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ORIGINAL REPORT

Predicting Treatment Response of Malignant Gliomas to Bevacizumab and Irinotecan by Imaging Proliferation With [^{18}F] Fluorothymidine Positron Emission Tomography: A Pilot Study

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ABSTRACT

Purpose
Evaluation of treatment effects in malignant brain tumors is challenging because of the lack of reliable response predictors of tumor response. This study examines the predictive value of positron emission tomography (PET) using [^{18}F] fluorothymidine (FLT), an imaging biomarker of cell proliferation, in patients with recurrent malignant gliomas treated with bevacizumab in combination with irinotecan.

Patients and Methods
Patients with recurrent malignant gliomas treated with biweekly cycles of bevacizumab and irinotecan were prospectively studied with FLT-PET at baseline, after 1 to 2 weeks, and after 6 weeks from start of treatment. A more than 25% reduction in tumor FLT uptake as measured by standardized uptake value was defined as a metabolic response. FLT responses were compared with response as shown by magnetic resonance imaging (MRI) and patient survival.

Results
Twenty-one patients were included, and 19 were assessable for metabolic response evaluation with FLT-PET. There were nine responders (47%) and 10 nonresponders (53%). Metabolic responders survived three times as long as nonresponders (10.8 *v* 3.4 months; $P = .003$), and tended to have a prolonged progression-free survival ($P = .061$). Both early and later FLT-PET responses were more significant predictors of overall survival (1 to 2 weeks, $P = .006$; 6 weeks, $P = .002$), compared with the MRI responses ($P = .060$ for both 6-week and best responses).

Conclusion
FLT-PET as an imaging biomarker seems to be predictive of overall survival in bevacizumab and irinotecan treatment of recurrent gliomas. Whether FLT-PET performed as early as 1 to 2 week after starting treatment is as predictive as the study indicates at 6 weeks warrants further investigation.

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INTRODUCTION

Bevacizumab, a recombinant humanized monoclonal antibody targeting vascular endothelial growth factor (VEGF), disrupts a critical process in the regulation of angiogenesis.[1,2] When administered as single agents, antiangiogenic drugs produce modest clinical or radiographic responses, but have not yielded survival benefits.[3] In contrast, when administered with chemotherapy, bevacizumab increased survival in previously treated or untreated colorectal and untreated lung cancer patients.[4,5] Improvement in progression-free survival but not overall survival was observed in previously untreated breast cancer patients.[6] It has been hypothesized that antiangiogenic agents may normalize the abnormal structure and function of blood vessels supplying the tumors to make them more efficient for oxygen and drug delivery.[7] Despite several active investigations, no reliable biomarkers predictive of treatment responses have been established.[3]

Glioblastoma cell lines secrete VEGF. [8] In situ analysis of tumor specimens demonstrated that production of VEGF is specifically induced in a subset of glioblastoma cells distinguished by their immediate proximity to necrotic foci and clustering of capillaries.[9] Moreover, critical tumor subpopulations within gliomas shared characteristics with neural

stem cells, and these stem cell–like glioma cells secreted elevated levels of VEGF, which were further induced by hypoxia.[10] Further, bevacizumab displayed potent antiangiogenic efficacy and suppressed the growth in mice xenografts derived from stem cell–like glioma cells.[10]

Significant changes in magnetic resonance imaging (MRI) contrast enhancement in 14 patients with recurrent malignant gliomas treated with bevacizumab in combination with various chemotherapeutic agents have been observed.[11] A response rate of 63% was seen in 32 patients with recurrent high-grade gliomas treated with bevacizumab and irinotecan.[12] The extent of MRI contrast enhancement in malignant gliomas has been used as an indicator of therapeutic response.[13] However, this approach is limited by the difficulty in distinguishing between tumor and treatment-induced necrosis,[14] and reliable prognostic information can be obtained only many weeks after treatment starts.

