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Phase II and pharmacogenomics study of enzastaurin plus temozolomide during and following radiation therapy in patients with newly diagnosed glioblastoma multiforme and gliosarcoma

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This open-label, single-arm, phase II study combined enzastaurin with temozolomide plus radiation therapy (RT) to treat glioblastoma multiforme (GBM) and gliosarcoma. Adults with newly diagnosed disease and Karnofsky performance status (KPS) ≥ 60 were enrolled. Treatment was started within 5 weeks after surgical diagnosis. RT consisted of 60 Gy over 6 weeks. Temozolomide was given at 75 mg/m^2 daily during RT and then adjuvantly at 200 mg/m^2 daily for 5 days, followed by a 23-day break. Enzastaurin was given once daily during RT and in the adjuvant period at 250 mg/day. Cycles were 28 days. The primary end point was overall survival (OS). Progression-free survival (PFS), toxicity, and correlations between efficacy and molecular markers analyzed from tumor tissue samples were also evaluated. A prospectively planned analysis compared OS and PFS of the current trial with outcomes from 3 historical phase II trials that combined novel agents with temozolomide plus RT in patients with GBM or gliosarcoma. Sixty-six patients were enrolled. The treatment regimen was well tolerated. OS (median, 74 weeks) and PFS (median, 36 weeks) results from the current trial were comparable to those from a prior phase II study using erlotininb and were significantly better than those from 2 other previous studies that used thalidomide or cis-retinoic acid, all in combination with temozolomide plus RT. A positive correlation between O-6-methylguanine-DNA methyltransferase promoter methylation and OS was observed. Adjusting for age and KPS, no other biomarker was associated with survival outcome. Correlation of relevant biomarkers with OS may be useful in future trials.

Keywords: adjuvant therapy, enzastaurin, glioblastoma multiforme, radiation therapy, temozolomide.

The standard of care for newly diagnosed glioblastoma multiforme (GBM) or gliosarcoma includes surgical resection, followed by radiation therapy (RT) with concurrent temozolomide, an alkylating agent, and by adjuvant temozolomide. In a definitive phase III trial, patients treated with the temozolomide plus RT regimen had significantly improved overall survival (OS), compared with patients who received RT alone. However, the 2-year survival rate for patients treated with temozolomide plus RT was only 26.5%.[1] Therefore, additional therapeutic strategies are needed

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to improve outcomes for patients with GBM and gliosarcoma.

Molecular studies have identified numerous genetic alterations associated with initiation and progression of brain cancer that may serve as targets for molecular therapies to further improve patient survival.[2,3] Enzastaurin is an example of a targeted agent that is a selective serine/threonine kinase inhibitor of protein kinase C (PKC). Enzastaurin disrupts the phosphotransferase activity of PKC isoforms via an interaction at the ATP binding site and displays selectivity in inhibiting the beta isoform.[4] The PKC family of enzymes is essential to tumor growth, proliferation, and apoptosis.[5,6] The beta isoform of PKC also lies in the signal cascade of vascular endothelial growth factor (VEGF) that is upregulated in GBM concomitant with overexpression of VEGF receptor;[7,8] inhibition of this pathway by enzastaurin blocks tumor angiogenesis and growth.[9] PKC activity is also thought to regulate AKT, a protein that has antiapoptotic effects and is involved in GBM proliferation.[10–12] Thus, inhibition of the AKT pathway by enzastaurin may lead to decreased cell growth and increased cell death.

