Published in final edited form as:

J Neurooncol. 2016 January ; 126(1): 185–192. doi:10.1007/s11060-015-1958-z.

A phase II study of bevacizumab and erlotinib after radiation and temozolomide in *MGMT* unmethylated GBM patients

J. J. Raizer¹, P. Giglio², J. Hu³, M. Groves⁴, R. Merrell⁵, C. Conrad⁴, S. Phuphanich³, V. K. Puduvalli², M. Loghin⁶, N. Paleologos⁷, Y. Yuan⁸, D. Liu⁸, A. Rademaker⁹, W. K. Yung⁶, B. Vaillant¹⁰, J. Rudnick³, M. Chamberlain¹¹, N. Vick⁵, S. Grimm¹, I. W. Tremont-Lukats⁶, J. De Groot⁶, K. Aldape¹², M. R. Gilbert¹³, and Brain Tumor Trials Collaborative

¹ Department of Neurology, Northwestern University, 710 North Lake Shore Drive, Abbott Hall, Room 1123, Chicago, IL 60611, USA

² James Cancer Hospital, Ohio State University, Columbus, OH, USA

³ Departments of Neurology and Neurosurgery, Cedars-Sinai Medical Center, Los Angeles, USA

⁴ Austin Brain Tumor Center, Austin, USA

⁵ Department of Neurology, NorthShore University Health System, Evanston, USA

⁶ Department of Neuro-Oncology, The University of Texas MD Anderson Cancer Center, Houston, USA

⁷ Department of Neurology, Rush University Medical Center, Chicago, USA

⁸ Department of Biostatistics, University of Texas MD Anderson Cancer Center, Houston, USA

⁹ Department of Preventive Medicine, Northwestern University, Chicago, USA

¹⁰ Dell Medical School, The University of Texas, Austin, USA

¹¹ Department of Neurology, University of Washington, Seattle, USA

¹² Department of Pathology, Princess Margaret Cancer Centre, Toronto, Canada

¹³ Neuro-Oncology Branch, NIH, Bethesda, USA

Abstract

J. J. Raizer jraizer@nm.org.

Author contributions Statistical Analysis was performed by (YY, DL and AR), Drafting/revising the manuscript-all authors. Study concept or design-JJR, AR, YY, MG. Analysis or interpretation of data-JJR, YY, DL, MG. Statistical Analysis was performed by (YY, DL and AR)

Compliance with ethical standards

Conflict of interest JJR-Research Support: Genentech, Novartis, Plexxikon, Celldex, Diffusion, Stemline; Advisory Board: Stemline, Novocure, Midatech, AbbVie, Proximagen; Speakers Bureau: Genentech. MG-Research Support: GSK. Speakers Bureau: Foundation Medicine; Advisory Board: Incyte, Genentech, Foundation Medicine. VKP-Advisory board and honoraria: Nektar therapeutics, Orbus Pharma, Celgene, Genentech, Foundation Medicine; Speaker: Depuy Synthes. AY-Consultant: Merck, Actelion, DNAtrix; Honoraria: Merck, Actalio; Research Fund, None. JR-Advisory Board: Novocure. SG-Advisory Board: AbbVie and Novocure, JD-Advisory Board: Genentech, Novartis, Celldex, Foundation Medicine, Celldex, Deciphera Pharmaceuticals. Research Support: Sanofi-Aventis, AstraZeneca, EMD-Serono, Eli Lilly, Novartis, Deciphera Pharmaceuticals. PG, JH, RM, CC, SP, ML, NP, YY, DL, AR, BV, MC, NV, IWT-L, KA, MRG—none.

Survival for glioblastoma (GBM) patients with an unmethylated *MGMT* promoter in their tumor is generally worse than methylated *MGMT* tumors, as temozolomide (TMZ) response is limited. How to better treat patients with unmethylated *MGMT* is unknown. We performed a trial combining erlotinib and bevacizumab in unmethylated GBM patients after completion of radiation (RT) and TMZ. GBM patients with an unmethylated *MGMT* promoter were trial eligible. Patient received standard RT (60 Gy) and TMZ (75 mg/m² × 6 weeks) after surgical resection of their tumor. After completion of RT they started erlotinib 150 mg daily and bevacizumab 10 mg/kg every 2 weeks until progression. Imaging evaluations occurred every 8 weeks. The primary endpoint was overall survival. Of the 48 unmethylated patients enrolled, 46 were evaluable (29 men and 17 women); median age was 55.5 years (29–75) and median KPS was 90 (70–100). All patients completed RT with TMZ. The median number of cycles (1 cycle was 4 weeks) was 8 (2–47). Forty-one patients either progressed or died with a median progression free survival of 9.2 months. At a follow up of 33 months the median overall survival was 13.2 months. There were no unexpected toxicities and most observed toxicities were categorized as CTC grade 1 or 2. The combination of erlotinib and bevacizumab is tolerable but did not meet our primary endpoint of increasing survival. Importantly, more trials are needed to find better therapies for GBM patients with an unmethylated *MGMT* promoter.

