Neuro-Oncology 15(10):1405–1412, 2013.
doi:10.1093/neuonc/not100
Advance Access publication August 1, 2013

Enzastaurin before and concomitant with radiation therapy, followed by enzastaurin maintenance therapy, in patients with newly diagnosed glioblastoma without *MGMT* promoter hypermethylation

Wolfgang Wick, Joachim P. Steinbach, Michael Platten, Christian Hartmann, Frederik Wenz, Andreas von Deimling, Peipei Shei, Valerie Moreau-Donnet, Clemens Stoffregen, and Stephanie E. Combs

University Clinic Heidelberg, Heidelberg, Germany (W.W., M.P., C.H., A.v.D., S.E.C.); German Cancer Consortium (DKTK), German Cancer Research Centre, Heidelberg, Germany (W.W., C.H., A.v.D.); J.W. Goethe University Hospital, Frankfurt am Main, Germany (J.P.S.); Hannover Medical School, Hannover, Germany (C.H.); University Medical Centre Mannheim, Mannheim, Germany (F.W.); Eli Lilly and Company, Lilly Corporate Center, Indianapolis, Indiana (P.S.); Lilly France, Neuilly Sur Seine Cedex, France (V.M-D.); Medical Department, Lilly Deutschland GmbH, Bad Homburg, Germany (C.S.)

Background. This study's primary objective was evaluation of the progression-free survival rate at 6 months (PFS-6) in patients with newly diagnosed glioblastoma without O^6-methylguanine-DNA-methyltransferase (*MGMT*) promoter hypermethylation postsurgically treated with enzastaurin before and concomitantly with radiation therapy, followed by enzastaurin maintenance therapy. PFS-6 of at least 55% was set to be relevant compared with the data of the EORTC 26981/22981 NCIC CE.3 trial.

Methods. Adult patients with a life expectancy of at least 12 weeks who were newly diagnosed with a histologically proven supratentorial glioblastoma without *MGMT* promoter hypermethylation were eligible. Patients were treated with enzastaurin prior to, concomitantly with, and after standard partial brain radiotherapy. Here we report on a multicenter, open-label, uncontrolled phase II study of patients with newly diagnosed glioblastoma without *MGMT* promoter hypermethylation treated with enzastaurin and radiation therapy within 4 study periods.

Results. PFS-6 was 53.6% (95% confidence interval [CI]: 39.8–65.6). The median overall survival was 15.0 months (95% CI: 11.9–17.9) for all patients, 3.9 months (95% CI: 0.8–9.0) for patients with biopsy, 15.4 months (95% CI: 10.1–17.9) for patients with partial resection, and 18.9 months (95% CI: 13.9–28.5) for patients with complete resection. The safety profile in this study was as expected from previous trials, and the therapy was well tolerated.

Conclusions. PFS-6 missed the primary planned outcome of 55%. The secondary exploratory analysis according to resection status of the different subgroups of patients with biopsies, partial resection, and complete resection demonstrates the strong prognostic influence of resection on overall survival.

Keywords: brain tumors, enzastaurin, glioblastoma, *MGMT*, radiotherapy.

Received February 25, 2013; accepted May 29, 2013.

Corresponding Author: Wolfgang Wick, MD, Department of Neurooncology, National Center for Tumor Diseases and Neurology Clinic, University Clinic Heidelberg and German Cancer Research Centre, Im Neuenheimer Feld 400, 69120-Heidelberg, Germany (wolfgang.wick@med.uni-heidelberg.de).

Glioblastoma is the most common primary malignant brain tumor in adults and among the most aggressive, making this disease a challenge to treat.[1,2] Historically, standard therapy was surgical resection[3] and postoperative radiation.[4] More recently, temozolomide (TMZ) as concomitant and adjuvant therapy to

radiotherapy increased the progression-free survival rate at 6 months (PFS-6; 53.9% vs 36.4%) and median overall survival (OS; 14.6 vs 12.1 mo).[5] Additionally, the 2-year survival rate was increased considerably[6] relative to radiotherapy alone. Although radiotherapy with concomitant and adjuvant TMZ is currently considered the standard of care,[1,7] subgroups of patients benefit only marginally and do not respond convincingly to this approach. The O^6-methylguanine-DNA-methyltransferase (MGMT) protein has DNA repair activity.[7] The activity of *MGMT* contributes to the resistance of cultured glioma cells and xenografts to alkylating agents. Hypermethylation of the *MGMT* promoter is associated with prolonged progression-free survival (PFS) and OS in glioblastomas and other gliomas.[7–9] Although patients with hypermethylated *MGMT* received a greater benefit from TMZ and radiotherapy than patients with unmethylated *MGMT* in a retrospective analysis of a phase III trial, patients with unmethylated *MGMT* promoter still appeared to derive some benefit from the combination.[6] As such, TMZ plus radiotherapy remains the standard of care for all patients with newly diagnosed glioblastoma, but improved treatments independent of *MGMT* status are needed, and even more so for patients with limited benefit from TMZ. Furthermore, this group of patients, best defined by the absence of *MGMT* promoter hypermethylation, allows the early evaluation of a compound with radiotherapy only, avoiding potential interaction biases and additional toxicity from the radiochemotherapy.