Preclinical studies have shown the antiproliferative and antiangiogenic activity of enzastaurin in tumor models, including glioma.[9,13] Enzastaurin has also been shown to enhance the efficacy of RT by preventing unwanted proinvasive and angiogenic effects and to enhance temozolomide-induced cell death in GBM cell lines.[14] Clinical studies in healthy volunteers and patients with solid tumors show that enzastaurin is well tolerated at doses that achieved a biologically active serum concentration.[15–17] Results from a phase II study of patients with recurrent high-grade gliomas demonstrated that enzastaurin was well tolerated, with possible antitumor activity, although not robust.[18] In addition, we previously reported on a phase I trial of enzastaurin given with temozolomide plus RT that also showed that the combination was well tolerated, and the results of that study formed the basis for dosing schedules tested in the current study.[19] On the basis of these promising data, we conducted a phase II study (designated ETRT) to determine the efficacy of enzastaurin in patients with newly diagnosed GBM or gliosarcoma who also received concomitant temozolomide and RT. Of note, the current study was developed and opened before the release of the results of a phase III study of enzastaurin, compared with lomustine, in the treatment of recurrent GBM; the phase III study reported that enzastaurin was well tolerated and had a better hematologic toxicity profile but did not have superior efficacy, compared with lomustine.

Materials and Methods

Patient Eligibility

Patients were at least 18 years of age with a histologically confirmed, newly diagnosed GBM or gliosarcoma. Biopsy or resection must have been performed $\leq$5 weeks before treatment. An MRI or CT scan of the brain was obtained after $\leq$14 days of treatment. Patients were required to have a Karnofsky performance status (KPS) score of $\geq$60 and an estimated survival time of $>$8 weeks. Patients were also required to have appropriate hematological, renal, and hepatic status. All patients were willing to practice birth control during and for 3 months after treatment.

Patients were excluded if they had prior cranial RT or any prior chemotherapy or Gliadel wafers for their brain tumors. Patients were not allowed use of enzyme-inducing antiepileptic drugs (EIAEDs). For patients previously treated with EIAEDs, use of the agent had to be stopped at least 2 weeks before treatment. Patients were excluded if they had a history of any other cancer (except nonmelanoma skin cancer or carcinoma in situ of the cervix), unless the cancer was in complete remission and the patient had not received any therapy for that disease for a minimum of 3 years. Patients receiving any anticoagulant therapy were ineligible. However, if a patient required anticoagulant therapy after starting treatment, the patient continued to receive study therapy but was monitored carefully. Patients with an electrocardiogram demonstrating clinically significant arrhythmia that was symptomatic or required treatment were excluded. Patients were also excluded if they were unable to swallow pills.

Study Design and Treatment Plan

This was a single–arm, open–label, phase II study of enzastaurin administered with temozolomide during and after RT for patients with newly diagnosed GBM or gliosarcoma. The current trial (and its phase I) was conducted at a single institution—the University of California, San Francisco. This study complied with the principles of Good Clinical Practice and the Declaration of Helsinki. An institutional review board approved the protocol, and all patients or their designated surrogates provided written informed consent before enrollment.

Treatment was started within 5 weeks after surgical diagnosis. The primary objective of the study was to determine the efficacy of enzastaurin in combination with temozolomide plus RT in patients with newly diagnosed GBM or gliosarcoma as measured by OS. Secondary objectives included the evaluation of progression-free survival (PFS), safety, and biomarkers relevant to enzastaurin and disease state and their correlation to clinical outcome.

RT was administered at a dose of $\sim$2.0 Gy/day for 5 days per week to a total dose of 60 Gy over a 6-week course. During RT, all patients received daily enzastaurin (250 mg/day) and daily temozolomide (75 mg/m^2/day). Two to 3 weeks after the completion of RT, patients were treated with temozolomide (200 mg/m^2/day) for 5 days of a 28-day cycle. On the basis of the results of our previous phase I trial,[19] all patients received continuous daily enzastaurin at 250 mg/day. Dose adjustments of enzastaurin were not allowed. Hematologic and liver toxicities were used as criteria

for dose adjustments of temozolomide. Planned adjuvant chemotherapy treatment was up to 12 cycles (or up to 12 months) unless disease progression or unacceptable toxicity occurred; additional treatment beyond 12 months was allowed at the discretion of the treating physician, assuming no significant toxicity, and with the consent of the patient.