[^{18}F]fluorodeoxyglucose (FDG) positron emission tomography (PET) has prognostic value in imaging brain tumors.[15] However, the high physiological glucose metabolic rate of the brain reduces the sensitivity of FDG-PET for lesion characterization.[16-18] [^{18}F]fluorothymidine (FLT) was developed as a noninvasive PET biomarker for imaging cell proliferation.[19] In a previously published study in 25 patients with gliomas,[20] FLT tumor uptake was well correlated with the proliferation index Ki-67. Thus, FLT-PET has the potential to serve as an imaging marker for treatment monitoring.

In this study, we sought to determine whether FLT-PET is predictive of the outcome of patients with recurrent malignant gliomas who were treated with bevacizumab and irinotecan; whether early and late PET studies provide similar information; and whether prognostic information obtained with MRI is comparable to that obtained from PET.

Table 1. Patient Characteristics

Characteristic		No.
Sex		
Male		11
Female		10
Age, years		
Median	58	
Range	26-78	
Tumor grade		
III		4
IV		17
Recurrences		
Median		2
1-2		14
3-4		6
5		1
Prior treatment regimens*		
Median		2
1-2		13
3-4		4
5-6		4
Dexamethasone treatment		
Absence		7
Presence		14
Days from prior radiation treatment		
Median		205
< 100		7
100-300		6
> 300		8

*Number of treatment regimens was defined as the number of specific treatment that each patient had received (eg, radiation and concomitant temozolamide was considered as one regimen).

PATIENTS AND METHODS

Patients

Twenty-one patients with recurrent high-grade gliomas were prospectively studied (Table 1). Seventeen had WHO grade 4 and four had WHO grade 3 glioma. Every patient had undergone surgical resection, radiation, and chemotherapy. All had tumor recurrence determined by MRI and/or neurological deterioration. The median number of recurrences and prior treatments was two (range, 1 to 5 and 1 to 6, respectively).

All patients gave written consent to participate in this study, which was approved by the University of California, Los Angeles (Los Angeles, CA), Office for Protection of Research Subjects. This was an imaging study. None of the patients were enrolled in treatment trials, and all were treated off-label with irinotecan and bevacizumab.

Treatment

Patients were treated every other week with bevacizumab (10 mg/kg) and irinotecan (125 mg/m^2 for patients not taking enzyme-inducing antiepileptic drugs and 350 mg/m^2 for patients taking enzyme-inducing antiepileptic drugs). Treatment continued until progression or unacceptable toxicity occurred. Fourteen patients were also treated with dexamethasone during the course of the FLT-PET study, with a median dose of 7.5 mg/d (range, 2 to 36 mg/d) at baseline and 5.2 mg/d (range, 1 to 20 mg/d) at the time of the last PET study. Eight patients were on either stable or tapering doses of dexamethasone, and six patients had a dose increase after the baseline MRI and PET studies were obtained.

PET Imaging

FLT was synthesized as published previously.[21] A baseline FLT-PET was performed within 1 week before the initiation of treatment, and follow-up FLT-PET was performed at 1 to 2 and at 6 weeks after start of treatment. PET was performed using a high-resolution full-ring PET scanner (ECAT HR+; Siemens/CTI, Munich, Germany), which acquires 63 contiguous slices simultaneously. No specific dietary instructions were given to the patients except for advising them to drink plenty of water before and after PET (to accelerate FLT excretion).

For FLT-PET imaging, a dynamic emission acquisition sequence in three-dimensional mode over 60 minutes was started with the intravenous injection of 2.0 MBq/kg FLT, followed by a 5-minute transmission scan for attenuation correction. PET emission data were reconstructed using iterative reconstruction ordered-subsets expectation maximization (eight iterations with six subsets) and a Gaussian filter with 5 mm full width at half maximum, using measured attenuation correction. The final reconstructed volume-set had a matrix size of 128 × 128 mm with a voxel size of 2.4 × 2.4 × 2.4 mm.

For region of interest (ROI) analysis, FLT data were summed between 30 and 60 minutes to obtain static images. The PET slice with the maximum tumor uptake was chosen for ROI analysis, and the two adjacent axial slices one plane above and one plane below the chosen slice were included to improve count statistics. A circular ROI (diameter 1.0 cm, 16 pixels) was placed over the area of the tumor. The radiotracer concentration in the ROIs was normalized to the injected dose per patient's body weight to derive the standardized uptake values (SUVs; g/mL).