Keywords

MGMT; Glioblastoma; Erlotinib; Bevacizumab

Introduction

Glioblastoma (GBM) remains one of the most lethal tumors with a median survival of 14–17 months despite extensive tumor resection followed by concomitant radiation (RT) and temozolomide (TMZ) and then 6–12 months of adjuvant TMZ [1–4]. Patients whose GBMs have a methylated *MGMT* promoter survive on average twice as long as those with unmethylated *MGMT* promoters when treated with TMZ [2, 3, 5]. The efficacy of concurrent and adjuvant TMZ in the unmethylated patient population is quite limited. Unfortunately, only one-third of patients have tumors with methylated *MGMT* promoter creating a significant unmet need for improved therapies for the remainder and majority of patients with GBM [5].

Vascular proliferation is one of the hallmarks of GBM that is most commonly associated with an increase in production of vascular endothelial growth factor (VEGF) subtype A [6, 7]. VEGF blockade can potentially decrease tumor growth by inhibiting pathway activation thereby preventing neoangiogenesis. Several anti-angiogenic agents targeting the VEGF pathway in recurrent GBM have been tried in clinical trials [8–11]. Only bevacizumab had positive data leading to an accelerated FDA approval in 2009 based on data from two trials [8, 9]. Two confirmatory trials in newly diagnosed patients failed to show a survival benefit with bevacizumab [3, 12].

Prominent abnormalities in signal transduction pathways are also characteristic of GBM. Based on the data from the TCGA, Epidermal Growth Factor is one of the most

overexpressed or mutated abnormalities in GBM [13]. EGF is mitogenic, an effect mediated by the binding of EGF to a cell surface EGF receptor (EGFR). To date, single agents targeting EGFR such as erlotinib, gefitinib or afatinib have not been successful in the treatment of recurrent glioblastoma [14–19].

The efficacy of single agent targeted therapies in malignant gliomas has been poor. However, pre-clinical data suggest that a multi-targeted approach may be more efficacious than single target inhibition [20–24]. Given the poor outcome of GBM patients with unmethylated *MGMT*, we designed a trial for this cohort of patients with the rationale of targeting post RT upregulation of VEGF and EGFR using bevacizumab and erlotinib after treatment with RT and TMZ, in order to improve OS [25–28].

Methods

Patients with newly diagnosed GBM or gliosarcoma were eligible for this IRB approved trial. Registration was a two-step process. After signing an informed consent, tissue was sent for central pathology review (KA) confirming the GBM diagnosis and then *MGMT* promoter methylation status was assessed by a central laboratory (Lab Corp, NC). Unmethylated patients then moved onto to the second step of the trial after completion of RT; methylated patients were not enrolled. Consent had to be signed before the onset of radiation. A 1 cm³ block of tissue was required for *MGMT* analysis thereby excluding biopsy only patients.

For eligibility, patients had to be ≥ 18 years of age with a Karnofsky performance status (KPS) ≥ 70. Tumor was required to be supratentorial and a post operative MRI was required no more than 72 h after surgery or >4 weeks (±7 days) after surgery. Evaluable or measurable disease following resection of tumor was not mandated. No prior treatment was allowed including the use of carmustine implant (Gliadel® Wafer) or radiosurgery.

All patients were treated with 60 Gy of radiation with concomitant TMZ at 75 mg/m²/day × 42 days (±3 days). Eligible patients started the experimental regimen of bevacizumab and erlotinib 4 weeks (±7 days) after the completion of RT + TMZ. Eligibility was reassessed, as step 2 of registration, prior to initiation of the experimental treatment. Within 7 days of registration, patients were required to have adequate bone marrow, liver and renal function, a prothrombin time/international normalized ratio (PT/INR) <1.4 for patients not on warfarin and they could not have proteinuria as demonstrated by a urine protein: creatinine (UPC) ratio ≤ 1.0 or urine dipstick for protein-uria ≤ 2+ (if ≥ 2+ proteinuria on dipstick, then a 24 h urine collection was required to show ≤ 1 g of protein in 24 h). Patients could not have any significant medical illnesses that in the investigator's opinion could not be adequately controlled with appropriate therapy, would compromise the patient's ability to tolerate this therapy, obscure toxicity or dangerously alter drug metabolism. Patients could not have inadequately controlled hypertension (systolic blood pressure >150 and/or diastolic blood pressure >100 mmHg), prior history of hypertensive crisis or hypertensive encephalopathy, New York Heart Association Grade II or greater congestive heart failure, history of cardio or cerebro-vascular events within 6 months prior to study enrollment, significant vascular disease (e.g. history of aortic dissection) or evidence of bleeding diathesis or coagulopathy.

Patients on full-dose anticoagulants (i.e. warfarin or low molecular weight heparin) were required to meet both of the following criteria: no active bleeding or pathological condition that carried a high risk of bleeding (e.g. tumor involving major vessels or known varices) and for those on warfarin the INR needed to be 2–3 or the patient needed to be on a stable dose of low molecular weight heparin. Patients could not have a major surgical procedure, open biopsy, or significant traumatic injury within 28 days of starting bevacizumab or the anticipation of need for major or minor surgical procedure during the course of the study, excluding placement of a vascular access device. Patients could not have a history of abdominal fistula, gastrointestinal perforation, or intra-abdominal abscess within the preceding 6 months and no serious, non-healing wound, ulcer, or bone fracture. Patients with known HIV disease were excluded from participating in the trial. Patients could not have a history of any other cancer (except non-melanoma skin cancer or carcinoma in situ of the cervix), unless in complete remission and off of all therapy for >3 years. Patients could not be pregnant or breast feeding; a negative pregnancy test was required within 7 days before registration. Effective contraception (men and women) had to be used in subjects of child-bearing potential while on trial and for 3 months after. Patients could only be on non-enzyme inducing anti-convulsants. If they were on an enzyme inducing anti-convulsant, they had to be converted to non-enzyme inducing anti-convulsants; a 2 weeks wash out period was required before starting bevacizumab and erlotinib.

