Cancer Therapy: Clinical

Phase II Study of Bevacizumab, Temozolomide, and Hypofractionated Stereotactic Radiotherapy for Newly Diagnosed Glioblastoma

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Abstract

Purpose: Bevacizumab is associated with decreased vascular permeability that allows for more aggressive radiotherapy schedules. We conducted a phase II trial in newly diagnosed glioblastoma utilizing a novel hypofractionated stereotactic radiotherapy (HFSRT) schedule combined with temozolomide and bevacizumab.

Experimental Design: Patients with tumor volume $\leq 60$ cc were treated with HFSRT ($6 \times 6$ Gy to contrast enhancement and $6 \times 4$ Gy to FLAIR hyperintensity with dose painting) combined with concomitant/adjuvant temozolomide and bevacizumab at standard doses. Primary endpoint was 1-year overall survival (OS): promising $\equiv 70\%$; nonpromising $\equiv 50\%$; $\alpha = 0.1$; $\beta = 0.1$.

Results: Forty patients were enrolled (median age: 55 years; methylated MGMT promoter: 23%; unmethylated: 70%). The 1-year OS was 93% [95% confidence interval (CI), 84–100] and median OS was 19 months. The median PFS was 10 months, with no pseudo-progression observed. The objective response rate (ORR) was 57%. Analysis of The Cancer Genome Atlas glioblastoma transcriptional subclasses (Nanostring assay) suggested patients with a proneural phenotype (26%) fared worse (ORR $= 14\%$, vs. 77% for other subclasses; $P = 0.009$). Dynamic susceptibility-contrast perfusion MRI showed marked decreases in relative cerebral blood volume over time ($P < 0.0001$) but had no prognostic value, whereas higher baseline apparent diffusion coefficient (ADC) ratios and persistent hypermetabolism at the 6-month FDG-PET predicted poor OS ($P = 0.05$ and 0.0001, respectively). Quality-of-life (FACT-BR-4) and neuropsychological test scores were stable over time, although some domains displayed transient decreases following HFSRT.

Conclusions: This aggressive radiotherapy schedule was safe and more convenient for patients, achieving an OS that is comparable with historical controls. Analysis of advanced neuroimaging parameters suggests ADC and FDG-PET as potentially useful biomarkers, whereas tissue correlatives uncovered the poor prognosis associated with the proneural signature in non–IDH-1–mutated glioblastoma. Clin Cancer Res; 20(19); 5023–31. ©2014 AACR.
Introduction

Glioblastoma, the most common and aggressive malignant glioma, is characterized not only by a short survival (1–3), but also by a particularly poor quality-of-life (QoL), as brain function is progressively compromised by both disease burden and effects of treatments (4, 5); new therapeutic options are clearly needed.

The standard of care for newly diagnosed glioblastoma was established in a phase III study testing focal radiotherapy (60 Gy in 2 Gy fractions over 6 weeks) with concomitant and adjuvant temozolomide versus radiotherapy alone (1). In that study, the chemoradiotherapy arm achieved a median overall survival (OS) of 15 months, compared with 12 months for radiotherapy alone. A post hoc tissue analysis (6) suggested that tumors with promoter methylation of the DNA repair enzyme O6-methylguanine-DNA methyltransferase (MGMT) had a better prognosis; in the chemotherapy arm, the median OS was 22 months for methylated versus 13 months for unmethylated tumors.

In the present study, we sought to optimize this standard first line regimen through two innovative approaches: (i) utilization of an alternative radiotherapy schedule based on aggressive hypofractionation, made possible with the adoption of stereotactic technology and (ii) addition of bevacizumab to prevent symptomatic radionecrosis and associated brain edema through decreased vascular permeability resulting from VEGF blockade (7). Exploratory correlative studies consisted of (i) advanced neuroimaging, including dynamic susceptibility contrast (DSC) MR perfusion, diffusion MRI, and FDG-PET; (ii) tissue correlates, including analysis of The Cancer Genome Atlas (TCGA) transcriptional subclasses (8, 9) and expression of angiogenesis and hypoxia-related genes; and (iii) evaluation of QoL and neuropsychological testing throughout treatment.

Patients and Methods

Patients

Main inclusion criteria (Supplementary Data S1, online) included histologically confirmed newly diagnosed glioblastoma, tumor volume ≤ 60 cc (approximately 5 cm maximum diameter), and age ≥ 18. Exclusion criteria included multicentric disease and contraindication to the use of bevacizumab. Therapeutic anticoagulation (e.g., for venous thromboembolism) was allowed.