Enzastaurin (LY317615) is an orally active protein kinase C and phosphoinositide-3 kinase/Akt inhibitor with apoptotic, antiproliferative, and anti-angiogenic activities.[10,11] It has anticancer and antiproliferative activity in cells and xenografts derived from solid tumors,[11,12] and there is preclinical evidence that enzastaurin and radiotherapy might act synergistically.[13,14] At the time that this protocol was approved, enzastaurin showed activity in solid tumors and was well tolerated in a phase I trial.[15] In this phase II trial, patients with unmethylated *MGMT* promoter, as determined by a method established by Esteller et al[16] and Hegi et al,[17] were treated with enzastaurin before, concomitantly with, and after radiotherapy to determine PFS-6.

Materials and Methods

Patient Eligibility

Patients ≥18 years old with newly diagnosed, histologically proven grade IV glioblastoma (based on the World Health Organization 2007 classification) were eligible. An Eastern Cooperative Oncology Group performance status (ECOG PS) of ≤2 and an estimated life expectancy of ≥12 weeks were mandatory. Other key eligibility criteria were availability of surgical or biopsy specimens for central pathology review and exploratory biomarker analysis; demonstration of an unmethylated *MGMT* promoter; disease evaluation by gadolinium-MRI within 72 h of surgery; ability to discontinue enzyme-inducing antiepileptic drugs ≥14 days prior to enrollment; adequate organ function; clinically normal cardiac function; and written informed consent.

Key exclusion criteria were inability to swallow tablets; planned surgery for other diseases; history of coagulation disorders involving bleeding, recurrent thrombotic events, or stroke; use of anticoagulant therapy at the time of study enrollment; and placement of polifeprosan 20 with carmustine implant wafer at surgery.

This study was conducted in accordance with the Declaration of Helsinki and applicable good clinical practice guidelines. Human investigations were performed after approval by a local human investigations committee. Written informed consent was obtained.

Molecular Methods

Only tissue samples with a histologically estimated tumor cell content of 80% or more underwent molecular analysis. DNA was extracted from paraffin-embedded tumor tissue using the DNeasy blood and tissue kit (Qiagen). A total of 200 ng of DNA from each tumor was treated with sodium bisulfite using the EZ DNA Methylation-Gold Kit (HISS Diagnostics). A172 glioma cells served as a positive control for *MGMT* promoter methylation. Genomic DNA extracted from peripheral blood leukocytes served as an unmethylated control. *MGMT* promoter methylation status was determined (C.H., A.v.D.) by a central quantitative methylation-specific PCR assay.[18]

Study Design and Treatment

The study was designed as a multicenter, open-label, uncontrolled phase II trial. Single arm designs are deemed acceptable in pilot efficacy assessment of novel agents when reliable historical datasets exist, as for our trial.[19] In study period I (safety run-in), 2 dose regimens were explored. Dose regimen 1 (DR1) was oral enzastaurin 500 mg once daily (qd), which has been used before as a monocompound, and dose regimen 2 (DR2) was enzastaurin 250 mg twice daily (b.i.d.), which was deemed to have superior pharmacokinetics. With both regimens, a loading dose of 1125 mg of enzastaurin was taken on day −7; doses from day −6 forward were either 500 mg qd or 250 mg b.i.d. Beginning on day 1, concurrent radiotherapy (CRT; 1.8- to 2.0-Gy fractions) was administered 5 days per week for 6 weeks.

Three patients were enrolled in DR1. If 1 dose-limiting toxicity (DLT) occurred in DR1, 3 additional patients were enrolled. If no DLT occurred in DR1 or a DLT occurred in ≤2 of 6 patients, DR2 was initiated. The planned enrollment in DR2 was 6 patients. If a DLT was observed in >2 of 6 patients in DR2, a decision would be made to close or modify the trial. DR2 began when all patients in DR1 completed a 2-week observation period following the enzastaurin plus radiation phase.

Study period II (treatment) started after the safety run-in demonstrated the feasibility of DR1 or DR2. If both regimens were feasible, the b.i.d. regimen would be chosen. Therefore, the full analysis set of this trial

consisted of patients who were treated with a loading dose of enzastaurin followed by enzastaurin 250 b.i.d. and CRT administered as previously described.

In study period III (maintenance), patients were treated with enzastaurin 250 mg b.i.d. until progression or unacceptable toxicity for a maximum of 3 years; however, if a patient benefited from therapy, treatment could have continued beyond 3 years with investigator and sponsor agreement.

In study period IV (follow-up), a 30-day safety follow-up was performed after enzastaurin treatment ended. Patients were followed for PFS and OS for a maximum of 2 years. For patients receiving enzastaurin for more than 3 years, the 30-day safety follow-up was to be performed, but long-term follow-up visits were not to occur.

DLTs were any of the following occurring during combination therapy: (i) any nonhematologic grade 3 toxicity per the Common Terminology Criteria for Adverse Events version 3.0 (CTCAE; excluding rapidly controlled alopecia, nausea, or vomiting); (ii) an absolute neutrophil count (ANC) of $<0.5 \times 10^9$/L lasting for 7 days; (iii) febrile neutropenia, defined as an ANC $<1.0 \times 10^9$/L and fever of at least 38.5°C; (iv) CTCAE grade ≥ 3 thrombocytopenia; (v) any grade 4 radiation-induced skin changes.