Patient Evaluations

Pretreatment evaluation included a complete history and physical and neurologic examination. Prestudy laboratory tests, obtained within 14 days after treatment, included a complete blood count (CBC) with differential and serum creatinine, total bilirubin, aspartate transaminase, alanine aminotransferase, alkaline phosphatase, blood urea nitrogen, glucose, potassium, sodium, and anticonvulsant levels (if applicable); serum pregnancy tests were performed for women of childbearing potential. Pathology slides from the most recent surgical material were submitted for retrospective pathology review to confirm the diagnosis and to evaluate molecular abnormalities in the tumor.

During RT, a CBC and differential were performed every 2 weeks, then at weeks 3 and 4 after the start of each 28-day temozolomide cycle. Blood chemistry tests were performed every 4 weeks. Safety evaluations were performed using the Common Terminology Criteria for Adverse Events (CTCAE, version 3).

Brain MRI was performed at baseline ($\leq$14 days before the start of treatment), 2–3 weeks after the completion of RT, and then every 8 weeks while patients were receiving treatment. The Macdonald Criteria were used to evaluate radiologic progression.[20]

All patients were observed for OS. Patients who experienced disease progression were observed for survival every 3 months.

Pharmacogenomics

Genomic DNA was isolated from formalin–fixed, paraffin–embedded tumor tissue or from blood samples with use of standard techniques and was subjected to bisulfite treatment, as described elsewhere.[21,22] The O-6-methylguanine-DNA methyltransferase (MGMT) methylation-specific polymerase chain reactions (MSPs) were performed using a 2-step approach, and the results were confirmed by a 1-step MSP in a subset of tumors.[23–26] Fetal brain tissue was used to generate positive and negative controls for the MSP with native fetal brain DNA positive for the unmethylated polymerase chain reaction (PCR) product and SssI-treated in vitro methylated fetal brain DNA serving as a positive control for the methylated MGMT PCR product. The PCR products were resolved on 4% agarose gels. Analysis of MSP data was performed by investigators who were blinded to the clinical data.

Immunohistochemistry (IHC) was used to evaluate molecular markers, including epidermal growth factor receptor (EGFR), phosphatase and tensin homolog (PTEN), phosphorylated–S6 ribosomal protein (S6) using the 2211 and 2215 antibodies, glycogen synthase kinase 3 beta (GSK3β), phosphorylated cAMP response element-binding protein (PCREB), VEGF, and mitogen–activated protein kinase (MAPK). The IHC assays were scored using a 0 to +3 scoring system. No positive staining was scored 0; at least 25% immunoreactivity of cells was scored +1; 26%–75% was scored +2; and $\geq$76% was scored +3. Results were analyzed using the actual IHC score, and the level of positivity was included as part of the assessment. Thus, any level of positivity was considered to be positive, but the range was taken into account.

Statistical Plan

The initial calculation of sample size was based on the goal of increasing survival compared with the historical median survival time of ~15 months.[1] Enrollment of 60 patients was estimated to provide 86% power with use of a 1-sided α of 0.1 for this comparison of the median survival using a parametric model, assuming a hazard ratio of 1.5 (historical/experimental). As described in the protocol, we recognized that a smaller hazard ratio might still be of interest, and thus, an analysis was planned using historical control information from prior University of California, San Francisco studies to estimate the potential therapeutic benefit, adjusting for age, sex, and extent of resection. Enrollment was expected to occur over a 12–15-month period, with follow-up for all patients of at least 15 months. Six patients treated using the same dosing regimen during the initial phase I trial were included for a total of 66 patients. If the discontinuation rate because of toxicity was $\geq$20% and the lower bound for the 1-tailed 95% confidence interval (CI) was >10%, the treatment strategy would not have been considered to be feasible and the study would be stopped.

Efficacy and safety analyses were conducted on the full analysis set, which included data from all patients who received at least 1 dose of the study drug. OS and PFS were estimated using the Kaplan-Meier method.[27] OS was defined as the time from the date of study registration to date of death from any cause. PFS was defined as the time from the date of study registration to the date of first observed progressive disease based on radiologic assessment, nonreversible neurologic progression, permanently increased corticosteroid requirement, or death from any cause, whichever came first. The comparative analysis of OS and PFS used a Cox proportional hazards model that included age, KPS, and extent of resection. For all efficacy analyses, 95% CIs were to be included with all point estimates.