Treatment

The study treatment schema is shown in Fig. 1. Starting 4 to 6 weeks from surgery, the hypofractionated stereotactic radiotherapy (HFSRT) was delivered in six treatments over 2 weeks, typically on a Monday, Wednesday, Friday schedule, as detailed in Supplementary Data S2 (online). Areas with contrast enhancement received 6 × 6 Gy and areas with FLAIR hyperintensity received 6 × 4 Gy, with dose painting for homogeneous dose distribution.

Chemotherapy concomitant with HFSRT consisted of bevacizumab 10 mg/kg i.v. on days 1 and 15, and temozolomide 75 mg/m² given orally daily throughout HFSRT, for a total of 2 weeks. Supportive antiemetic therapy (e.g., ondansentron) and pneumocystis prophylaxis were recommended. Corticosteroids were given at the discretion of the treating physician.

Adjuvant chemotherapy started approximately 3 weeks after HFSRT, consisting of 28-day cycles of temozolomide given at conventional doses (150–200 mg/m²) on days 1 to 5, combined with bevacizumab 10 mg/kg on days 1 and 15. A total of six adjuvant cycles were mandated per protocol; treatment continuation beyond six cycles was left to the treating physician.

Figure 1. Study treatment schema and comparison with the European Organization for Research and Treatment of Cancer/National Cancer Institute of Canada (EORTC/NCIC) standard glioblastoma regimen (1). BEV, bevacizumab; RT, radiotherapy; TMZ, temozolomide.

Conclusion

The study treatment schema established in a randomized phase III trial (1) was adopted as the standard of care for newly diagnosed glioblastoma, both in the United States and internationally. The study schema was designed to optimize patient selection, and provided new insights into the biology of bevacizumab and hypofractionated radiotherapy in glioblastoma. Analysis of the glioblastoma transcriptional subclasses according to The Cancer Genome Atlas (TCGA) through the Nanostring assay showed that patients with a proneural phenotype achieved unexpectedly poor outcomes, suggesting that in the absence of IDH-1 mutation, this phenotype is a marker of poor, and not better prognosis as previously thought. Dynamic susceptibility-contrast perfusion MRI showed marked decreases in relative cerebral blood volume over time, suggesting a role as a biomarker of antiangiogenic effects. However, meaningful antitumor effects were better captured by imaging techniques based on cell proliferation and metabolism, such as apparent diffusion coefficient and FDG-PET, which were of prognostic value in this trial.

Translational Relevance

We describe a new hypofractionated radiotherapy schedule for newly diagnosed glioblastoma, made possible through antipermeability and corticosteroid-like effects of bevacizumab. The resulting treatment schedule (six treatments in 2 weeks) is more convenient than the standard 30 treatments in 6 to 7 weeks, and was associated with comparable survival. Comprehensive exploratory correlative studies were performed to optimize patient selection, and provided new insights into the biology of bevacizumab and hypofractionated radiotherapy in glioblastoma. Aggressive hypofractionation, made possible with the adoption of stereotactic technology and addition of bevacizumab to prevent symptomatic radionecrosis and associated brain edema through decreased vascular permeability resulting from VEGF blockade (7). Exploratory correlative studies consisted of (i) advanced neuroimaging, including dynamic susceptibility contrast (DSC) MR perfusion, diffusion MRI, and FDG-PET; (ii) tissue correlates, including analysis of The Cancer Genome Atlas (TCGA) transcriptional subclasses (8, 9) and expression of angiogenesis and hypoxia-related genes; and (iii) evaluation of QoL and neuropsychological testing throughout treatment.
treated physician’s discretion. Weekly CBCs and monthly comprehensive metabolic panel and urine/plasma creatinine ratio were obtained.

Advanced neuroimaging evaluation

In addition to standard MRI, all patients underwent DSC perfusion MRI at baseline, as well as postradiotherapy (preadjuvant chemotherapy), and every 2 months thereafter. Supplementary Data S3 (online) detail imaging processing and acquisition parameters (8–13). Commercially available software (FuncTools 2.6.9, GE Healthcare) was used to determine perfusion parameters such as relative cerebral blood volume (rCBV), relative peak height (rPH), and peak signal recovery (PSR), as well as apparent diffusion coefficient (ADC). In addition, a dedicated brain FDG-PET scan was obtained after six adjuvant cycles for characterization of disease activity and correlation with outcome.