Pretreatment evaluation, within 7 days prior to registration step 2, included a complete history and neurologic examination with neuroimaging as noted. CBC with differential and platelets, PT, PTT, INR, electrolytes, BUN/Cr, LFTs, calcium, phosphorus, magnesium, serum pregnancy test for women of childbearing potential, UPC ratio or urine dipstick screening. EKG was required with 14 days prior to Step 2 registration.

Treatment with bevacizumab and erlotinib started on week 4 after completion of concurrent RT and TMZ. A baseline contrast enhanced MRI with optional perfusion imaging was required. Bevacizumab was dosed at 10 mg/kg every 2 weeks (± 2 days) as an intravenous infusion over 30–90 min. Premedication with Tylenol 650 mg and Benadryl 50 mg was administered for infusion reactions. Erlotinib was administered orally and was dosed at 150 mg each day continuously and toxicities were managed per guidelines in the package insert.

During treatment, patients underwent a neurologic examination and assessment of KPS approximately every 4 weeks. Repeat MR imaging was performed after every two cycles (approximately 8 weeks). CBC with differential and platelets, electrolytes/BUN/Cr was done within 72 h before each dose of bevacizumab. Urine was collected to determine the UPC ratio and proteinuria assessed within 72 h before each dose of Bevacizumab. Patients remained on treatment until disease progression based on Macdonald criteria (>25 % increase in size), unacceptable toxicity or clinically significant intratumoral hemorrhage (petechial hemorrhage did not affect treatment) on MRI. All patients were followed until death. Patients were evaluated for adverse events defined by the Common Toxicity Criteria version 3.0 at each study visit for the duration of their participation in the study. Patients discontinued from the treatment for any reason were evaluated for safety ~30 days (28–42 days) after the decision to discontinue treatment.

Statistics

The primary endpoint was the overall survival (OS), and secondary endpoints include the progression free survival (PFS) and response rate. OS and PFS were analyzed using the Kaplan–Meier product limit curve. Response rates were summarized as proportions with 95 % exact binomial confidence intervals. In the study by Hegi et al. [5], patients without *MGMT* methylation, treated with RT plus temozolomide, had a median overall survival of 12.7 months and a median progression-free survival of 5.3 months. Assuming exponential survival and a study duration of 48 months, a sample size of 50 patients will have 80 % power to detect a median survival of 17.9 months in this group of patients receiving RT + TMZ followed by bevacizumab + erlotinib, compared with 12.7 months in the historical RT + TMZ group (1). A one-tailed test and a Type I error rate of 5 % was used assuming that the addition of bevacizumab + erlotinib would not decrease median survival.

Results

One hundred and fifteen patients were screened to reach an enrollment of 48 patients whose tumors were *MGMT* unmethylated between 03/04/2009 and 11/07/2011. The remaining two patients were not enrolled, as they would not have lead to a positive trial. Two patients were not evaluable due to protocol ineligibility leaving 46 patients (29 men and 17 women) for treatment analysis. The median age was 55.5 years (range 29–75) and median KPS was 90 (range 70–100). At time of initial surgery 2 patients had undergone an open biopsy, 24 patients a gross total resection (including 1 who had a prior biopsy) and 20 a sub-total resection (including 2 who had a prior biopsy). Extent of surgery for the 46 eligible patients did not impact PFS ($p = 0.85$) or OS ($p = 0.9$). All patients completed standard RT with concomitant TMZ ($75 \text{ mg/m}^2/\text{day} \times 6$ weeks) and went on to receive treatment with bevacizumab and erlotinib. The number of cycles of bevacizumab and erlotinib ranged from 2 to 47 (median 8) with a single patient remaining on active treatment. Twenty-six patients received 8 or more cycles, 16 patients received 12 or more cycles and 4 patients received >20 cycles.

At the time of this report, 41 patients either progressed or died with a median PFS of 9.2 months [95 % CI (6.4, 11.3)]. At a median follow up of 33 months the median OS was 13.2 months [95 % CI (10.8, 19.6)] with 38 patients having died. The cause of death was due to tumor in all patients except 1 who had a pulmonary embolism. Using the median age of 65 as a cut-off for “elderly patients”, those <65 years of age had a median PFS and OS of 9.2 and 13.6 months and those ≥ 65 years of age had a median PFS and OS of 6.4 and 12.7 months (non-significant difference). In patients with a KPS of ≥ 90, the median PFS and OS were 9.3 and 14.8 months respectively, and for patients with KPS <90, the median PFS and OS were 7.3 and 11.8 months (not significant). Comparing follow up MRIs with the post-RT baseline imaging assessed response. Best response was a complete response in 4 patients, partial response in 12 patients and stable disease in 28 patients; 2 patients had no measurable disease on their post RT MRI and therefore not evaluable for response.