For the final analysis, survival data were compared with results from 3 earlier prospective phase II clinical trials that treated similar patient populations with other chemotherapy agents in combination with temozolomide during and after RT. All trials were conducted at the University of California, San Francisco. The first study (TTRT) used thalidomide with temozolomide

Table 1. Baseline patient characteristics by study protocol

Parameter	ETRT	RTRT	OTRT	TTRT
Patients, *n*	66	61	65	67
Age, median years (range)	57 (25–80)	54 (28–74)	55 (22–77)	51 (22–77)
KPS, median (range)	90 (60–100)	90 (60–100)	90 (60–100)	90 (60–100)
Biopsy, *n* (%)	10 (15)	10 (16)	8 (13)	13 (19)
Subtotal resection, *n* (%)	39 (59)	34 (56)	31 (48)	33 (49)
Gross total resection, *n* (%)	17 (26)	17 (28)	25 (39)	21 (31)
MGMT methylated, *n* (%)	13 (20)	N/A	16 (25)	N/A
MGMT unmethylated, *n* (%)	30 (45)	N/A	28 (43)	N/A
Methylation unknown, *n* (%)	23 (35)	N/A	21 (32)	N/A

ETRT — Current trial.
TTRT — Phase II study of temozolomide and thalidomide with radiation therapy for newly diagnosed glioblastoma multiforme.[28]
RTRT — A phase II study of concurrent temozolomide and cis-retinoic acid with radiation for adult patients with newly diagnosed supratentorial glioblastoma.[29]
OTRT — Phase II study of erlotinib plus temozolomide during and after radiation therapy in patients with newly diagnosed glioblastoma multiforme or gliosarcoma.[30]
Abbreviations: *MGMT*, O-6-methylguanine-DNA methyltransferase; *n*, number of patients.

plus RT;[28] the second study (RTRT) used cis-retinoic acid with temozolomide plus RT;[29] the third study (OTRT) used erlotinib with temozolomide plus RT.[30]

An exploratory analysis of biologic correlative data using Cox proportional hazard models was performed to increase our knowledge of how the potential benefit of enzastaurin might be influenced by genetic characteristics of the tumor.

Results

Patient Characteristics

A total of 66 eligible patients were registered from September 2006 through October 2008. Table 1 shows the baseline characteristics for the current trial (ETRT) and for the other historical control groups. Median age for ETRT was 57 years; median KPS was 90 (range, 60–100); median survival follow-up for 19 censored patients at the time of analysis was 103 weeks (range, 70–175 weeks). The historical control group included 193 patients treated in the 3 prior phase II studies. Median age for the combined historical control group was 54 years; median KPS was 90 (range, 60–100). For the historical group, 14 patients were censored with median survival follow-up time of 234 weeks (range, 129–279 weeks). The extent of surgical resection was similar among the current and historical groups.

Efficacy and Toxicity

Efficacy results are shown in Fig. 1 and Tables 2 and 3. In the current study (ETRT), median OS was 74 weeks (95% CI, 62–83 weeks) and median PFS was 36 weeks (95% CI, 30–49 weeks). Under the assumption of an exponential distribution and using the approximate normality of the estimated hazard, the observed OS was improved, compared with the historical 15-month value. Multivariate analysis correcting for age, KPS, and extent of resection showed that the PFS and OS results of the current study were significantly greater, compared with those of both RTRT and TTRT historical trials (see Table 3). However, there was no significant difference for PFS and OS results comparing ETRT with the OTRT trial.