Response Evaluation and Follow-Up

Contrast and noncontrast brain MRI for treatment monitoring were acquired in all patients within 1 week before and in 6-week intervals after the start of treatment. Studies were interpreted by an experienced neuroradiologist (W.P.). All patients were either not receiving or receiving a stable dose of corticosteroids for at least 5 days before the baseline MRI study. MRIs were evaluated based on the Macdonald criteria.[22] In addition, T2 and/or

Table 2. Patient Characteristics, Tumor FLT Uptake, and Response to Treatment (N = 21)

Patient No.	Sex	Age (years)	Diagnosis	Baseline SUV	Post-Treatment SUV (days)		SUV Change (%)		MRI		TTP (months)	OS (months)
					1-2 Weeks	6 Weeks	1-2 Weeks	6 Weeks	6 Weeks	Best Response		
1	Female	38	AA	0.74	0.59	0.78	−20	5	SD	PR	5.1	8.5
2	Male	78	GBM	1.57	2.28	ND	45	NA	PD	PD	0.1	0.8
3	Male	64	GBM	0.88	0.62	0.63	−30	−28	SD	PR	2.3	7.7
4	Female	45	GBM	0.74	0.82	1.67	11	126	SD	SD	1.8	11.4
5	Male	37	GBM	1.56	1.39	1.45	−11	13	SD	PR	3.9	4.0
6	Male	65	AA	0.83	0.44	056	−47	−33	SD	SD	3.7	4.7
7	Male	65	AMG	0.64	0.54	ND	−16	NA	PD	PD	0.8	2.6
8	Female	61	GBM	1.69	1.16	1.15	−31	−32	SD	PR	4.5	13.1
9	Male	69	GBM	0.45	0.45	0.39	0	−13	SD	SD	2.7	2.7
10	Male	26	GBM	0.75	0.74	1.08	−1	44	SD	SD	3.1	11.5
11	Female	65	GBM	0.69	0.60	1.20	−13	74	PD	PD	2.6	3.6
12	Female	35	GBM	0.72	0.33	0.35	−54	−52	PR	PR	2.1	10.4
13	Female	62	GBM	0.59	0.37	0.39	−37	−34	PR	PR	9.0	13.7+
14	Male	28	AA	0.81	0.46	0.60	−43	−26	PD	PD	1.6	12.6+
15	Female	68	GBM	1.34	1.14	ND	−15	NA	PD	PD	1.1	1.6
16	Female	47	GBM	2.35	1.42	1.33	−40	−43	SD	SD	5.8	11.4+
17	Female	54	GBM	1.22	0.61	0.65	−50	−47	PR	PR	7.4	10.8+
18	Male	58	GBM	4.79	1.77	5.24	−63	9	SD	SD	1.5	3.2
19	Male	46	GBM	1.81	0.82	0.62	−55	−66	PR	PR	2.4	10.4+
20	Male	28	GBM	1.66	ND	ND	NA	NA	PD	PD	0.9	8.6
21	Female	73	GBM	1.07	ND	ND	NA	NA	PD	PD	1.9	7.5

Abreviations: SUV, standardized uptake value; MRI, magnetic resonance imaging; TTP, time to tumor progression; OS, overall survival; AA, anaplastic astrocytoma; GBM, glioblastoma multiforme; AMG, anaplastic mixed glioma; SD, stable disease; PD, progressive disease; PR, partial response; ND, not done; NA, not applicable.

fluid-attenuated inversion-recovery images were evaluated. For example, for a patient to be classified as a partial responder, the contrast images need to show a more than 50% decrease in the area of enhancement and stable or decreased disease on T2 and fluid-attenuated inversion recovery images while the patient was on stable or decreased steroid dose. Both MRIs at 6 weeks after starting treatment and the MRI with best overall response were evaluated.