A total of 928 adverse events were reported that had a suspected relationship to the bevacizumab and erlotinib regimen. Most (885) were either grade 1 or 2. There were only 40

grade 3 and 3 grade 4 events, demonstrating that this regimen was well tolerated. Grade 3 and 4 toxicities are listed in Table 1. One patient each had a grade 4 stroke and bowel perforation, which is a known toxicity of our post radiation treatment.

At time of recurrence, 14 patients did not have any further treatment. The remaining patients received additional therapy including 9 undergoing repeat surgery, 2 had stereotactic radiosurgery and 6 had repeat RT. The number of times a systemic salvage chemotherapy regimen was used was 1 in 29 patients, 2 in 12 patients, 3 in 5 patients, and 5 in 1 patient.

Discussion

The current study assessed the benefit of using the combination of bevacizumab and erlotinib in newly diagnosed patients with unmethylated *MGMT* promoter GBM after completion of RT and TMZ as maintenance therapy post RT. The patients with unmethylated GBM were chosen because the benefit of adjuvant TMZ in this group is not clearly documented and given the poor survival, alternative treatment strategies are needed [1, 4]. There may be synergistic effects of combining TMZ with RT [29, 30] therefore the concurrent treatment was a component of the trial. Regardless, the study did not demonstrate an improvement in OS for this patient population as the observed 13.2 month median OS was only marginally better than the historical results of 12.7 months [5].

Given the poor prognosis for patients with *MGMT* unmethylated GBM, there has been increasing interest in eliminating TMZ from the standard treatment regimen and substituting other experimental treatments for these tumors (Table 2). For example, Wick et al. [31] performed a single arm trial of enzastaurin in newly diagnosed unmethylated GBM patients. Patients started enzastaurin 7 days prior to radiation, during RT (60 Gy) and then as maintenance treatment for up to 3 years. The PFS-6 was 53.6 % with a median PFS of 6.6 months; by pre-specified criteria was a negative study. The PFS rate at 12 and 24 months was 14.9 and 3.7 %. Median OS was 15 months with the 6, 12 and 24 month OS rates of 87.7, 63 and 27 % (Fig. 1).

The “CORE” trial (Cilengitide, Temozolomide, and Radiation Therapy in Treating Patients with Newly Diagnosed Glioblastoma and Unmethylated *MGMT* Gene Promoter Status) reported data only in abstract form [32]. In that trial, unmethylated GBM patients were treated with RT + TMZ or RT + TMZ plus standard or high-dose cilengitide. The OS for the groups were very similar to the results of our study with median survivals of 13.4, 16.3 and 14.5 months, respectively. The “Glarius” trial compared RT + TMZ followed by either TMZ or bevacizumab + irinotecan (B-I) in a randomized trial for unmethylated GBM patients [33]. Patients in the B-I arm received four cycles of bevacizumab over 6 weeks of radiation and then received B-I every 2 weeks until disease progression. Patients in the TMZ arm received six monthly courses of standard dose post-RT TMZ. 6 months following random assignment, PFS was significantly higher for patients receiving B-I (9.74 vs. 5.99 months for TMZ; $p < 0.0001$). The OS endpoint was significantly longer for patients receiving B-I (16.6 vs. 14.8 months for TMZ; $p = 0.031$).

Two large randomized trials stratified patients based on their *MGMT* status. RTOG 0525 compared dose dense (DD) to standard dose (SD) TMZ after RT and TMZ [2]. The median OS was 14.0 months for tumors with unmethylated *MGMT* gene promoter with a median PFS of 8.7 months. For patients with *MGMT* unmethylated tumors the median OS was 14.6 months for SD and 13.3 for the DD arm. Median PFS was 5.1 months for the SD arm and 6.0 months for the DD arm. RTOG 0825 was a randomized placebo controlled trial of RT + TMZ + bevacizumab or placebo that showed no improvement in PFS or OS [3]. For the patients who were unmethylated, the median OS was 14.3 months (95 % CI 13.6–15.3) and the median PFS was 8.2 months (95 % CI 7.5–9.2). The Avaglio trial was similar to RTOG 0825 and assessed outcome based on methylation status but this was not a stratification factor for randomization [12]. For the unmethylated patients with GBM, the median PFS was 5.8 months for the placebo group and 10 months for the bevacizumab treated group, while OS was 14.6 and 15.1 months, respectively.

Finally, a clinical trial at UCSF explored the combination of bevacizumab and erlotinib in patients with newly diagnosed GBM [34]. This study administered the combination therapy during RT in conjunction with TMZ then continued with all three agents (TMZ, bevacizumab and erlotinib) post-RT. The median OS was 17.5 months for unmethylated patients was not significantly different from local institution historical controls.