Radiotherapy

Target volumes were determined based on pre- and posttherapeutic diagnostic T1-weighted MRI scans or contrast-enhanced CT scans in axial slicing. The target volume comprised the contrast-enhancing tumor, edema, and a safety margin of 2 cm. Three-dimensional inverse treatment planning was performed in all patients according to International Commission on Radiation Units and Measurements (ICRU) Report 50 standards. Isocenter definition was done by either virtual or conventional simulation. Radiotherapy was performed according to a conventional fractionation regimen (5 fractions of 2.0 Gy ICRU reference point) administered weekly for 6 weeks up to a total target volume dose of 60.0 Gy. If parts of the brainstem or optic chiasma were in the radiation field, the single doses were reduced to 1.8 Gy, the organs at risk were limited to 54.0 Gy, and the total dose was reduced to 59.4 Gy. Corticosteroids were administered at the discretion of the treating physicians.

Dose Adjustments

Enzastaurin administration was omitted for the following adverse events (AEs) until event resolution: ANC $<0.5 \times 10^9$/L for >7 days; ANC $<1.0 \times 10^9$/L with fever (38.5°C); platelet count $<25 \times 10^9$/L; CTCAE grade 4 transaminase elevations; and clinically relevant CTCAE grade 3 or 4 nonhematologic toxicity (nausea and vomiting were managed with antiemetics). If the event resolved to grade 1 or baseline, therapy was resumed, but at 250 mg per day (qd or b.i.d. depending on the regimen). If, after restarting therapy, the event did not recur after 14 days, the dose could be re-escalated to the full dose at investigator discretion. If the event did not resolve to grade 1 or baseline within 2 weeks, or another event occurred at the reduced dose, the patient was discontinued from enzastaurin and received radiotherapy only.

Radiotherapy was interrupted if absolute granulocyte counts were below 0.5×10^9/L or platelet counts were below 25×10^9/L. Radiotherapy resumed when the levels exceeded these cutoffs. In case of any grade 4 radiation-induced skin changes, radiotherapy was interrupted until the event resolved.

Patient Evaluations

Within 14 days prior to the start of therapy, patients had a complete medical history, physical examination (including ECOG PS and assessment for steroid use), Mini Mental Status Examination (MMSE), neurologic function status test, and slit lamp examination. Pre-study tests performed within 14 days prior to therapy included electrocardiogram (ECG), hematology, blood chemistry, neurologic functional status, and (in women) a pregnancy test. Baseline MRIs were performed within 21 days prior to start of therapy and no more than 72 h after start of therapy.

During visit 1, occurring during enzastaurin therapy, a physical examination (including ECOG PS and concomitant medication assessment) and CTCAE grading were performed. During visit 2, occurring during the enzastaurin + radiation phase, a physical examination (including ECOG PS and concomitant medication assessment), CTCAE grading, ECG, hematology, blood chemistry, neurologic functional status, and MMSE were performed. During visit 3, occurring during the maintenance phase, brain scans were performed starting 4 weeks after completion of radiation, and then after every 6 weeks ($\pm$ 1 wk) of treatment. The same imaging method as baseline was used. During visit 3, the patient had a physical examination with CTCAE grading, ECG, blood chemistry, neurologic examination, and MMSE. This visit was repeated every 6 weeks. Until 6 months postoperatively, scans were repeated every 6 weeks. After visit 5, scans were repeated every second visit (every 3 months $\pm$ 1 wk). A slit lamp ocular examination was repeated every 6 months, if the patient's status allowed.

Confirmation of response occurred not less than 4 weeks from the first evidence of response. Thereafter, responding patients were followed every 6 weeks ($\pm$1 wk).

Statistical Considerations

The primary objective was PFS-6. In an agent not expected to interfere with contrast enhancement, PFS and PFS-6 are accepted surrogate endpoints.[19,20] Secondary objectives were safety and tolerability, neurologic status, overall response rate, OS, biomarkers relevant to enzastaurin and disease state, and correlation to clinical outcome.

PFS was the time from the randomization date to the date of objectively determined progressive disease (based on radiologic assessment) or death from any cause, whichever came first. PFS was derived using the Kaplan–Meier method. Planned enrollment was 60 patients; of these, 54 patients were planned for study period II. The sample size calculation assumed that PFS followed an exponential distribution. Forty-three events of progression or death were needed to have an 80% power to detect an improvement between the null hypothesis PFS-6 of 40%, which was the PFS-6 rate achieved with radiochemotherapy in patients with unmethylated *MGMT* promoter in the European Organisation for Research and Treatment of Cancer (EORTC) 26981/22981 National Cancer Institute of Canada (NCIC) CE.3 trial and the alternative hypothesis of PFS-6 of 55% at a significance level of 0.05. Assuming a censoring rate of 20%, the study needed to enroll 54 patients, who were followed ≥6 months. This allowed detection of a clinically relevant increase of PFS for the investigational regimen over the historical PFS profile of a standard treatment for the same indication; assumptions were based on published data.[5,17] All statistical tests were conducted at a 2-sided alpha level of 0.05 unless otherwise stated.

Tumor responses were measured and recorded using the Macdonald criteria.[21] For complete response (CR) or partial response (PR), best response was confirmed; the second assessment was performed ≥28 days after the first evidence of response. Two objective status determinations of CR before progression were required for a best response of CR, and 2 determinations of PR or better before progression, but not qualifying for a CR, were required for a best response of PR.