Survival time was the interval from the date of the start of the treatment to the date of death. Time to tumor progression was the time interval from the date of the start of treatment to the date of first documented evidence of disease progression, based on MRI and/or neurological deterioration. The median time from baseline FLT-PET to the start of the treatment was 2 days. Patients were followed for survival until death. No patient was lost to follow-up.

Statistical Analysis

On the basis of literature in metabolic imaging with FLT and FDG, the intrasubject variability for measuring FLT uptake is less than 20%.[23-25] A reduction in FDG activity by more than 25% has been considered a threshold for treatment responses for various tumors by the European Organisation for Research and Treatment of Cancer (EORTC).[26] On the basis of these considerations, a population of 19 patients was required to detect with a power of 80% significant differences in metabolic changes among responders and nonresponders at a 5% level.[27]

Differences between groups of patients were tested by the *t* test. Patients who were alive at the date of the last follow-up were excluded on that date. Patients who died without progressive disease (PD) documented were considered to have had PD at the time of death. Receiver operating characteristic (ROC) curves analysis was used to identify the optimum cutoff value of the tumor FLT uptake reduction for differentiation of responding and nonresponding patients. Survival estimates were calculated according to the Kaplan-Meier method.[28] Statistical analyses of multiple variables were performed with the Cox proportional hazards model.[29] Variables reaching a significance of $P < .05$ by univariate analysis were included in the multivariate analysis.

Table 3. FLT-PET Responses at 1-2 and 6 Weeks

Group	Change in SUV (%)					
	1-2 Weeks			6 Weeks		
	Change	SE	No. of Patients	Change	SE	No. of Patients
FLT responders	−43	9	10	−40	12	9
FLT nonresponders	−2	20	9	27	61	7
Overall	−18	38	19	−15	37	16

Abbreviations: FLT, [^{18}F] fluorothymidine; PET, positron emission tomography; SUV, standardized uptake value.

Table 4. Cox Regression Analysis of Overall Survival of Predictive Factors

Predictive Factor	Univariate		Multivariate	
	P	HR	*P*	HR
Age	.068	1.037		
No. of recurrences	.02	1.935	.888	
No. of prior treatments	.012	1.513	.054	1.503
Tumor grade	.81			
Dexamethasone treatment	.499			
Time from prior radiation	.937			
Lack of FLT reduction at 6 weeks	.005	6.594	.02	4.955
Lack of FLT reduction at 1-2 weeks	.01	4.746		
FLT uptake at baseline	0.713			
FLT uptake at 1-2 weeks	0.15			
FLT uptake at 6 weeks	.12			

Abbreviations: HR, hazard ratio; FLT, [^{18}F] fluorothymidine.

RESULTS

Changes of FLT-PET Uptake During Treatment

Twenty-one patients were registered for the FLT-PET study between June 2005 and February 2006. All 21 patients underwent baseline FLT-PET scan. These data were used to determine whether baseline FLT-PET was predictive of patient outcome.

Two patients did not complete the follow-up PET study (Table 2), and cannot be analyzed for the metabolic response. Thus, 19 patients were assessable for metabolic responses (Fig 1). The baseline FLT-PET was performed 2.0 ± 1.7 days before start of treatment, and follow-up scans were performed at 12 ± 6 days (1.7 ± 0.8 weeks) and 43 ± 12 days (6.1 ± 1.7 weeks) after start of treatment. Three patients (patients 2, 7, and 15) did not undergo the third FLT-PET because of clinical deterioration, with one death occurring before the third scan. Therefore, early PET after 1 to 2 weeks was available in 19 patients, and PET after 6 weeks was available in 16 patients.

SUVs from the tumor ROIs were obtained from each FLT-PET (Table 2). ROC curve analysis was performed using the clinical criteria of 6 months survival to separate responders from those with PD. The area under the ROC curve was 0.761 ± 0.11, with a sensitivity of 72.7% and a specificity of 87.5%. A 25% reduction in FLT-PET uptake at 6 weeks served as the optimal threshold for response. Using this threshold, nine (43%) of the 19 patients sustained a metabolic response at 6 weeks, whereas 10 patients (57%) did not. The metabolic responders demonstrated a 43% ± 9% decrease of FLT uptake from baseline to the first follow-up scan and a 40% ± 13% decrease of FLT uptake from baseline to the second follow-up study (Table 3). Among the 10 nonresponders, FLT uptake increased by 2% ± 20% at the time of the first follow-up scan and by 27% ± 61% at the second follow-up scan.