Given the high incidence of alterations of EGFR in GBM, including amplification, mutation, and over-expression, there has been great interest in EGFR inhibitors [35–37]. Some studies shown that response to EGFR inhibitors is greatest with co-expression of PTEN and EGFRvIII [35] and high levels of EGFR expression and low levels of phosphorylated protein kinase B (PKB)/Akt phosphorylation [36]. Conversely, the effect of EGFR inhibitors on EGFR phosphorylation and downstream signaling was ineffective when assessed in patients pre-treated with EGFR inhibitors [37]. Interestingly, in the UCSF trial [34] described above, there was no correlation of PTEN expression and EGFR amplification with OS. However, there was an improved OS for EGFR amplification compared to historical controls (13.3 vs. 7.4 months) and a trend for improvement in survival (19.8 vs. 15.5 months).

The data from our trial, and that of others, suggest that irrespective of the treatment used median survival in GBM patients with an unmethylated *MGMT* promoter is on the order of 13–15 months. Modest advances have been made in treating patients with GBM but those with an unmethylated *MGMT* promoter have fared less well. Moving forward, new therapeutic approaches are needed for this poorer prognosis group. In fact, this need has led to consideration of forgoing the use of temozolomide in this population to expand the range of new therapies that can be considered in the newly diagnosed setting. In the enzastaurin trial, omission of TMZ did not lead to a decreased survival compared to other trials where TMZ is used in unmethylated patients (Table 2). Finally, whether newer generation EGFR inhibitors might have therapeutic utility remains to be seen but many are currently under investigation.

Acknowledgments

Trial was supported by Genentech and “Head for the Cure Foundation”.

References

1. Stupp R, Hegi ME, Mason WP, van den Bent MJ, Taphoorn MJ, Janzer RC, Ludwin SK, Allgeier A, Fisher B, Belanger K, Hau P, Brandes AA, Gijtenbeek J, Marosi C, Vecht CJ, Mokhtari K, Wesseling P, Villa S, Eisenhauer E, Gorlia T, Weller M, Lacombe D, Cairncross JG, Mirimanoff RO, European Organisation for R, Treatment of Cancer Brain T, Radiation Oncology G, National Cancer Institute of Canada Clinical Trials G. 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 EORTCNCIC trial. *Lancet Oncol.* 2009; 10:459–466. doi:10.1016/S1470-2045(09)70025-7. [PubMed: 19269895]
2. Gilbert MR, Wang M, Aldape KD, Stupp R, Hegi ME, Jaeckle KA, Armstrong TS, Wefel JS, Won M, Blumenthal DT, Mahajan A, Schultz CJ, Erridge S, Baumert B, Hopkins KI, Tzuk-Shina T, Brown PD, Chakravarti A, Curran WJ Jr, Mehta MP. Dose-dense temozolomide for newly diagnosed glioblastoma: a randomized phase III clinical trial. *J Clin Oncol.* 2013; 31:4085–4091. doi:10.1200/JCO.2013.49.6968. [PubMed: 24101040]
3. Gilbert MR, Dignam JJ, Armstrong TS, Wefel JS, Blumenthal DT, Vogelbaum MA, Colman H, Chakravarti A, Pugh S, Won M, Jeraj R, Brown PD, Jaeckle KA, Schiff D, Stieber VW, Brachman DG, Werner-Wasik M, Tremont-Lukats IW, Sulman EP, Aldape KD, Curran WJ Jr, Mehta MP. A randomized trial of bevacizumab for newly diagnosed glioblastoma. *N Engl J Med.* 2014; 370:699–708. doi:10.1056/NEJMoa1308573. [PubMed: 24552317]
4. Stupp R, Mason WP, van den Bent MJ, Weller M, Fisher B, Taphoorn MJ, Belanger K, Brandes AA, Marosi C, Bogdahn U, Curschmann J, Janzer RC, Ludwin SK, Gorlia T, Allgeier A, Lacombe D, Cairncross JG, Eisenhauer E, Mirimanoff RO. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. *N Engl J Med.* 2005; 352:987–996. [PubMed: 15758009]
5. Hegi ME, Diserens AC, Gorlia T, Hamou MF, de Tribolet N, Weller M, Kros JM, Hainfellner JA, Mason W, Mariani L, Bromberg JE, Hau P, Mirimanoff RO, Cairncross JG, Janzer RC, Stupp R. MGMT gene silencing and benefit from temozolomide in glioblastoma. *N Engl J Med.* 2005; 352:997–1003. doi:10.1056/NEJMoa043331. [PubMed: 15758010]
6. Gupta K, Radotra BD, Banerjee AK, Nijhawan R. Quantitation of angiogenesis and its correlation with vascular endothelial growth factor expression in astrocytic tumors. *Anal Quant Cytol Histol.* 2004; 26:223–229. [PubMed: 15457675]
7. Maia AC Jr, Malheiros SM, da Rocha AJ, da Silva CJ, Gabbai AA, Ferraz FA, Stavale JN. MR cerebral blood volume maps correlated with vascular endothelial growth factor expression and tumor grade in nonenhancing gliomas. *AJNR Am J Neuroradiol.* 2005; 26:777–783. [PubMed: 15814920]
8. Friedman HS, Prados MD, Wen PY, Mikkelsen T, Schiff D, Abrey LE, Yung WK, Paleologos N, Nicholas MK, Jensen R, Vredenburgh J, Huang J, Zheng M, Cloughesy T. Bevacizumab alone and in combination with irinotecan in recurrent glioblastoma. *J Clin Oncol.* 2009; 27(28):4733–4740. [PubMed: 19720927]
9. Kreisl TN, Kim L, Moore K, Duic P, Royce C, Stroud I, Garren N, Mackey M, Butman JA, Camphausen K, Park J, Albert PS, Fine HA. Phase II trial of single-agent bevacizumab followed by bevacizumab plus irinotecan at tumor progression in recurrent glioblastoma. *J Clin Oncol.* 2009; 27:740–745. [PubMed: 19114704]
10. Batchelor TT, Mulholland P, Neyns B, Nabors LB, Campone M, Wick A, Mason W, Mikkelsen T, Phuphanich S, Ashby LS, Degroot J, Gattamaneni R, Cher L, Rosenthal M, Payer F, Jurgensmeier JM, Jain RK, Sorensen AG, Xu J, Liu Q, van den Bent M. Phase III randomized trial comparing the efficacy of cediranib as monotherapy, and in combination with lomustine, versus lomustine alone in patients with recurrent glioblastoma. *J Clin Oncol.* 2013; 31:3212–3218. doi:10.1200/JCO.2012.47.2464. [PubMed: 23940216]
11. de Groot JF, Lamborn KR, Chang SM, Gilbert MR, Cloughesy TF, Aldape K, Yao J, Jackson EF, Lieberman F, Robins HI, Mehta MP, Lassman AB, Deangelis LM, Yung WK, Chen A, Prados MD, Wen PY. Phase II study of aflibercept in recurrent malignant glioma: a North American Brain Tumor Consortium study. *J Clin Oncol.* 2011; 29:2689–2695. doi:10.1200/JCO.2010.34.1636. [PubMed: 21606416]