The Kaplan–Meier curves of the overall PFS time and the OS time were to be generated and the quartiles and appropriate point probabilities calculated. For event rates, the point estimates as well as the 95% confidence intervals (CIs) were to be presented. All efficacy and safety analyses were performed on all patients receiving at least 1 dose of enzastaurin at the dose level chosen for study period II. The clinical data of those patients in study period I who were treated with the same enzastaurin dose as patients in study period II were included in the efficacy analysis of study period II.

Results

Between 23 October 2007 and 28 July 2011, 60 patients in 10 German centers were entered in this trial. Of these, 3 patients receiving qd enzastaurin were not considered in the full analysis set. The remaining 57 patients receiving b.i.d. enzastaurin were enrolled and made up the full analysis set. Table 1 shows baseline demographics. Figure 1 shows the flow diagram for the trial.

Efficacy

The primary efficacy measure was the rate of patients showing PFS-6 after diagnosis. PFS was defined as the time from the surgical diagnosis date to the date of objectively determined progressive disease (based on radiologic assessment) or death from any cause, whichever came first. The PFS-6 was 53.6% (Table 2). The median PFS was 6.6 months. The 12-month PFS rate was 14.9%, and the 24-month PFS rate was 3.7%. Further exploratory subgroup analysis by baseline neurosurgical intervention showed that those patients with complete resection of their tumors had a PFS-6 rate of 72.0% versus 45.5% and 22.2% for patients who had partial resection and biopsy, respectively. At first progression, 49/55 (89%) patients received TMZ and 2 a nitrosourea.

Median OS was 15.0 months; the 6-month, 1-year, and 2-year OS rates were 87.7%, 63.0%, and 27.0%, respectively (Table 2).

In a Cox regression analysis with covariates for gender, surgery with complete resection, and ECOG PS (0 vs 1, 2), surgery (yes vs no) with complete resection was significantly associated with improved OS (hazard ratio [HR] = 0.43 [95% CI: 0.23–0.79]; $P = .0066$). Additionally, ECOG PS was significantly associated with decreased OS (HR = 2.42 [95% CI: 1.29–4.55]; $P = .0059$).

Patients undergoing biopsy ($n = 9$) had a median OS of 3.9 months (95% CI: 0.8–9.0). The 6-month survival rate in this group was 44.4% (95% CI: 13.6–71.9); at 1 year, there were no survivors. Of the 23 patients undergoing partial resection, 18 had events. The median OS in this group was 15.4 months (95% CI: 10.1–17.9). The 6-month survival rate in this group was 91.3% (95% CI: 69.5–97.8); at 1 year, the survival rate was 69.3% (95% CI: 46.1–84.0); and at 2 years, the survival rate was 18.5% (95% CI: 5.8–36.7). Of the 26 patients undergoing complete resection, 19 had events. The median OS in this group was 18.9 months (95% CI: 13.9–28.5). The 6-month survival rate in this group was 100.0% (95% CI: 100.0–100.0); at 1 year, the survival rate was 80.0% (95% CI: 58.4–91.1); and at 2 years, the survival rate was 44.0% (95% CI: 24.5–61.9).

The overall response rate was 7.0% (95% CI: 2.0–17.0). There was 1 (1.8%) CR and 3 (5.3%) PRs. Approximately half (54.4%) of the patients in the study experienced stable disease.

Safety

Overall, 53 (93%) patients experienced at least 1 treatment-emergent adverse event (TEAE); of these, 38 (66.7%) patients had TEAEs possibly related to enzastaurin, 33 (57.9%) had TEAEs possibly related to radiation, 16 (28.1%) had TEAEs possibly related to enzastaurin + radiation, and 6 (10.5%) had TEAEs possibly related to study procedure. Twenty-six (45.6%) patients experienced at least 1 grade 3/4 TEAE; of these, 10 (17.5%) had TEAEs possibly related to enzastaurin, 5 (8.8%) had TEAEs possibly related to radiation, 4 (7.0%) had TEAEs possibly related to enzastaurin + radiation, and 1 (1.8%) had TEAEs possibly related to study procedure.

Serious TEAEs that were possibly related to the study are listed in Table 3. Four patients discontinued due to serious AEs; these were cerebral aspergillosis,

Table 1. Baseline demographics of enrolled patients

	Enzastaurin b.i.d. ($n = 57$)
Median age, y (range)	57.6 (29.4–79.8)
Gender, *n* (%)	
Male	36 (63.2)
Female	21 (36.8)
ECOG performance status, *n* (%)	
0	39 (68.4)
1	16 (28.1)
2	2 (3.5)
Mean Mini Mental State Test total score (SD)	28.3 (2.52)
Neurologic function, *n* (%)	
No neurologic symptoms, fully active	31 (54.4)
Minor neurologic symptoms, fully active	18 (31.6)
Moderate neurologic symptoms, fully active	6 (10.5)
Moderate neurologic symptoms, less than fully active	2 (3.5)
Severe neurologic symptoms, totally inactive	0
Unknown	0
Mental status, *n* (%)	
Normal function	49 (86.0)
Minor mental confusion	7 (12.3)
Gross confusion but awake	1 (1.8)
Unknown	0
Patients receiving steroid therapy at baseline, *n* (%)	20 (35.1)
Dexamethasone	6 (10.5)
Mean dose (SD)	7.2 (5.1)
Fortecortin	14 (24.6)
Mean dose (SD)	5.4 (3.2)
Prior brain surgery, *n* (%)	57 (100)
Partial resection	23 (40.4)
Complete resection	25 (43.9)
Biopsy	9 (15.8)

pneumonia, convulsion, and pulmonary embolism. Three patients discontinued due to nonserious AEs; these were fatigue, elevated gamma-glutamyltransferase, hyponatremia, and deep vein thrombosis.