Response Evaluation and Patient Survival

At the time of this writing, 16 patients had died. Overall 6-month survival was 62% and 65% for patients with grade 4 glioma. Patients with a metabolic response had a significantly longer survival compared with patients without metabolic response (median survival, 10.8 *v* 3.4 months; $P = .003$). Of note, five (56%) of the nine metabolic responders were still alive, whereas none of the 10 nonresponders (0%) were. FLT response at 6 weeks predicted patient survival, as demonstrated by Kaplan-Meier survival analysis ($P = .002$; Fig 2). FLT-PET at 1 to 2 weeks was also significant for overall survival ($P = .006$). A trend for improved progression-free survival of metabolic responders was seen ($P = .061$).

We also examined whether single measurements of FLT SUVs at any time point were predictive of patient outcome. Using various cutoff values, the maximum FLT SUVs were at no time significant predictors of patients' survival (Kaplan-Meier log-rank $P = .752$, .412, and .231 for SUVs at baseline, 1 to 2 weeks, and 6 weeks, respectively). Likewise, neither baseline tumor to normal background FLT uptake ratios nor their temporal changes were predictive of survival ($P = .446$, .495, and .685, respectively).

Response by MRI was evaluated at approximately 6 weeks (5.8 ± 1.0 weeks) after the start of the treatment and was available for 20 patients (except for patient 2, who died before 6 weeks). By MRI, four (19%) of 21 patients had partial response (PR), 10 (48%) of 21 had stable disease (SD), and 7 (33%) of 21 had PD. An MRI response at 6 weeks tended to predict prolonged survival ($P = .060$). Best response predictions assessed with MRI occurred at 7 weeks (range, 2.1 to 18.6 weeks). At this time point, twice as many patients (eight of 21, 38%) demonstrated PR, seven (33%) of 21 had PD, and six (29%) of 21 had SD. Best response by MRI also tended to predict prolonged survival ($P = .060$).

Multiple clinical variables were tested in univariate and multivariate analyses (Table 4). In univariate analysis, survival was better if patients had fewer recurrences or fewer prior treatments. Other clinical variables such as age, tumor grade, dexamethasone treatments, and time from prior radiation therapy did not predict survival. In the multivariate analysis, the number of prior treatment was the only clinical variable to significantly predict survival.

In considering all variables (clinical and imaging based), a lack of reduction in FLT uptake at 6 weeks after start of treatment was the strongest predictor of death (hazard ratio [HR] = 5.0), whereas a higher number of prior treatments had an HR for death of 1.5.

DISCUSSION

This prospective study provides the first evidence that FLT-PET can serve as an imaging biomarker for predicting treatment responses in patients with recurrent malignant gliomas. Changes in FLT tumor