Tissue correlates

As detailed in Supplementary Data S4, TCGA glioblastoma transcriptional subclasses (refs. 14 and 15; proneural, mesenchymal, classical, and neural) were assessed from mRNA extracted from formalin-fixed paraffin-embedded tissue obtained at initial surgery, using a gene expression profiling assay (Nanostring nCounter) based on 81 genes selected from the initial TCGA publication (14). Genes for which expression levels are known to directly reflect copy number alterations (Supplementary Data S4) were also added to the probe set, along with VEGF target genes (ANGPTL4, CA-9, EGLN-3, GLUT1, and PDK1).

In a separate analysis, tumor MGMT promoter methylation status was determined utilizing real-time methylation-specific PCR, as described previously (6). IDH1 R132H mutation status was determined by IHC (Dianova; 1:30).

Neuropsychological evaluation

Prospective neuropsychological evaluations (16–18) were performed in consenting, progression-free patients at baseline and 12, 8, and 12 months. Raw test scores were compared with published normative values according to age and education, and converted into z-scores; a z-score ≤ −1.5 represents impairment. Evaluated domains included Executive Function (Trail Making; Brief Test of Attention; Controlled Oral Word Association), Verbal Memory (Hopkins Verbal Learning Test-Revised), and Visual Memory (Brief Visuospatial Memory Test-Revised). QoL was evaluated with the Functional Assessment of Cancer Therapy-Brain Cancer v4 (FACT-BR-4). Fatigue was evaluated with the Functional Assessment of Chronic Illness Therapy-Fatigue Subscale 4 (FACT-FS) and depression with the Beck Depression Inventory II (BDI).

Statistical analysis

The primary endpoint was survival probability at one year (1y-OS). A single-stage binomial design was utilized, with 1y-OS of 70% considered promising and 50% nonpromising: α = 0.1; β = 0.1. Forty patients were to be enrolled; if ≥24 were alive at one year, the regimen would be considered worthy of further study. Secondary endpoints consisted of PFS, response rate (Macdonald; ref. 19; and RANO criteria; ref. 20), and toxicity profile (NCI-CTCAE v3). Exploratory endpoints included feasibility and preliminary evaluation of the prognostic value of advanced neuroimaging parameters and tissue-based biomarkers, as well as QoL and neuropsychological evaluation. Neuropsychological test scores were summarized using descriptive statistics, and longitudinal trajectories evaluated using linear mixed models (LMM) controlling for age and fatigue. Exact follow-up assessment times from baseline were calculated, and both linear and quadratic terms were estimated by the LMMs.

This investigation was performed after approval by the Institutional Review Board and in accordance with an assurance filed with and approved by the U.S. Department of Health and Human Services. Informed consent was obtained from each subject or subject’s guardian.

Results

Patient characteristics

Table 1 shows the characteristics of the 40 patients enrolled.

Treatment and toxicity

Treatment was generally well tolerated and followed the established toxicity profile of each drug (Supplementary Data S5, online). One patient discontinued bevacizumab because of thrombotic microangiopathy with irreversible grade 4 renal failure. One patient developed grade 4 surgical wound infection without dehiscence but was able to resume treatment. Two patients had grade 4 pulmonary embolism and one experienced a late ischemic stroke. One patient with a history of difficult to control seizures died suddenly during sleep, while on treatment; autopsy found no tumor hemorrhage or thromboembolic event. Central nervous system bleeding was reported in two patients, both asymptomatic: one grade 1 intratumoral hemorrhage and one experienced a late ischemic stroke. One patient had grade 4 pulmonary embolism and one experienced a late ischemic stroke. Central nervous system bleeding was reported in two patients, both asymptomatic: one grade 1 intratumoral hemorrhage and one grade 1 hemorrhage in a preexisting cavernoma, in a patient receiving concomitant full-dose anticoagulation.

A total of 11 patients elected to continue treatment beyond the required six adjuvant cycles. Those patients received a median of 12 cycles in total (range, 7–23 cycles).