Seven patients experienced 9 TEAEs leading to enzastaurin dose adjustments. These TEAEs were nausea, impaired healing, pneumonia, wound infection, increased gamma-glutamyltransferase level, convulsion, dizziness, hemiparesis, and deep vein thrombosis. With the exception of nausea and hemiparesis, which occurred during induction, these events occurred during radiotherapy.

Overall, the most common possibly drug-related TEAE was urine color change (19.3% of patients, grade 1 only) followed by grade 1 fatigue (8.8% of patients). Grades 2 and 3 fatigue were experienced by 1.8% of patients each. The most common possibly radiation-related TEAEs were grade 1 alopecia (22.8% of patients) and grade 1 radiation dermatitis (12.3% of patients). Grade 2 radiation-related alopecia and radiation dermatitis were experienced by 1.8% of patients each. The only drug- and radiation-related TEAE occurring in >5% of patients was grade 1 fatigue (5.3% of patients). Thrombosis and embolism were the only grades 3–5 TEAEs to occur in ≥2 patients (3.5% of patients).

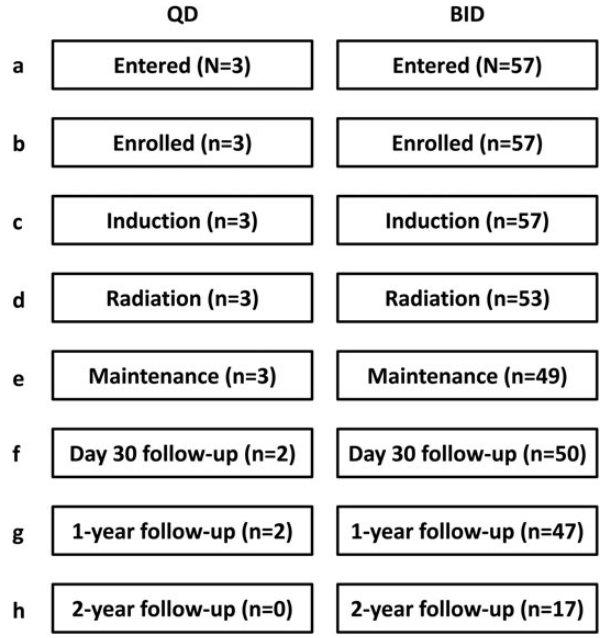

Fig. 1. Flow diagram. a = signed the informed consent; b = patients receiving at least 1 dose of enzastaurin; c = patients included in the first treatment period, which encompassed the first enzastaurin dose to first irradiation; d = patients included in the radiation period, which encompassed the first irradiation to last irradiation; e = patients in the maintenance period, which encompassed the day after last irradiation to the last enzastaurin dose; f = duration of follow-up period was ≥1 day; g = duration of follow-up period was >30 days; h = duration of follow-up period was >365 days.

Overall, 7 patients died during the study's drug therapy or within 30 days of discontinuation. Of these, 2 patients died from progressive disease during the maintenance period. One drug-related death occurred during induction (intracranial tumor hemorrhage). Four deaths were due to AEs (2 sepsis and 1 ventricular fibrillation during radiotherapy and 1 pneumonia during maintenance therapy).

Neurologic Status

Figure 2 shows MMSE scores over time for the full analysis set. During the entire study period, 29 (50.9%) patients required at least 1 steroid dose increase. Twenty patients required a dose increase due to deterioration of performance or mental status, and 11 patients required a dose increase due to other reasons.

Discussion

MGMT promoter hypermethylation is a promising molecular biomarker in glioblastoma[9] and is associated

Table 2. Analyses of efficacy measures

	All Patients	Biopsies	Partial Resections	Complete Resections
PFS-6 (95% CI)	53.6% (39.8–65.6)	22.2% (3.4–51.3)	45.5% (24.4–64.3)	72.0% (50.1–85.6)
Median PFS (95% CI)	6.6 mo (4.6–8.2)	–	–	–
12-mo PFS	14.9% (7.0–25.6)	–	–	–
24-mo PFS	3.7% (0.7–11.4)	–	–	–
Median OS	15.0 mo (11.9–17.9)	3.9 mo (0.8–9.0)	15.4 mo (10.1–17.9)	18.9 mo (13.9–28.5)
6-mo OS	87.7% (76.0–93.9)	44.4% (13.6–71.9)	91.3% (69.5–97.8)	100.0% (100.0–100.0)
1-y OS	63.0% (49.1–74.1)	0%	69.3% (46.1–84.0)	80.0% (58.4–91.1)
2-y OS	27.0% (16.2–39.0)	0%	18.5% (5.8–36.7)	44.0% (24.5–61.9)