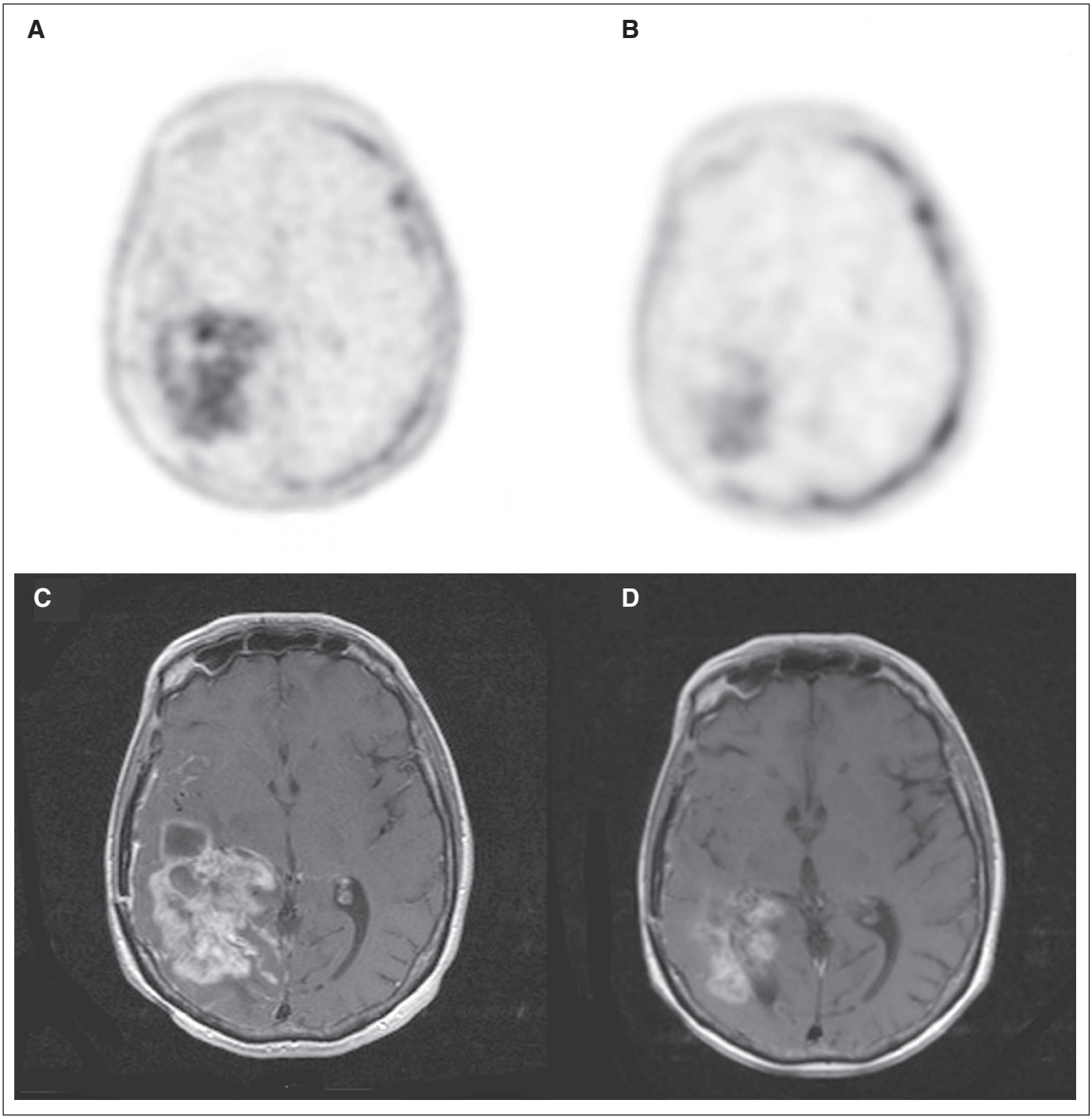

Fig 1. Patient (No. 8) classified by FLT-PET as responder at 1 week but stable disease by magnetic resonance imaging (MRI) at 6 weeks. Response by MRI occurred at 3 months. Upper panel shows [^{18}F] fluorothymidine (FLT) positron emission tomography (PET) slices (A) before and (B) 1 week after starting treatment. Lower panel shows MRI images (C) before and (D) 3 months after starting treatment.

uptake stratified the patient population into two subgroups; FLT responders (nine of 19, 43%) had a median survival of 10.8 months, whereas nonresponders (10 of 19, 57%) lived one third as long, with a median survival of only 3.4 months ($P = .003$). The majority (56%) of patients with metabolic response, but none (0%) of the nonresponders, were alive at the end of the study. Changes in FLT uptake were predictive as early as 1 to 2 weeks (average, 12 days) after starting treatment ($P = .006$). Conventional imaging criteria based on MRI changes that demonstrated a PR rate of 19% (four of 21) at 6 weeks after start of treatment and 38% (eight of 21) at time of best response relatively weakly predicted survival ($P = .060$ for both).