Table 1. Patient characteristics (N = 40)

<table>
<thead>
<tr>
<th>Age</th>
<th>Median: 55 (range, 17–75)</th>
</tr>
</thead>
<tbody>
<tr>
<td>KPS</td>
<td>Median: 90 (range, 70–100)</td>
</tr>
<tr>
<td>Women</td>
<td>14 (35%)</td>
</tr>
<tr>
<td>Men</td>
<td>26 (65%)</td>
</tr>
<tr>
<td>Complete surgical resection</td>
<td>10 (25%)</td>
</tr>
<tr>
<td>Partial resection or biopsy</td>
<td>30 (75%)</td>
</tr>
<tr>
<td>MGMT promoter methylation</td>
<td></td>
</tr>
<tr>
<td>Methylated</td>
<td>9 (23%)</td>
</tr>
<tr>
<td>Unmethylated</td>
<td>28 (70%)</td>
</tr>
<tr>
<td>Unknown</td>
<td>3 (8%)</td>
</tr>
</tbody>
</table>
Efficacy

Thirty-seven patients were alive after one year [1-yr OS: 93%; 95% confidence interval (CI), 84–100], meeting the study’s primary endpoint (Fig. 2A). The median OS was 19 months (95% CI, 15–23). The median follow-up of survivors was 42 months. The objective response rates (ORR; complete + partial responses) were 87% (Macdonald) and 57% (RANO; Table 2). The median PFS was 10 months (95% CI, 8–11) and 1-year PFS = 28% (95% CI, 14–41); the results were identical using RANO criteria, with median PFS of 10 months (95% CI, 8–11) and 1-year PFS of 28% (95% CI, 14–41; Fig. 2B). Younger patients (age ≤50) survived longer (median 26 months) than patients aged >50 (median 17 months, P = 0.02; Fig. 2C). Patients undergoing gross
total resection achieved median OS of 26 months, as compared with 16 months in patients undergoing partial resection or biopsy ($P = 0.01$).

No patient developed worsening of symptoms or radiographic pseudoprogression within the first 4 months following radiotherapy. In patients free of progression, the Karnofsky Performance Status (KPS) was maintained throughout treatment, and the use of corticosteroids significantly decreased over time ($P < 0.0001$; Fig. 3B). The mean daily dexamethasone dose at baseline was 2.8 mg; following radiotherapy: 1.5 mg; at 6 months: 0.2 mg. Three patients thought to have tumor progression manifest by increased FLAIR hyperintensity without increased contrast enhancement underwent surgical resection; on pathology, tumor progression was confirmed in one patient, and two had mostly necrotic tissue.

The pattern of radiographic progression (21) was determined in 29 patients during the trial. Among those, 25 (86%) had local progression, defined as focus of enhancing or nonenhancing tumor at, or within 3 cm of the primary site resection cavity. Four patients (14%) had distant progression, defined as new single focus of enhancement or FLAIR, developing more than 3 cm from the primary site. No patient displayed the so-called diffuse pattern of progression (21) defined as recurrence centered or extending more than 3 cm from the primary site with poorly defined margins, or a multifocal pattern.

**Exploratory tissue correlates**

MGMT promoter methylation status could be determined in 37 patients, and was methylated in 23% and unmethylated in 70%. There was no association between MGMT status and response ($P = 1.0$), PFS ($P = 0.39$), or OS ($P = 0.56$; Fig. 2D).

The transcriptional glioblastoma subclass was determined in 31 patients (Table 3): Proneural: 26%; mesenchymal: 42%; classical: 29%; and neural: 3%. Patients with proneural tumors had lower response rate compared with other phenotypes ($P = 0.009$), and survived a median of 15 months, as compared with 21 months for other phenotypes ($P = 0.56$). There were no statistically significant differences in age distribution according to glioblastoma subclass or MGMT promoter methylation.

Among the 25 patients with sufficient tissue for IHC, none displayed the IDH-1 R132H mutation, including all of the evaluated proneural tumors.

The expression levels of angiogenesis and hypoxia-related genes had no prognostic value. Similarly, we found no correlations between inferred copy number alterations based on gene expression data and patient outcome.

**Exploratory imaging correlates**

Analysis of DSC MR perfusion and diffusion-weighted imaging (DWI) parameters is shown in Fig. 3A and Supplementary Data S6 (online). Initiation of treatment resulted in a progressive reduction in mean rCBV over time: mean rCBV was 3.4 at baseline, versus 2.3 after radiotherapy ($P < 0.0001$), 1.5 after 2 months, and 1.4 after 4 months.

