

## Phase II trial of erlotinib with temozolomide and radiation in patients with newly diagnosed glioblastoma multiforme

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**Abstract** Approximately 40–50% of glioblastomas (GBM) overexpress epidermal growth factor receptor (EGFR). Erlotinib is a specific and potent EGFR tyrosine kinase inhibitor active against refractory GBM. Patients with non-small cell lung cancer and  $\geq$  grade 2 erlotinib-induced rash have improved survival. This phase 2 study assessed the efficacy and safety of concurrent radiation therapy (RT) and temozolomide with pharmacodynamic dose escalation of erlotinib in patients with newly diagnosed GBM. Patients received RT 60 Gy in 30 fractions with concurrent temozolomide 75 mg/m<sup>2</sup>/day  $\times$  42 days, followed in four weeks by temozolomide 150–200 mg/m<sup>2</sup>/day  $\times$  5, every 28 days for 12 cycles. Patients received erlotinib, 50 mg/day and increased by 50 mg/day every 2 weeks until the occurrence of grade 2 rash or to a maximum dose of 150 mg/day,

from day 1 until disease progression. Twenty-seven patients were treated in this study. Twenty-two (81%) patients came off study for progressive disease (18 [67%]) or adverse events (4 [15%]). Eighteen patients (67%) have died. Median progression-free survival was 2.8 months, and the median overall survival was 8.6 months. Five patients remain on study with a median follow-up of 16 months. Grade 3/4 toxicities included thrombocytopenia, anemia, lymphopenia, fatigue, and febrile neutropenia. There were four deaths on study, three definitely treatment-related; therefore, the trial was terminated after accrual of 27 of 30 planned patients. Erlotinib co administered with RT and temozolomide was not efficacious and had an unacceptable toxicity.

**Keywords** EGFR inhibitor · Temozolomide · Erlotinib · Glioblastoma multiforme · Newly diagnosed · Chemotherapy · Efficacy · Phase II

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### Introduction

Glioblastoma multiforme (GBM) is an aggressive primary tumor of the central nervous system accounting for about 50% of all adult gliomas [1]. Based on a significant survival advantage provided by the use of concurrent chemoradiotherapy and adjuvant chemotherapy with temozolomide demonstrated in a recent phase III trial, this regimen is now considered to be standard of care for newly diagnosed GBM [2]. Despite an improvement in overall survival (OS) with this regimen, the prognosis of patients with GBM remains poor with a median OS of 9 to 15 months and a 2-year survival rate of 9–26% [2, 3].

Approximately 40–50% of GBM overexpress epidermal growth factor receptor (EGFR), a type I receptor tyrosine

kinase correlated with an aggressive phenotype [4–6]. EGFR activates an intracellular tyrosine kinase that leads to a signal transduction cascade that enhances survival and infiltration of GBM cells in vitro [7, 8]. Overexpression of EGFR correlates with increased cellular proliferation, tumorigenesis, decreased apoptosis, and a poor prognosis [5, 7, 9]. In addition, EGFR overexpression in GBM correlates with radioresistance [10, 11].

Erlotinib (Tarceva™) is a specific, potent inhibitor of EGFR tyrosine kinase with antitumor activity in lung and pancreatic cancer [12, 13]. In preclinical studies in GBM cell lines, erlotinib suppressed anchorage-independent growth, had antiproliferative and apoptotic effects, and had activity against cell lines that harbor the EGFRvIII mutant receptor [14, 15]. Erlotinib alone or with temozolomide has antitumor activity in phase I or II studies with patients with stable or refractory GBM [16, 17]. Erlotinib was well tolerated in phase I trials of patients with solid malignancies, including newly diagnosed GBM; grade 3 rash, fatigue, diarrhea, or stomatitis occurred in 5–15% of patients [16, 18].

In this study, we investigated the efficacy and safety of erlotinib in combination with the standard regimen of temozolomide and radiotherapy (RT) in patients with newly diagnosed GBM. In studies of erlotinib in patients with non-small cell lung cancer, survival correlated with development of grade 2 skin rash, suggesting a physiologic marker for maximal dose [19]. The primary objective of this trial was to determine progression free survival (PFS) and OS. Toxicity and the feasibility of pharmacodynamic dose escalation were secondary objectives.