Toxicity for the 66 patients who received at least 1 dose of enzastaurin (250 mg) was modest and tolerable. Forty-two patients discontinued treatment because of disease progression. Six patients (9.1%) discontinued because of an adverse event (AE), including 2 patients as a result of low platelet counts. Sixty-six patients experienced at least 1 treatment-emergent AE. Forty-four patients (66.7%) experienced at least 1 CTCAE grade 3–4 treatment-emergent AE, and 37 patients (56.1%) experienced a CTCAE grade 3–4 CTCAE that was considered by the investigator to be possibly related to study drug. The most common grade 3/4 CTCAE was lymphopenia (26 [39.4%] regardless of causality and 25 [37.9%] possibly related to study drug). Three (4.5%) and 4 (6.1%) grade 3/4 CTCAEs of platelet count decrease and platelet counts (unspecified) were reported, respectively. Twenty-two patients (33.3%) experienced a serious AE, and 9 (13.6%) patients experienced a serious AE that was considered to be possibly related to study drug, including 1 case of thrombocytopenia. The 2 most common serious AEs classified as possibly related to study drug were pneumonia (3 [4.5%]; none were attributed to pneumocystis carinii) and urinary tract infection (2 [3.0%]). No deaths were reported during therapy, and 5 were reported within 30 days after therapy discontinuation, all because of tumor progression.

Molecular Analyses

Molecular marker analyses were possible for most patient samples, although assured conclusions are obviously limited by the study size (MGMT; $n = 43$; EGFR, $n = 51$; PTEN, $n = 49$; S6 analyzed with 2211

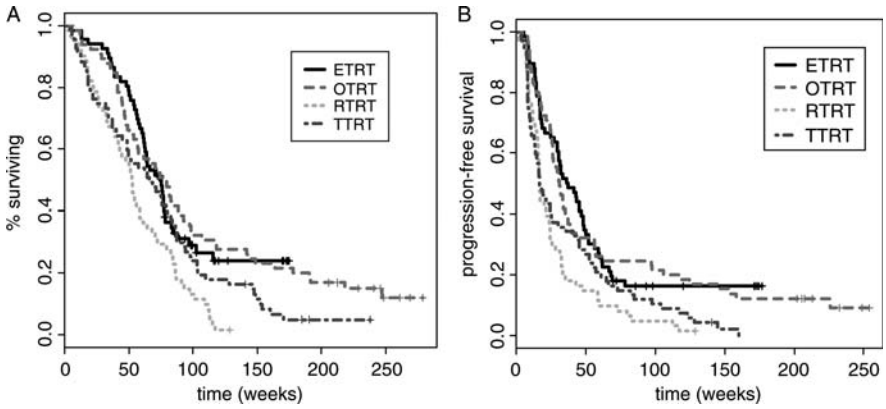

Fig. 1. Kaplan-Meier analyses of overall and progression-free survival by study protocol. (A) Overall survival. For historical controls, 14 patients were censored with a median survival follow-up of 234 weeks (range: 129–279 weeks). For ETRT, 19 patients were censored with median survival follow-up of 103 weeks (range: 70–175 weeks). (B) Progression-free survival. For historical controls ($n = 193$), 9 patients were censored with median survival follow-up of 207 weeks (range: 129–254 weeks). For ETRT, 11 patients were censored with median survival follow-up of 120 weeks (range: 70–177 weeks). ETRT — Current trial. TTRT — Phase II study of temozolomide and thalidomide with radiation therapy for newly diagnosed glioblastoma multiforme.[28] RTRT — A phase II study of concurrent temozolomide and cis-retinoic acid with radiation for adult patients with newly diagnosed supratentorial glioblastoma.[29] OTRT — Phase II study of erlotinib plus temozolomide during and after radiation therapy in patients with newly diagnosed glioblastoma multiforme or gliosarcoma.[30] Abbreviation: *n*, number of patients.