Table 2. Response rates according to Macdonald and RANO criteria

<table>
<thead>
<tr>
<th></th>
<th>Macdonald</th>
<th>RANO</th>
</tr>
</thead>
<tbody>
<tr>
<td>N = 30*</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>CR</td>
<td>27%</td>
<td>12–46</td>
</tr>
<tr>
<td>PR</td>
<td>60%</td>
<td>41–77</td>
</tr>
<tr>
<td>SD</td>
<td>7%</td>
<td>1–22</td>
</tr>
<tr>
<td>PD</td>
<td>7%</td>
<td>1–22</td>
</tr>
<tr>
<td>ORR</td>
<td>87%</td>
<td>69–96</td>
</tr>
</tbody>
</table>

*N = 10 patients had no measurable disease postoperatively and accordingly were not evaluable for radiographic response.
However, DSC MR perfusion parameters such as baseline rCBV, change in rCBV after radiotherapy, rPH, and PSR did not predict PFS or OS. Conversely, lower baseline ADC was associated with prolonged OS (HR = 0.25; P = 0.05), but not PFS (RANO P = 0.13). The association between ADC ratios and OS was maintained even after patients with a complete resection were excluded (P = 0.03). There was no association between ADC parameters and response.

The presence of hypermetabolism on FDG-PET performed at the 6-month time point was associated with poor OS (P < 0.0001; Fig. 2E). Patients with tumors displaying hypermetabolism on the 6-month FDG-PET survived a median of 7 months from the date of the exam, as compared with 14 months in patients without hypermetabolism.

**Exploratory neurocognitive findings**

A total of N = 37 (93%) patients agreed to undergo neuropsychological evaluations (Supplementary Data S7, online). At baseline, cognitive test z-scores were in the low average to average range on most tests. The LMMs showed significant linear improvement in verbal fluency over time (COWA; P = 0.001). There were no changes in graphomotor speed (TMTA), attention (BTA), or visuospatial delayed recall (BVMT-R-DEL). After decreasing at the 4-month assessment, verbal learning (HVLT-R-TL) and verbal recognition memory (HVLT-R-DI) scores improved on subsequent evaluations to baseline levels or higher (quadratic time P = 0.01 and 0.08, respectively). Cognitive flexibility scores (TMTB) decreased at the 4-month assessment (linear time P = 0.023) before somewhat stabilizing (quadratic time P = 0.07) below baseline levels at the 8 and 12-month assessments. Visuospatial learning (BVMT-R-TL) was stable at 4-months, with a possible indication of decline at subsequent follow-ups (quadratic time P = 0.08). There were no significant changes in mood, self-reported QoL, or fatigue across the study period.

**Discussion**

In this phase II trial, patients with newly diagnosed glioblastoma were treated with a hypofractionated radiotherapy schedule in combination with bevacizumab and temozolomide, and achieved a 1y-OS of 93%, median OS of 19 months, and median PFS of 10 months. As compared with standard chemoradiotherapy, this regimen constituted a more convenient treatment option for patients, given the decreased use of corticosteroids and a notably shorter radiotherapy schedule, consisting of six treatments within 2 weeks, instead of the standard 30 treatments delivered daily over 6 to 7 weeks.

The value of adding bevacizumab to standard chemoradiotherapy for newly diagnosed glioblastoma is currently a matter of debate. Prospective phase II studies testing this combination reported a median PFS of 10 to 14 months and median OS of 19 to 21 months (22, 23). More recently, preliminary results of two phase III trials examining standard chemoradiotherapy with or without bevacizumab, AVAGLIO (24), and RTOG 0825 (25), suggested that adding bevacizumab resulted in improvements in PFS (AVA-GLIO: 11 vs. 6 months; RTOG 0825: 11 vs. 7 months), but not in OS (AVA-GLIO: 17 months in both arms; RTOG 0825: 16 months in both arms).