Table 3. Serious TEAEs possibly related to study (patients with ≥1 event)

	Drug-Related ($n = 57$)		Radiation-Related ($n = 57$)		Drug- and Radiation-Related ($n = 57$)	
	Events	n (%) 8 (14.0)	Events	n (%) 4 (7.0)	Events	n (%) 3 (5.3)
Total events	19	–	7	–	6	–
Ear and labyrinth disorders	1	1 (1.8)	0	0 (0)	0	0 (0)
Vertigo	1	1 (1.8)	0	0 (0)	0	0 (0)
Gastrointestinal disorders	2	1 (1.8)	0	0 (0)	0	0 (0)
Nausea	1	1 (1.8)	0	0 (0)	0	0 (0)
Vomiting	1	1 (1.8)	0	0 (0)	0	0 (0)
General disorders and administrative site conditions	1	1 (1.8)	0	0 (0)	0	0 (0)
Fatigue	1	1 (1.8)	0	0 (0)	0	0 (0)
Infections and infestations	7	4 (7.0)	4	2 (3.5)	4	2 (3.5)
Pneumonia	2	2 (3.5)	0	0 (0)	0	0 (0.0)
Bronchopulmonary aspergillosis	1	1 (1.8)	1	1 (1.8)	1	1 (1.8)
Cerebral aspergillosis	1	1 (1.8)	1	1 (1.8)	1	1 (1.8)
Empyema	1	1 (1.8)	1	1 (1.8)	1	1 (1.8)
Pneumocystis jiroveci infection	1	1 (1.8)	1	1 (1.8)	1	1 (1.8)
Investigations	1	1 (1.8)	0	0 (0)	0	0 (0)
Hepatic enzyme increased	1	1 (1.8)	0	0 (0)	0	0 (0)
Neoplasms	1	1 (1.8)	0	0 (0)	0	0 (0)
Intracranial tumor hemorrhage	1	1 (1.8)	0	0 (0)	0	0 (0)
Nervous system disorders	2	2 (3.5)	3	3 (5.3)	2	2 (3.5)
Brain edema	0	0 (0)	1	1 (1.8)	0	0 (0)
Convulsions	1	1 (1.8)	1	1 (1.8)	1	1 (1.8)
Hemiparesis	1	1 (1.8)	1	1 (1.8)	1	1 (1.8)
Respiratory, thoracic, and mediastinal disorders	2	2 (3.5)	0	0 (0)	0	0 (0)
Pulmonary embolism	2	2 (3.5)	0	0 (0)	0	0 (0)
Vascular disorders	2	2 (3.5)	0	0 (0)	0	0 (0)
Deep vein thrombosis	2	2 (3.5)	0	0 (0)	0	0 (0)

with prolonged PFS and OS in glioblastomas and anaplastic gliomas.[7–9,22] In the EORTC 26981/22981 CE.3 trials, patients with methylated *MGMT* received substantial benefit from the addition of TMZ to radiotherapy compared with patients with unmethylated *MGMT* even in the long-term follow-up[6]; however, patients with unmethylated *MGMT* still appeared to derive some benefit from the combination.[6] Further, testing of *MGMT* has been shown to be difficult and neither fully sensitive nor reliable.[9] Therefore, TMZ + radiotherapy remains the standard of care for all patients with newly diagnosed glioblastoma with median PFS and OS of ~7 and 15 months, respectively.[5,6] Despite this standard of clinical practice, the limited efficacy of TMZ in patients with unmethylated *MGMT* promoter led to the development of a series of clinical trials that restricted entry to patients with a glioblastoma with unmethylated *MGMT* promoter. For enzastaurin, the rationale was based on