12. Chinot OL, Wick W, Mason W, Henriksson R, Saran F, Nishikawa R, Carpentier AF, Hoang-Xuan K, Kavan P, Cernea D, Brandes AA, Hilton M, Abrey L, Cloughesy T. Bevacizumab plus radiotherapy-temozolomide for newly diagnosed glioblastoma. *N Engl J Med*. 2014; 370:709–722. doi:10.1056/NEJMoa1308345. [PubMed: 24552318]
13. Network CGAR. Comprehensive genomic characterization defines human glioblastoma genes and core pathways. *Nature*. 2008; 455(7216):1061–1068. [PubMed: 18772890]
14. Raizer JJ, Abrey LE, Lassman AB, Chang SM, Lamborn KR, Kuhn JG, Yung WK, Gilbert MR, Aldape KA, Wen PY, Fine HA, Mehta M, Deangelis LM, Lieberman F, Cloughesy TF, Robins HI, Dancey J, Prados MD. A phase II trial of erlotinib in patients with recurrent malignant gliomas and nonprogressive glioblastoma multiforme postradiation therapy. *Neuro-oncol*. 2010; 12:95–103.
15. Peereboom DM, Shepard DR, Ahluwalia MS, Brewer CJ, Agarwal N, Stevens GH, Suh JH, Toms SA, Vogelbaum MA, Weil RJ, Elson P, Barnett GH. Phase II trial of erlotinib with temozolomide and radiation in patients with newly diagnosed glioblastoma multiforme. *J Neurooncol*. 2010; 98:93–99. doi:10.1007/s11060-009-0067-2. [PubMed: 19960228]
16. Rich JN, Reardon DA, Peery T, Dowell JM, Quinn JA, Penne KL, Wikstrand CJ, Van Duyn LB, Dancey JE, McLendon RE, Kao JC, Stenzel TT, Ahmed Rasheed BK, Tourt-Uhlig SE, Herndon JE 2nd, Vredenburgh JJ, Sampson JH, Friedman AH, Bigner DD, Friedman HS. Phase II trial of gefitinib in recurrent glioblastoma. *J Clin Oncol*. 2004; 22:133–142. doi:10.1200/JCO.2004.08.110. [PubMed: 14638850]
17. Yung WK, Vredenburgh JJ, Cloughesy TF, Nghiemphu P, Klencke B, Gilbert MR, Reardon DA, Prados MD. Safety and efficacy of erlotinib in first-relapse glioblastoma: a phase II open-label study. *Neuro-oncol*. 2010; 12:1061–1070. doi:10.1093/neuonc/noq072. [PubMed: 20615922]
18. van den Bent MJ, Brandes AA, Rampling R, Kouwenhoven MC, Kros JM, Carpentier AF, Clement PM, Frenay M, Campone M, Baurain JF, Armand JP, Taphoorn MJ, Tosoni A, Kletzl H, Klughammer B, Lacombe D, Gorlia T. Randomized phase II trial of erlotinib versus temozolomide or carmustine in recurrent glioblastoma: EORTC brain tumor group study 26034. *J Clin Oncol*. 2009; 27:1268–1274. doi:10.1200/JCO.2008.17.5984. [PubMed: 19204207]
19. Eisenstat DD, Nabors LB, Mason WP, Perry JR, Shapiro WR, Kavan P, Phuphanich S, Fu Y, Cong XJ, Shahidi M, Reardon DA. A phase II study of daily afatinib (BIBW 2992) with or without temozolomide (21/28 days) in the treatment of patients with recurrent glioblastoma. *J Clin Oncol* (Meeting Abstracts). 2011; 29(15_suppl) 2010.
20. Goudar RK, Shi Q, Hjelmeland MD, Keir ST, McLendon RE, Wikstrand CJ, Reese ED, Conrad CA, Traxler P, Lane HA, Reardon DA, Cavenee WK, Wang XF, Bigner DD, Friedman HS, Rich JN. Combination therapy of inhibitors of epidermal growth factor receptor/vascular endothelial growth factor receptor 2 (AEE788) and the mammalian target of rapamycin (RAD001) offers improved glioblastoma tumor growth inhibition. *Mol Cancer Ther*. 2005; 4:101–112. [PubMed: 15657358]
21. Fan QW, Specht KM, Zhang C, Goldenberg DD, Shokat KM, Weiss WA. Combinatorial efficacy achieved through two-point blockade within a signaling pathway—a chemical genetic approach. *Cancer Res*. 2003; 63:8930–8938. [PubMed: 14695210]
22. Wang MY, Lu KV, Zhu S, Dia EQ, Vivanco I, Shackelford GM, Cavenee WK, Mellinghoff IK, Cloughesy TF, Sawyers CL, Mischel PS. Mammalian target of rapamycin inhibition promotes response to epidermal growth factor receptor kinase inhibitors in PTEN-deficient and PTEN-intact glioblastoma cells. *Cancer Res*. 2006; 66:7864–7869. doi:10.1158/0008-5472.CAN-04-4392. [PubMed: 16912159]
23. Zhu Y, Shah K. Multiple lesions in receptor tyrosine kinase pathway determine glioblastoma response to pan-ERBB inhibitor PF-00299804 and PI3 K/mTOR dual inhibitor PF-05212384. *Cancer Biol Ther*. 2014; 15:815–822. doi:10.4161/cbt.28585. [PubMed: 24658109]
24. Verreault M, Wepler SA, Stegeman A, Warburton C, Strutt D, Masin D, Bally MB. Combined RNAi-mediated suppression of Rictor and EGFR resulted in complete tumor regression in an orthotopic glioblastoma tumor model. *PLoS ONE*. 2013; 8:e59597. doi:10.1371/journal.pone.0059597. [PubMed: 23555046]
25. Lammering G, Valerie K, Lin PS, Mikkelsen RB, Contessa JN, Feden JP, Farnsworth J, Dent P, Schmidt-Ullrich RK. Radiosensitization of malignant glioma cells through overexpression of