Table 2. Overall and progression-free survival by study protocol

Outcome	ETRT	RTRT	OTRT	TTRT
Overall survival[a]				
Patients, *n* (*n*, censored)	66 (19)	61 (1)	65 (9)	67 (4)
% surviving (95% CI)[b]				
26 weeks (6 months)	94 (88–100)	75 (65–87)	92 (86–99)	75 (65–86)
39 weeks (9 months)	83 (75–93)	66 (55–79)	85 (76–94)	67 (57–79)
52 weeks (12 months)	76 (66–87)	52 (41–67)	65 (54–77)	55 (45–69)
65 weeks (15 months)	55 (44–68)	34 (24–49)	57 (46–70)	51 (40–64)
Median survival in weeks (95% CI)	74 (62–83)	52 (42–64)	80 (57–97)	66 (49–84)
Progression-free survival[a]				
Patients, *n* (*n*, censored)	66 (11)	61 (1)	65 (7)	67 (1)
% PFS (95% CI)[b]				
13 weeks (3 months)	88 (80–96)	70 (60–83)	82 (73–92)	64 (54–77)
26 weeks (6 months)	65 (55–78)	30 (20–44)	60 (49–73)	37 (27–51)
39 weeks (9 months)	49 (38–62)	18 (11–31)	35 (25–49)	34 (25–48)
Median PFS in weeks (95% CI)	36 (30–49)	17 (16–24)	31 (26–39)	17 (14–30)

[a]Estimated from Kaplan-Meier curves.
[b]Measured from time of study registration.
ETRT — Current trial.
TTRT — Phase II study of temozolomide and thalidomide with radiation therapy for newly diagnosed glioblastoma multiforme.[28]
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OTRT — Phase II study of erlotinib plus temozolomide during and after radiation therapy in patients with newly diagnosed glioblastoma multiforme or gliosarcoma.[30]
Abbreviations: CI, confidence interval; *n*, number of patients; PFS, progression-free survival.

antibody, $n = 49$; S6 analyzed with 2215 antibody, $n = 49$; VEGF, $n = 47$; GSK3β, $n = 49$; PCREB, $n = 48$; and MAPK, $n = 49$). Not all patients had available paraffin blocks, and in some cases, there was not enough tissue remaining to accomplish each of the correlative studies. Figure 2 and Table 4 show the results of the MSP and IHC assays. After controlling for patient age and KPS, promoter methylation of MGMT had the strongest correlation with both OS and PFS; unmethylated MGMT was associated with increased hazard of earlier death (hazard ratio [HR] = 5.19; 95% CI, 1.88–14.31; $P = .001$) and with increased hazard of earlier progression (HR = 3.07; 95% CI, 1.31–7.23; $P = .01$).

Table 3. Multivariate cox proportional hazard analysis of overall and progression-free survival

Parameter	Overall Survival		Progression-Free Survival	
	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
vs ETRT				
OTRT	0.98 (0.66–1.47)	.93	0.99 (0.68–1.44)	.94
RTRT	2.09 (1.41–3.09)	.0002	2.12 (1.46–3.07)	<.001
TTRT	1.51 (1.02–2.24)	.04	1.82 (1.27–2.62)	.001
Age	1.03 (1.02–1.05)	<.001	1.02 (1.01–1.03)	.01
KPS	0.98 (0.96–1.00)	.02	0.99 (0.87–1.01)	.25
Extent of resection (vs subtotal)				
Biopsy	1.82 (1.27–2.64)	.001	2.5 (1.72–3.65)	<.001
Gross total	0.89 (0.66–1.21)	.45	0.74 (0.55–1.00)	.05

ETRT — Current trial.
TTRT — Phase II study of temozolomide and thalidomide with radiation therapy for newly diagnosed glioblastoma multiforme.[28]
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OTRT — Phase II study of erlotinib plus temozolomide during and after radiation therapy in patients with newly diagnosed glioblastoma multiforme or gliosarcoma.[30]
Abbreviations: CI, confidence interval; HR, hazard ratio.