## Methods

### Patients

Patients with histologically-proven, newly diagnosed GBM who were within 28 days of their diagnostic biopsy or resection, were at least 18 years old, and with a Karnofsky performance score of at least 60 were eligible for this IRB-approved study. Prior administration of temozolomide, tyrosine kinase inhibitors, or cranial radiation, or concurrent administration of hepatic cytochrome P-450 enzyme-inducing antiepileptic drugs (EIAEDs) such as phenytoin, carbamazepine, phenobarbital, felbamate, oxycarbazepine, or topiramate were not allowed. Patients on EIAEDs were transitioned to levetiracetam prior to starting the trial. An absolute neutrophil count  $>1.5 \times 10^9$  per liter, a platelet count  $>100 \times 10^9$  per liter; hemoglobin  $>90$  g/l, serum creatinine and total serum bilirubin  $<1.5$  times the upper limit of normal, aspartate aminotransferase or alanine

aminotransferase  $<2.5$  times the upper limit of normal, and alkaline phosphatase  $<2.5$  times the upper limit of normal were required. Patients of reproductive potential were required to use effective contraception. Written informed consent was required prior to treatment.

Patients with a severe underlying disease, including human immunodeficiency virus or chronic hepatitis B or C infection were excluded. Other antineoplastic therapy during study drug administration was prohibited. Women who were pregnant or breast feeding were excluded. Patients with any malignancy within 3 years with the exception of surgically cured carcinoma-in situ of the cervix, non-melanoma skin cancer, or adequately treated stage I or II cancer in complete remission were excluded. Demonstration of EGFR expression by the tumor or presence of measurable disease on neuroimaging was not required for enrollment in the trial.

### Study design

All patients were treated at the Cleveland Clinic. RT was administered in 2 Gy/day fractions, 5 days per week, to a total of 60 Gy over six weeks. The target volume for the initial and cone-down volume was based on the post-operative MRI. The initial target volume included the edema on T2 sequences plus a 2.0-cm margin; if no edema was present, a 2.5 cm margin beyond the contrast-enhanced lesion and resection cavity was treated. The initial target volume was treated with 46 Gy in 23 fractions. The cone-down tumor volume included the resection cavity with contrast-enhancing lesion plus a 2.5-cm margin.

Temozolomide administration began on day 1 of RT at 75 mg/m<sup>2</sup>/day and continued for 42 days. Temozolomide was taken while fasting, 1 h before RT, and in the morning on days without RT. All patients received a single inhaled dose of pentamidine for prophylaxis against pneumocystis pneumonia during weeks 1 and 4 of RT. The initial dose of erlotinib was 50 mg/day, beginning on Day 1 of RT, with escalation by 50 mg/day every 2 weeks until the occurrence of an erlotinib-induced grade 2 or greater rash outside the radiation field or a maximum dose of 150 mg/day. Erlotinib was continued until disease progression. Patients who developed seizures were treated with non-EIAEDs. Grade 2 or higher skin rash was treated with minocycline, topical tetracycline, topical retinoids or oral antibacterial agents.

An MRI was obtained four weeks after completion of RT as a baseline. Patients received temozolomide daily for 5 days during every 28 day cycle for up to 12 cycles. Patients received temozolomide 150 mg/m<sup>2</sup>/day for 5 days for cycle 1. In the absence of grade 3 or 4 neutropenia or

thrombocytopenia during cycle 1, patients received temozolomide 200 mg/m<sup>2</sup>/day for 5 days for 11 additional cycles. CBCs were monitored weekly during weeks 3–6 of radiation therapy and subsequently on day 1 of each post-radiation cycle of therapy. Compliance with this schedule of monitoring was estimated to be at least 95%.