Although meaningful antitumor effects and survival benefit with anti-VEGF therapies remain to be demonstrated in glioblastoma, the effects on vascular permeability are unquestionable, as demonstrated by rapid reductions in contrast enhancement and peritumoral edema following treatment initiation (26). This "corticosteroid-like" effect made possible the hypofractionated schedule used in this trial, designed to produce radiobiological effects comparable with the standard regimen of 60 Gy/1.8-2 Gy fractions. Advances in radiotherapy techniques, particularly IMRT and stereotactic technology, have allowed for remarkable improvements in spatial targeting, but the development of hypofractionated schedules has been limited (27). In addition to convenience, the biologic rationale for hypofractionation is based on potentially enhanced direct cell kill and reduction in the accelerated tumor cell repopulation (28), although late-responding neural tissue injury and toxicity may increase. To date, the best studied use of hypofractionated or abbreviated radiotherapy schedules has been in poor prognosis populations, such as the elderly or poor performance status patients, who have been treated with biologic doses that are equivalent to, or lower than standard schedules. Such studies have found median OS of 5 to 12

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**Table 3. Distribution of transcriptional glioblastoma subclasses and corresponding age, response rate (RANO), and OS**

<table>
<thead>
<tr>
<th>Class</th>
<th>N (%)</th>
<th>Median age</th>
<th>ORR by RANO (95% CI)</th>
<th>Median OS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proneural</td>
<td>8 (26%)</td>
<td>62</td>
<td>14%* (9%–53%)</td>
<td>15 m*</td>
</tr>
<tr>
<td>Mesenchymal</td>
<td>13 (42%)</td>
<td>55</td>
<td>70% (35%–83%)</td>
<td>20 m</td>
</tr>
<tr>
<td>Classical</td>
<td>9 (29%)</td>
<td>58</td>
<td>83% (36%–100%)</td>
<td>22 m</td>
</tr>
<tr>
<td>Neural</td>
<td>1 (3%)</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>All patients with evaluable tissue</td>
<td>31</td>
<td>57</td>
<td>58% (37%–78%)</td>
<td>20 m</td>
</tr>
</tbody>
</table>

Abbreviation: N/A, not applicable (only one patient presented with this phenotype).

*aP = 0.009 (Fisher exact test, proneural vs. others).

*bP = 0.56 (log-rank test, proneural vs. others).
months (27, 29–33), which in small randomized trials (29, 31) did not appear inferior to standard schedules, although noninferiority trial designs have not been used. Also under investigation is the use of hypofractionation and IMRT in escalation studies to deliver higher tumor biologic effective doses, although an increased corticosteroid requirement and radionecrosis are major deterrents to the wide application of such schedules (27, 34–38). In our study, the addition of bevacizumab seems to have offset the development of symptoms and radiographic signs of radionecrosis typically associated with hypofractionation, decreasing the need for corticosteroids. Interestingly, pathology in some of our reoperated patients showed signs of significant tissue damage; it is possible that other patients thought to have nonenhancing tumor progression in fact had nonenhancing treatment effects. It must also be noted that results of our exploratory neuropsychological testing showed some transient radiotherapy effects on neurocognitive functions, which seemed less marked in comparison with standard fractionation schedules (39), although definitive conclusions are limited by our small patient number, lack of a control arm, and confounding effects of bevacizumab. Analysis of RTOG 0825 (25) suggested that the addition of bevacizumab resulted in more frequent cognitive decline as compared with standard chemoradiotherapy, although interpretation of such findings is limited by the fact that progressing patients were not evaluated, and patients remained longer on study in the bevacizumab arm, which implies that the cognitive decline could represent unrecognized tumor progression, rather than direct bevacizumab effects.

The survival curve in our trial followed the typical pattern observed in bevacizumab glioblastoma studies, with a decrease in early death rates, but no improvement in later survival endpoints in comparison with contemporary phase II historical controls, which have reported median OS of 18 to 19 months (40). Therefore, identifying imaging and tissue-based biomarkers and subgroups of patients who benefit from, or who are harmed by, this type of treatment remains crucial (41). We examined bevacizumab effects on tumor blood perfusion as measured by DSC-MRI, and found a nearly universal decrease in blood perfusion over time, demonstrating that the intended targeting of angiogenesis was accomplished. However, for prognostic purposes, diffusion-based parameters were more helpful. Tumors with the lowest ADCs seemed to benefit most perhaps representing highly hypoxic, or densely cell-packed phenotypes, therefore constituting optimal candidates for antiangiogenic treatments (42). Interestingly, we found the FDG-PET at the 6-month time point to be a reliable marker of treatment failure (Fig. 2E), even when other imaging modalities suggested stability. Taken together, our findings support the use of imaging techniques based on cell proliferation and metabolism, rather than perfusion and vasculature imaging, to guide future development of antiangiogenic therapies.