To our knowledge, this is the first published study demonstrating the ability of a PET imaging biomarker for predicting survival benefit in response to anti-VEGF therapy. No noninvasive

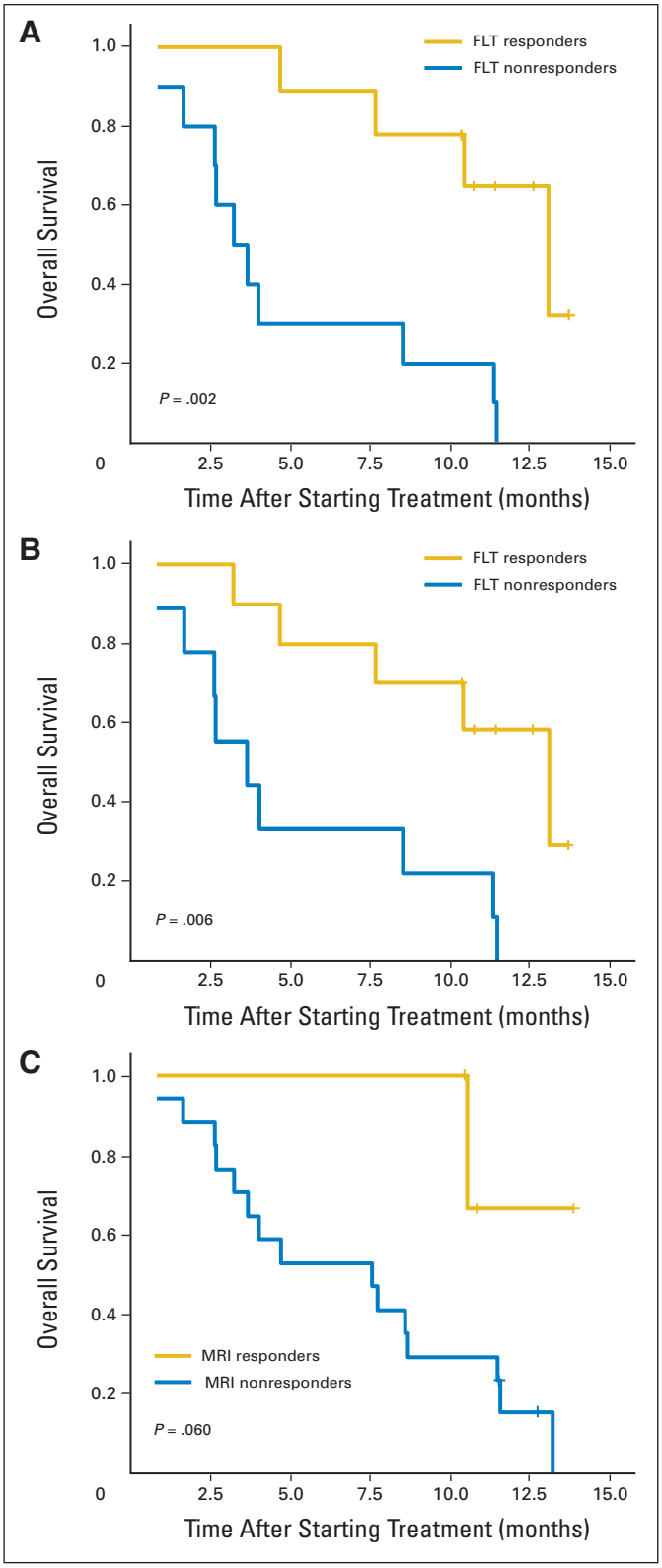

Fig 2. Overall survival by [^{18}F] fluorothymidine (FLT) positron emission tomography (PET) response at (A) 6 weeks and (B) 1 to 2 weeks. A significant difference between patients with and without metabolic response is observed ($P = .002$ at 6 weeks and $P = .006$ at 1 to 2 weeks). A trend is noted between patients with and without response by magnetic resonance imaging (MRI) at 6 weeks (C, $P = .060$).