- dominant-negative epidermal growth factor receptor. *Clin Cancer Res.* 2001; 7:682–690. [PubMed: 11297265]
26. Bowers G, Reardon D, Hewitt T, Dent P, Mikkelsen RB, Valerie K, Lammering G, Amir C, Schmidt-Ullrich RK. The relative role of ErbB1-4 receptor tyrosine kinases in radiation signal transduction responses of human carcinoma cells. *Oncogene.* 2001; 20:1388–1397. doi:10.1038/sj.onc.1204255. [PubMed: 11313882]
 27. Hovinga KE, Stalpers LJ, van Bree C, Donker M, Verhoeff JJ, Rodermond HM, Bosch DA, van Furth WR. Radiation-enhanced vascular endothelial growth factor (VEGF) secretion in glioblastoma multiforme cell lines—a clue to radioresistance? *J Neurooncol.* 2005; 74:99–103. doi: 10.1007/s11060-004-4204-7. [PubMed: 16193379]
 28. Lund EL, Hog A, Olsen MW, Hansen LT, Engelholm SA, Kristjansen PE. Differential regulation of VEGF, HIF1alpha and angiopoietin-1, -2 and -4 by hypoxia and ionizing radiation in human glioblastoma. *Int J Cancer.* 2004; 108:833–838. doi:10.1002/ijc.11662. [PubMed: 14712484]
 29. Wedge SR, Porteous JK, Glaser MG, Marcus K, Newlands ES. In vitro evaluation of temozolomide combined with X-irradiation. *Anticancer Drugs.* 1997; 8:92–97. [PubMed: 9147618]
 30. van Rijn J, Heimans JJ, van den Berg J, van der Valk P, Slotman BJ. Survival of human glioma cells treated with various combination of temozolomide and X-rays. *Int J Radiat Oncol Biol Phys.* 2000; 47:779–784. [PubMed: 10837964]
 31. Wick W, Steinbach JP, Platten M, Hartmann C, Wenz F, von Deimling A, Shei P, Moreau-Donnet V, Stoffregen C, Combs SE. Enzastaurin before and concomitant with radiation therapy, followed by enzastaurin maintenance therapy, in patients with newly diagnosed glioblastoma without MGMT promoter hypermethylation. *Neurooncol.* 2013; 15:1405–1412. doi:10.1093/neuonc/not100.
 32. Nabors LB, Fink KL, Mikkelsen T, Grujicic D, Tarnawski R, Nam do H, Mazurkiewicz M, Salacz M, Ashby L, Zagonel V, Depenni R, Perry JR, Hicking C, Picard M, Hegi ME, Lhermitte B, Reardon DA. Two cilengitide regimens in combination with standard treatment for patients with newly diagnosed glioblastoma and unmethylated MGMT gene promoter: results of the open-label, controlled, randomized phase II CORE study. *Neurooncol.* 2015; 17:708–717. doi:10.1093/neuonc/nou356.
 33. Herrlinger U, Schaefer N, Steinbach JP, Weyerbrock A, Hau P, Goldbrunner R, Friedrich F, Stockhammer F, Ringel F, Braun C, Kohnen R, Leutgeb B, Belka C, Urbach H, Stummer W, Glas M. Bevacizumab, irinotecan, and radiotherapy versus standard temozolomide and radiotherapy in newly diagnosed, MGMT-nonmethylated glioblastoma patients: First results from the randomized multicenter GLARIUS trial. *ASCO Annual Meeting Proceedings.* 2013; 31(18_suppl):LBA2000.
 34. Clarke JL, Molinaro AM, Phillips JJ, Butowski NA, Chang SM, Perry A, Costello JF, Desilva AA, Rabbitt JE, Prados MD. A single-institution phase II trial of radiation, temozolomide, erlotinib, and bevacizumab for initial treatment of glioblastoma. *Neurooncol.* 2014 doi:10.1093/neuonc/nou029.
 35. Mellinghoff IK, Wang MY, Vivanco I, Haas-Kogan DA, Zhu S, Dia EQ, Lu KV, Yoshimoto K, Huang JH, Chute DJ, Riggs BL, Horvath S, Liau LM, Caveness WK, Rao PN, Beroukhi R, Peck TC, Lee JC, Sellers WR, Stokoe D, Prados M, Cloughesy TF, Sawyers CL, Mischel PS. Molecular determinants of the response of glioblastomas to EGFR kinase inhibitors. *N Engl J Med.* 2005; 353:2012–2024. doi:10.1056/NEJMoa051918. [PubMed: 16282176]
 36. Haas-Kogan DA, Prados MD, Tihan T, Eberhard DA, Jelluma N, Arvold ND, Baumber R, Lamborn KR, Kapadia A, Malec M, Berger MS, Stokoe D. Epidermal growth factor receptor, protein kinase B/Akt, and glioma response to erlotinib. *J Natl Cancer Inst.* 2005; 97:880–887. doi: 10.1093/jnci/dji161. [PubMed: 15956649]
 37. Lassman AB, Rossi MR, Raizer JJ, Abrey LE, Lieberman FS, Greife CN, Lamborn K, Pao W, Shih AH, Kuhn JG, Wilson R, Nowak NJ, Cowell JK, DeAngelis LM, Wen P, Gilbert MR, Chang S, Yung WA, Prados M, Holland EC. Molecular study of malignant gliomas treated with epidermal growth factor receptor inhibitors: tissue analysis from North American Brain Tumor Consortium Trials 01-03 and 00-01. *Clin Cancer Res.* 2005; 11:7841–7850. doi: 10.1158/1078-0432.CCR-05-0421. [PubMed: 16278407]