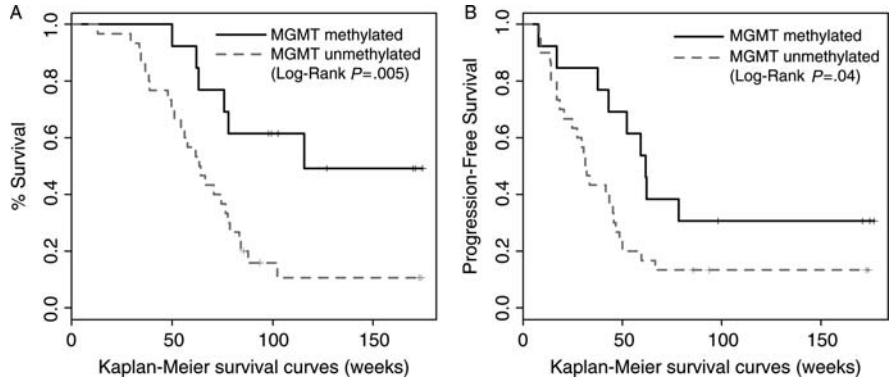

Fig. 2. Clinical outcomes by *MGMT* methylation status. (A) Overall survival. Based on Cox models, unmethylated *MGMT* was associated with increased hazard of earlier death, adjusting for the effect of age and KPS (HR = 5.19, 95% CI: 1.88–14.31, $P = .001$). (B) Progression-free survival. Similarly, unmethylated *MGMT* was associated with increased hazard of earlier progression, adjusting for the effect of age and KPS (HR = 3.07, 95% CI: 1.31–7.23, $P = .01$). Abbreviations: CI, confidence interval; HR, hazard ratio; *MGMT*, O-6-methylguanine-DNA methyltransferase.

As shown in Table 4, univariate Cox proportional hazard analysis of survival in relation to the molecular marker expression showed a significant relationship between S6 score (using either S6-specific antibody, 2211, or 2215) and OS; specifically, higher S6 score was significantly associated with greater hazard of death ($P = .032$ for the 2211 antibody and $P = .02$ for the 2215 antibody). No other molecular marker displayed such a relationship. However, multivariate analysis adjusting for age and KPS showed that expression of S6, measured by both 2211 and 2215 antibodies, was not significantly associated with OS ($P = .24$ and $P = .13$, respectively).

Discussion

The current standard of care for treating patients with newly diagnosed GBM continues to be based on a randomized phase III trial published by Stupp et al. in 2005.[1] In that study, patients were treated either with RT alone or with RT and concurrent temozolomide followed by adjuvant temozolomide given for 6 months. Median OS for the temozolomide-plus-RT arm was 14.6 months, compared with 12.1 months for the RT-only arm. The present study was powered for a primary end point of increasing survival at the historical median survival time of ~15 months (see Table 2). In the current study, OS for patients treated with enzastaurin and temozolomide plus RT was slightly more favorable (74 weeks; 17.1 months) compared with the Stupp et al. historical standard. There is presumptive evidence that molecular profiles of brain cancers may predict response to molecular inhibitors in at least some subgroups of patients.[2,3] A posthoc subgroup analysis of patients treated in the Stupp et al. trial, correlating MGMT promoter methylation with survival, was conducted in an attempt to define patient groups that may be more or less sensitive to treatment.[21] According to that analysis, patients with MGMT promoter methylation had significantly improved OS, compared with patients with

Table 4. Analysis of overall survival with respect to molecular markers

Molecular Marker	HR (95% CI)	*P*-value
S6 2211 score	1.7 (1.05, 2.77)	.032
S6 2215 score	1.69 (1.08, 2.66)	.02
GSK3β score	0.95 (0.62, 1.45)	.81
PCREB score	0.95 (0.51, 1.77)	.87
EGFR score	0.83 (0.61, 1.13)	.23
EGFR intensity	0.54 (0.24, 1.2)	.13
PTEN score	0.89 (0.66, 1.21)	.46
PTEN intensity	0.59 (0.2, 1.79)	.36
VEGF score	0.95 (0.71, 1.26)	.71
VEGF intensity	0.63 (0.26, 1.52)	.30
MAPK score	1.03 (0.64, 1.65)	.91

