following standard chemotherapies. In addition, it has been suggested that bevacizumab treatment is more effective against enhancing compared with nonenhancing tumor (26,32). Therefore it would be of interest to determine if perfusion data could improve the stratification of patients in their response to bevacizumab treatment. Additionally this combination of perfusion and diffusion data might be more accurate in assessing nonenhancing tumor following bevacizumab treatment in comparison with standard MR imaging alone.

Our analysis has several potential limitations. One consideration is the effect of radiation necrosis. Zeng et al (33) reported that radiation necrosis has higher ADCs than does recurrent tumor. Therefore, enrichment of either early radiation necrosis (pseudoprogression) or late radiation necrosis in patients with high ADCs could pose an important confound. Even when controlling for the effects of radiation necrosis, we found that a significant progression-free survival advantage was maintained in bevacizumab-treated patients with high versus low ADC. Furthermore, whether high ADCs in patients diagnosed with recurrent GBM are associated with endogenous tumoral necrosis, radiation-induced necrosis, or a combination of the two, the predictive value of ADC analysis of pretreatment MR images remains unaffected.

Another caveat to our findings is that there was a high degree of treatment variability for our patients. In particular, multiple chemotherapy regimens were used in combination with bevacizumab therapy, and there were some differences in radiation doses and timing between the bevacizumab-treated and control patients. Steroid doses at baseline MR imaging also varied. It has been reported that steroid treatment reduces mean ADC by 7% in brain tumors (34). We found no significant difference between ADCs in patients treated with steroids versus those not treated with steroids. Potentially, patients with more edema were more likely to receive steroid treatment, and so the two factors offset one another. Follow-up analysis of patients with more homogeneous steroid, chemotherapy, and radiation treatments will be necessary to validate our results.

In conclusion, our study shows the potential utility of enhancing-tumor ADC

Table 4

Multivariate Cox Model for Overall Progression-free Survival for All Patients

Variable	Hazard Ratio	Standard Error	*P* Value	95% Confidence Interval
$ADC_L < 1201$	2.8	0.81	<.001	1.6, 4.9
LCP > 0.68	2.4	0.71	.003	1.3, 4.3
Age ≥ 55 years	1.4	0.37	.16	0.9, 2.4
Initial enhancing tumor volume	1.0	0.14	>.99	0.8, 1.3
No bevacizumab*	3.0	1.36	.01	1.3, 7.3
Site†				
A	1.9	1.08	.25	0.6, 5.8
B	0.9	0.48	.90	0.3, 2.6
C	1.2	0.84	.83	0.3, 4.8
D	0.4	0.45	.41	0.0, 3.5
E	1.5	0.81	.46	0.5, 4.3

Note.—Test of proportional hazards assumption ($P = .47$); results are from bevacizumab-treated and control patients combined ($n = 82$). $P < .05$ indicates a significant difference.

* Indicates patients not treated with bevacizumab.

† Location where patient MR images were obtained.

histogram analysis in stratifying response to salvage chemotherapy with bevacizumab-based regimens in patients with recurrent GBM. The observation that presalvage chemotherapy ADC analysis was more accurate than measuring tumor response at first follow-up in stratifying patients on the basis of progression-free survival at 6 months demonstrates the utility of this approach and may allow for earlier decisions in treatment strategy for individual patients.

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