Fig. 1.

Progression free and overall survival in patients with unmethylated *MGMT* promoter treated with RT and TMZ and then bevacizumab and erlotinib

Table 1

Grade 3 and 4 toxicities

Adverse term/event	Grade 3	Grade 4
Hyperbilirubinemia	1	0
Cerebrovascular ischemia	0	1
Dehydration	1	0
Dermatology/skin	1	0
Hypertension	3	0
Fatigue	3	0
Leukocytes	1	0
Lymphopenia	12	0
Heartburn/dyspepsia	1	0
Pain NOS	1	0
Pain abdomen	1	0
Pain head/headache	1	0
Bowel perforation	0	1
Hypophosphatemia	0	1
Rash/desquamation	5	0
Rash/acneform	2	0
Syncope	1	0
Thrombosis/embolism	2	0
Wound complication	1	0

Table 2PFS and OS in patients with *MGMT*unmethylated promoter

	Agent	PFS (m)	OS (m)
Wick [31]	RT + enzastaurin	6.6	15
Nabors [32]	RT + TMZ and adjuvant TMZ		13.4
	RT + TMZ + Standard dose celengitide		16.3
	RT + TMZ + high dose celengitide		14.5
Herrlinger [33]	RT + TMZ and adjuvant TMZ	5.99	14.8
	RT + TMZ and bevacizumab + irinotecan	9.74	16.6
RTOG 0525 [2]	RT + TMZ adjuvant standard dose TMZ	5.1	14.6
	RT + TMZ adjuvant dose dense TMZ	6.0	13.3
RTOG 0825 [3]	RT + TMZ adjuvant TMZ + placebo (favorable/unfavorable molecular profile)	7.3/5.4	14.6/14.6
	RT + TMZ adjuvant TMZ + bevacizumab (Favorable/unfavorable molecular profile)	10.1/9.8	13.9/14.0
Avagliano [12]	RT + TMZ adjuvant TMZ + placebo	5.8	14.6
	RT + TMZ adjuvant TMZ + bevacizumab	10	15.1
Clarke [34]	RT + TMZ + erlotinib + bevacizumab	12.4	17.5
Curent trial	RT + TMZ adjuvant erlotinib + bevacizumab	9.2	13.2