Note: Adjusting for age and KPS, S6 expression (measured by 2211 and 2215 antibodies) was no longer significantly associated with OS ($P = .24$ and $P = .13$, respectively).
Abbreviations: HR, hazard ratio; CI, confidence interval; KPS, Karnofsky performance status; EGFR, epidermal growth factor receptor; GSK3β, glycogen synthase kinase 3 beta; MAPK, mitogen-activated protein kinase; PCREB, phosphorylated cAMP response element-binding protein; OS, overall survival; PTEN, phosphatase and tensin homolog; S6, S6 ribosomal protein; VGEF, vascular endothelial growth factor.

unmethylated MGMT (21.7 months vs 15.3 months). Because the analysis was performed retrospectively, prospective validation is required before MGMT methylation can be used for clinical decisions about treatment with temozolomide. In the current study, we also found a significant difference in outcome between patients with promoter-methylated MGMT and those with unmethylated MGMT. Although not providing direct evidence, these results suggest that some patient groups may be identified and possibly have their treatment tailored by this specific molecular signature. These observations will require more testing, particularly in larger, prospective, controlled clinical studies, and currently should not be used to stratify patients.

The molecular correlative analyses in the current trial also suggest an association between enzastaurin and S6 biomarker status. However, these results must be viewed with the caution that this was a small phase II study conducted in a single institution. There are several other potential molecular markers that could affect cellular response to enzastaurin, temozolomide, or RT and serve as predictors of outcome. These preliminary results suggest that molecular correlative studies should be considered as part of future multimodal combination therapy trials for patients with GBM or gliosarcoma.

In the current study, we also performed a planned comparison of the survival outcome for patients given enzastaurin in combination with temozolomide plus RT (ETRT) to several historical phase II studies of other novel agents administered with temozolomide plus RT that were conducted at our institution and published in 2004 (TTRT; thalidomide),[28] in 2005 (RTRT; cis–retinoic acid),[29] and in 2009 (OTRT; erlotinib).[30] The current trial produced significantly greater survival times, compared with both TTRT and RTRT. However, the survival results produced by the ETRT regimen in the current trial were comparable to those from our more recent phase II trial (OTRT; erlotinib). The 3 historical studies (OTRT, RTRT, and TTRT) were chosen as comparator trials on the basis of the similar use of a noncytotoxic agent with temozolomide therapy during and after RT. There were also similarities for these trials in terms of patient characteristics, eligibility requirements, and imaging standards. The differences observed between the current trial and the 3 historical control studies may have resulted from the novel agents themselves or changing patterns of care for GBM and gliosarcoma. Until these differences in survival outcomes are further clarified and understood, comparisons of phase II studies with published survival data for patients treated with temozolomide plus RT should be interpreted with caution. In fact, recent results for patients with newly diagnosed GBM treated with temozolomide plus RT and either single-agent talampanel, poly-ICLC, or cilengitide showed significantly longer survival, compared with patients treated with only temozolomide plus RT.[26] The current study (ETRT) continues this trend by demonstrating encouraging OS and PFS results, compared with previous studies (RTRT and TTRT). However, it is unclear whether the novel agent or the improved care of patients with GBM has led to this trend or whether subsequent treatments, such as bevacizumab, have influenced results. Of course, the current study and the comparator trials done at the University of California, San Francisco, are limited by the nature of their design, which is single-arm phase II trials, rather than randomized phase II trials with an experimental treatment arm compared with a standard RT and temozolmide arm.

In conclusion, enzastaurin given in combination with temozolomide plus RT was well tolerated. The paucity of significant drug-associated toxicities associated with enzastaurin, the ease of use by oral administration, and the clinical data shown here as compared with a historical 15-month survival value, make enzastaurin a potentially attractive agent to evaluate in combination with other antiangiogenic and cytotoxic agents in patients with malignant glioma. However, when compared with other similar phase II trials that have a survival approaching 20 months, the combination of enzastaurin and temozolomide does not appear to provide additional benefit, and thus, future studies appear unrealistic. In addition, it is unclear whether the present study's improved survival compared with the 15-month historical standard is attributable to enzastaurin in combination with temozolomide, an improvement in care of patients with GBM, or subsequent treatment agents, such as bevacizumab. Future similar studies will need to consider whether a historical survival standard beyond 15 months is appropriate.

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