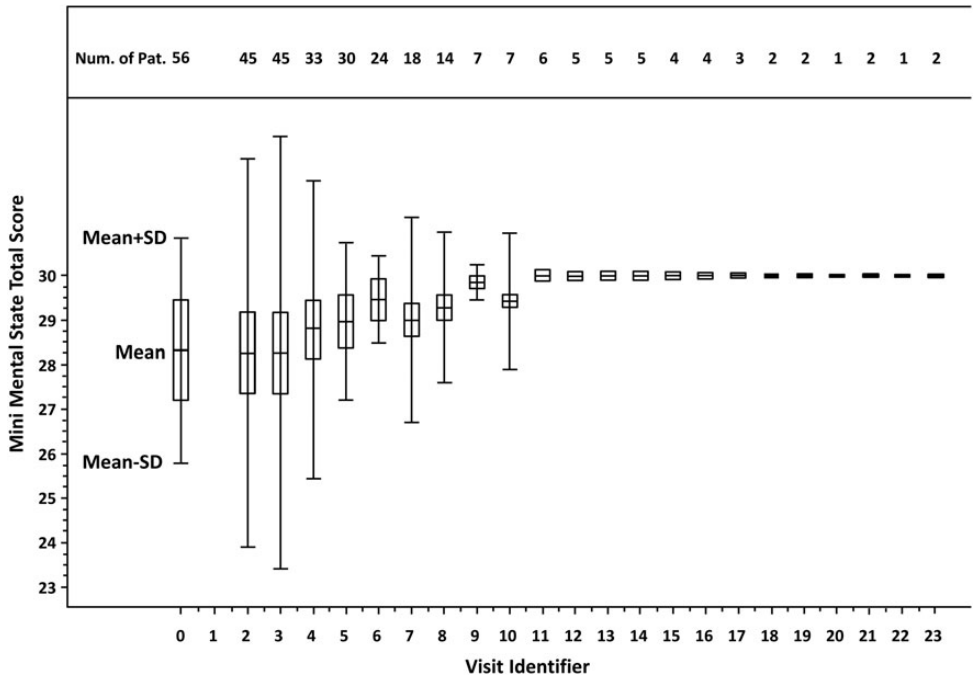

Fig. 2. Mini Mental State total score, full analysis population. The area within the rectangle is proportional to the number of patients with Mini Mental State Exam total score at respective visits. Abbreviations: Num, number; Pat, patients.

good tolerability but limited efficacy as a monocompound[23] as well as clear signals from a preclinical animal model where the combination of enzastaurin and radiotherapy demonstrated synergistic efficacy independent of *MGMT* status.[14]

This was the first trial of a prospectively planned study in a molecularly defined patient subpopulation with a poor prognosis. The safety profile of enzastaurin demonstrated in this study was expected and showed that the combination of enzastaurin and radiotherapy is tolerated. The combination also allowed for a stable cognitive function of patients on trial as determined by Mini Mental State Examination.

The PFS-6 for this trial was 53.6%, which is less than the planned primary outcome of 55%. However, this PFS-6 is similar to that seen in patients treated with TMZ + radiotherapy (53.9%) and is greater than historical data of radiotherapy alone (36.4%). The PFS-6 of 53.6% in this trial is also interesting compared with the subsets of patients without *MGMT* promoter hypermethylation in the pivotal EORTC/NCIC trial. In that trial, PFS-6 rates in the *MGMT* unmethylated population were 35.2% for patients receiving radiotherapy alone and 40.0% for patients receiving radiochemotherapy.[5]

PFS-6 by baseline extent of resection demonstrated a clear delineation among tumor biopsy, partial resection, and complete resection (22.2%, 45.5%, and 72.0%, respectively). Though restricted to patients with unmethylated *MGMT* promoter, the number of patients with a complete resection and good performance status and the number of patients receiving steroids at study entry reflect a relatively good prognosis group of patients compared with the EORTC 26981 trial. Another limitation is the use of PFS-6 as the primary efficacy endpoint. Many trials no longer use PFS-6 as the primary endpoint and focus more on OS.[24–26] This trial is also limited by being open-label and having only historical data for alternative treatment comparison. Further, the PFS data may be impacted by pseudoprogression, a concept that received attention[27] primarily when this trial was already enrolling. However, comparative analysis of median OS of the present trial (15 mo) is relevant compared not only with the median OS with radiochemotherapy (11.8 mo) in patients without *MGMT* promoter hypermethylation, but also with the 12.1 months achieved with primary radiotherapy or the 14.6 months for radiochemotherapy with TMZ in the full trial population of the EORTC/NCIC trial.[6] This is further underscored by the unexpected data on subgroups for extent of resection. The OS data for patients with a biopsy only (3.9 mo), partial resection (15.4 mo), and complete resection (18.9 mo) further support a potential prognostic value of neurosurgical interventions.

In conclusion, despite these limitations, enzastaurin in combination with radiotherapy yielded promising results in a molecularly diagnosed group of poor prognosis patients with glioblastoma. This trial marks the first controlled trial that suggests differences in OS using the prognostic factor of tumor resection type in unmethylated patients. The concept is now followed in the development of cilengitide, separately for patients with newly diagnosed glioblastoma with a methylated (CENTRIC trial) or unmethylated (CORE trial) *MGMT* promoter. The OS data show that it is reasonable to focus further

development strategies for enzastaurin on patients who undergo partial or complete resection.

Acknowledgments

The authors thank Michelle Mynderse, PhD, and Joseph Durrant, both of inVentiv Health Clinical, for writing and editorial support, respectively.

Funding

This trial was supported by Eli Lilly and Company.

Conflict of interest statement. Dr Wick is a board member of MSD and Roche, serves as a consultant for Magforce and a speaker for Roche and MSD, and has provided educational presentations for Prime Oncology. Dr Steinbach is a board member of Roche, a consultant for Boehringer, has grants through Merck, and has attended meetings using funds from Medac. Dr von Deimling has served as a reviewer for Clinical Cooperation Unit Neuropathology, DKFZ, Heidelberg. Drs Platten, Hartmann, Wenz, and Combs have no conflicts of interest to report.