biomarkers have yet been identified as being predictive of the outcome of bevacizumab treatment despite significant advances in identifying such candidate markers.[30] Biomarkers are needed to identify responsive patients, to optimize treatment doses, and to predict treatment efficacy.[31] Previous studies have demonstrated that, in rectal cancers, bevacizumab caused reduction in tumor perfusion, vascular volume, microvascular density, and interstitial fluid pressure as measured by functional computed tomography.[32-33] However, whether these changes during treatment correlate with clinical outcome is not yet known. Uptakes of FLT correlates with activity of thymidine kinase-1 (TK1), an enzyme expressed during the DNA synthesis phase of the cell cycle.[34] Phosphorylation of FLT intracellularly by TK1 results in trapping of the negatively charged FLT monophosphate.[35-36] FLT tumor uptake is correlated with Ki-67, a common proliferation index used ex vivo, as demonstrated in a number of extracranial cancers.[37-42] FLT uptake has also been investigated in brain tumors,[43-44] and similar correlations have been observed.[20,45,46] The current study suggests that early changes in FLT uptake could serve as a biomarker for predicting treatment effects in brain tumors, as has been recently reported for patients with breast cancer.[47]

A 6-month survival of 65% for glioblastoma patients was seen in our study. For a disease that historically has been generally associated with a bleak outcome, the high percentage of patients who showed a favorable response to treatment was remarkable. This favorable response argues against the hypothesis that bevacizumab-based chemotherapy in brain tumors only changes the permeability of the blood-brain barrier without a significant true antitumor effect. Multivariate analysis demonstrated that FLT response was the most powerful independent predictor of survival among all variables tested, including age, number of recurrences, number of prior treatments, tumor grade, dexamethasone treatment, and time from radiation therapy. The number of prior treatments was the only significant clinical variable with a lower HR (5.0 *v* 1.5). Although all of our patients had recurrent disease and had been treated previously with various regimens, the median recurrence was two, with 67% having two or fewer recurrences; the median number of prior treatment was two with 62% having two of fewer prior treatments. All patients were naïve to irinotecan, consistent with the recent report that, for patients with advanced colorectal cancer, adding bevacizumab to previously failed regimens did not lead to significant response or survival.[48]

In contrast to our previous study,[20] baseline FLT SUVs were not predictive of patient survival. Several explanations might account for this finding. First, our prior study included patients with low- and high-grade gliomas as well as stable patients in long-term remission, whereas our current population consisted of patients with recurrent high-grade gliomas only. Further, the prior study was a study of imaging characteristics and not a study of treatment response. Thus, this lack of baseline FLT SUV in predicting outcome in the current study might reflect the lack of potential interaction of baseline SUV and treatment response.

Through ROC curve analysis, a metabolic response of greater than 25% reduction in tumor FLT uptake was found to be the threshold with best predictive power for overall survival. This finding is consistent with literatures on criteria of metabolic

response.[26] Although those previous studies were performed in patients withextracranial tumors and with FDG-PET, the present study demonstrates that a metabolic response with FLT according to this definition also correlates with survival in patients with brain tumors.

This is a small exploratory study with limitations. For example, FLT-PET response at 6 weeks after starting treatment was a more significant predictor of survival than response at 1 to 2 weeks ($P = .002$ *v* .006), but this difference was attributable to a single patient (patient 18). A larger study is warranted to establish whether FLT-PET as early as 1 to 2 weeks after starting treatment is just as predictive of overall survival as the 6-week FLT-PET.

In conclusion, this prospective study demonstrated that bevacizumab and irinotecan treatment in patients with recurrent high-grade gliomas resulted in a significant survival benefit. Secondly, metabolic response is much more powerful in predicting overall survival than is response by anatomic imaging. Further, metabolic response as early as 1 to 2 weeks after starting treatment can be predictive. Because of difficulties in assessing response by conventional imaging modalities, the use of FLT-PET for prediction of response and survival appears promising and warrants validation with larger prospective series.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

AUTHOR CONTRIBUTIONS

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