Finally, we evaluated a range of potential tissue-based biomarkers implicated in glioblastoma and angiogenesis. A previous study in recurrent malignant gliomas suggested that high VEGF expression as determined by IHC was associated with increased response to bevacizumab but did not predict survival, whereas expression of CA9 was associated with poor survival outcome (41). In our study, we focused on applying the TCGA-based transcriptional classification, under the hypothesis that the marked differences in oncogenic and angiogenic drivers across distinct expression signatures might translate into differential responses to anti-VEGF therapy. Unexpectedly, results suggested that proneural glioblastomas were associated with worse outcomes as compared with other subclasses. Proneural glioblastomas had previously been associated with dysregulated PDGF signaling (14, 43, 44) and a relatively favorable prognosis (43), in sharp contrast with our findings. Subsequent work, however, suggested that the majority, if not the entirety, of this prognostic advantage derived from the enrichment of the proneural subclass with IDH-mutant tumors, a well-known predictor of prolonged survival (14, 15). None of the proneural tumors in our study expressed mutant IDH-1, which may explain their poor outcome in this group. On the basis of our results, a post hoc analysis of tissue collected in the AVAGLIO study has been initiated, utilizing our Nanostring-based methodology (45). Preliminary results showed that proneural glioblastomas without IDH-1 mutation were associated with a poor prognosis in the placebo arm, confirming our finding that this molecular signature is associated with a poor prognosis, and suggesting this is not associated with a detrimental effect of bevacizumab in these patients. In fact, additional analysis suggested that adding bevacizumab may actually improve survival in these poor prognosis patients, a finding that will require further validation in larger, prospective studies. Interestingly, in our trial, patients with mesenchymal tumors benefitted from treatment and did not fare worse than other subclasses, which is consistent with their high VEGF expression. This finding contrasts with a preliminary analysis of the RTOG 0825 study, which found that some mesenchymal-associated genes conferred a worse prognosis in patients exposed to bevacizumab (46). Further studies are needed to determine whether this discrepancy is explained by our small number of patients, differing tissue study techniques, or to an effect unique to the hypofractionated radiotherapy schedule utilized in this study.

Our study has a number of limitations. Results are applicable to unifocal tumors, with a volume of 60 cc or less. However, for the most part, our population of patients had prognostic factors comparable with historical controls (40). Many trials used as historical controls have also excluded multifocal tumors (2, 25) and larger tumors are also often excluded because of poor KPS. Another limitation is that a baseline FDG-PET was not obtained, and therefore it is not possible to determine whether the hypermetabolism observed at the 6 month was already present at the time of treatment start, or if developed throughout treatment. Finally, our correlative studies and neuropsychological testing are limited by small patient numbers, and few long-term
survivors. Our analyses should be regarded as exploratory and hypothesis-generating: further investigations in larger, randomized studies will be necessary to validate our findings and determine whether potential biomarkers are of a predictive or prognostic value.

In summary, we describe a new use for bevacizumab in newly diagnosed glioblastoma, capitalizing on the anti-permeability effects to develop a convenient hypofractionated radiotherapy schedule. In our hands, this regimen was found to be safe, associated with minimal detrimental effects on QoL, and with survival results comparable with other regimens. Our prospective correlative studies provide new insights into the biology of bevacizumab and hypofractionated radiotherapy in glioblastoma, and uncover candidate imaging and tissue-based biomarkers with either prognostic or predictive value that warrant further investigation in adequately powered randomized studies.

Disclosure of Potential Conflicts of Interest

A.M.P. Omuro is a consultant/advisory board member for CarThera, Novocure, and Roche. A. Lassman is a consultant/advisory board member for Amgen, Abbott, Agenus, Celgene, Genentech, Kyowa Hakko Kirin Pharma, Mediatech, Novartis, RadMD, Roche, Sciente, Sigma Tau, Stemline, Synapse, and Venture Infections, and reports receiving lecture fees from American Physician Institute for Advanced Professional Studies, Merck Sharp & Dohme, and OmniPlex America. No potential conflicts of interest were disclosed by the other authors.

References


Authors’ Contributions


Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): A.M.P. Omuro, S. Karimi, L.M. DeAngelis, T.A. Chan, R. Barradas-Panchal, J. Zhang, G. Fadare, L.E. Abrey, J.T. Huse


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Hypofractionated Radiotherapy, Temozolomide, and Bevacizumab for GBM


Phase II Study of Bevacizumab, Temozolomide, and Hypofractionated Stereotactic Radiotherapy for Newly Diagnosed Glioblastoma

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