References

1. Clarke J, Butowski N, Chang S. Recent advances in therapy for glioblastoma. *Arch Neurol*. 2010;67(3):279–283.
2. Preusser M, de Ribaupierre S, Wohrer A, et al. Current concepts and management of glioblastoma. *Ann Neurol*. 2011;70(1):9–21.
3. Hess KR. Extent of resection as a prognostic variable in the treatment of gliomas. *J Neurooncol*. 1999;42(3):227–231.
4. Walker MD, Strike TA, Sheline GE. An analysis of dose-effect relationship in the radiotherapy of malignant gliomas. *Int J Radiat Oncol Biol Phys*. 1979;5(10):1725–1731.
5. Stupp R, Mason WP, van den Bent MJ, et al. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. *N Engl J Med*. 2005;352(10):987–996.
6. Stupp R, Hegi ME, Mason WP, et al. Effects of radiotherapy with concomitant and adjuvant temozolomide versus radiotherapy alone on survival in glioblastoma in a randomised phase III study: 5-year analysis of the EORTC-NCIC trial. *Lancet Oncol*. 2009;10(5):459–466.
7. Silber JR, Bobola MS, Blank A, Chamberlain MC. O(6)-methylguanine-DNA methyltransferase in glioma therapy: promise and problems. *Biochim Biophys Acta*. 2012;1826(1):71–82.
8. von Deimling A, Korshunov A, Hartmann C. The next generation of glioma biomarkers: MGMT methylation, BRAF fusions and IDH1 mutations. *Brain Pathol*. 2011;21(1):74–87.
9. Weller M, Stupp R, Reifenberger G, et al. MGMT promoter methylation in malignant gliomas: ready for personalized medicine? *Nat Rev Neurol*. 2010;6(1):39–51.
10. Chen YB, LaCasce AS. Enzastaurin. *Expert Opin Investig Drugs*. 2008;17(6):939–944.
11. Graff JR, McNulty AM, Hanna KR, et al. The protein kinase C beta-selective inhibitor, enzastaurin (LY317615.HCl), suppresses signaling through the AKT pathway, induces apoptosis, and suppresses growth of human colon cancer and glioblastoma xenografts. *Cancer Res*. 2005;65(16):7462–7469.
12. Keyes KA, Mann L, Sherman M, et al. LY317615 decreases plasma VEGF levels in human tumor xenograft-bearing mice. *Cancer Chemother Pharmacol*. 2004;53(2):133–140.
13. Tabatabai G, Frank B, Wick A, et al. Synergistic antiglioma activity of radiotherapy and enzastaurin. *Ann Neurol*. 2007;61(2):153–161.
14. Willey CD, Xiao D, Tu T, et al. Enzastaurin (LY317615), a protein kinase C beta selective inhibitor, enhances antiangiogenic effect of radiation. *Int J Radiat Oncol Biol Phys*. 2010;77(5):1518–1526.
15. Carducci MA, Musib L, Kies MS, et al. Phase I dose escalation and pharmacokinetic study of enzastaurin, an oral protein kinase C beta inhibitor, in patients with advanced cancer. *J Clin Oncol*. 2006;24(25):4092–4099.
16. Esteller M, Hamilton SR, Burger PC, Baylin SB, Herman JG. Inactivation of the DNA repair gene O6-methylguanine-DNA methyltransferase by promoter hypermethylation is a common event in primary human neoplasia. *Cancer Res*. 1999;59(4):793–797.
17. Hegi ME, Diserens AC, Gorlia T, et al. MGMT gene silencing and benefit from temozolomide in glioblastoma. *N Engl J Med*. 2005;352(10): 997–1003.
18. Vlassenbroeck I, Califice S, Diserens AC, et al. Validation of real-time MSP to determine MGMT promoter methylation in glioma. *J Mol Diagn*. 2008;10:332–337.
19. Galanis E, Wu W, Cloughesy T, et al. Phase 2 trial design in neuro-oncology revisited: a report from the RANO group. *Lancet Oncol*. 2012;13(5):e196–e204.
20. Reardon DA, Galanis E, DeGroot JF, et al. Clinical trial end points for high-grade glioma: the evolving landscape. *Neuro Oncol*. 2011;13(3): 353–361.
21. Macdonald DR, Cascino TL, Schold SC, Jr, Cairncross JG. Response criteria for phase II studies of supratentorial malignant glioma. *J Clin Oncol*. 1990;8(7):1277–1280.
22. Wick W, Hartmann C, Engel C, et al. NOA-04 randomized phase III trial of sequential radiochemotherapy of anaplastic glioma with procarbazine, lomustine, and vincristine or temozolomide. *J Clin Oncol*. 2009;27(35):5874–5880.
23. Wick W, Puduvalli VK, Chamberlain MC, et al. Phase III study of enzastaurin compared with lomustine in the treatment of recurrent intracranial glioblastoma. *J Clin Oncol*. 2010;28(7):1168–1174.
24. Wick W, Platten M, Meisner C, et al. Temozolomide chemotherapy alone versus radiotherapy alone for malignant astrocytoma in the elderly: the NOA-08 randomised, phase 3 trial. *Lancet Oncol*. 2012;13(7):707–715.
25. Minniti G, Lanzetta G, Scaringi C, et al. Phase II study of short-course radiotherapy plus concomitant and adjuvant temozolomide in elderly patients with glioblastoma. *Int J Radiat Oncol Biol Phys*. 2012;83(1):93–99.
26. Stummer W, Meinel T, Ewelt C, et al. Prospective cohort study of radiotherapy with concomitant and adjuvant temozolomide chemotherapy for glioblastoma patients with no or minimal residual enhancing tumor load after surgery. *J Neurooncol*. 2012;108(1):89–97.
27. Wen PY, Macdonald DR, Reardon DA, et al. Updated response assessment criteria for high-grade gliomas: Response Assessment in Neuro-Oncology working group. *J Clin Oncol*. 2010;28(11):1963–1972.