Hematopoietic growth factors were only used in patients with persistent refractory neutropenia. Prophylactic antiemetics were used during RT at the discretion of the investigator. Anticonvulsants and corticosteroids were administered as needed with EIAEDs avoided if possible. Patients who inadvertently received EIAEDs during the study transitioned to non-EIAEDs as soon as feasible.

#### Dose reductions

During RT, temozolomide was held and not restarted for an ANC <1500/μl or platelets <100,000/μl. There was no dose reduction for temozolomide during RT. During post-RT temozolomide, the cycle was initiated once ANC ≥1500 and platelets ≥100,000. In patients with grade 3 temozolomide-related toxicity, temozolomide was held until the toxicity returned to grade 0–1. If the ANC nadir from the previous cycle was <500 and/or platelet nadir was <20,000, the temozolomide dose was decreased by 25% for the subsequent cycle. For reversible, grade 4 non-hematological temozolomide-related toxicity, the temozolomide dose was reduced to 80% of the dose for the previous cycle for all subsequent cycles. If temozolomide-related toxicity did not resolve within 2 weeks or if the toxicity was not tolerated, temozolomide was discontinued, but any remaining RT and erlotinib was given.

Erlotinib was held for grade ≥3 rash until improvement to grade ≤2 and resumed with a 50 mg/day dose reduction. If erlotinib-related toxicity did not resolve within 2 weeks or the toxicity was intolerable, erlotinib was discontinued but any remaining RT and temozolomide was given.

#### Assessment of response and safety

Patients were removed from the study for unacceptable toxicity or toxicity leading to discontinuation of both temozolomide and erlotinib. Other criteria for study discontinuation were patient choice, noncompliance, serious concurrent illness, or progressive disease. Progressive disease was defined as ≥25% increase in the sum of the products of all T1 weighted gadolinium-enhancing measurable disease compared with the baseline measurement, or the appearance of new lesions. A PET scan, blood volume MRI or tissue biopsy were attempted, if necessary, to distinguish tumor growth from pseudo-progression. Cases of possible progression were also reviewed as necessary at a multidisciplinary brain tumor conference.

Physical examinations, assessment of routine labs, and evaluation of toxicity were conducted prior to the study and at scheduled intervals throughout the study. A gadolinium-enhanced MRI of brain was performed within 3 weeks before starting radiation and before cycles 1, 3, 5, 7, 9, and 11 of post radiation temozolomide. An MRI was obtained every 2 months during the first year after completion of temozolomide and every 3 months thereafter.

#### Statistical methods

The primary endpoints of the study were PFS and OS. Assuming a 6-month PFS of 50%, based on historical controls, it was estimated that 30 patients would need to be assessed and followed for at least six months in order to have 80% power, based on a 2-sided  $P = .05$  exact binomial test, to detect an increase in PFS to 75% [20–22].

With 30 patients, the incidence of specific toxicities, and key quantiles such as 6-month and 1-year PFS and OS could be estimated using 95% confidence intervals with maximum half-widths of 19%. PFS and OS were calculated from the date of study entry to the date of disease progression, death, or final follow-up and were summarized using the method of Kaplan and Meier. If the underlying incidence for a specific toxicity was ≤5%, there was a ≥79% chance of observing the toxicity in at least one of the 30 patients enrolled in the study. Statistical significance was defined as  $P < 0.05$  and statistical tests were two-sided. All analyses were conducted using SAS<sup>®</sup> version 8.0 software (SAS Institute, Inc., Cary, NC).

#### Methyl guanine methyl transferase (MGMT) promoter methylation analysis

Nested polymerase chain reaction (PCR) was used for amplification of bisulfite treated genomic DNA. First round PCR MGMT primers: forward is TTTTTTTGTTTTTTTTTGGTTTT and reverse is AACCTAAACTAACACCTAAA. The annealing temperature was 45°C. The second round PCR MGMT primers: forward is CCTAATGTTGGGATAGTT, reverse is CAACATCACTAACACCTAACC, and the annealing temperature was 60°C. Second reverse primer sequences are derived from Parkinson et al. and from the NCBI sequence site; primer sequences are available on request [23]. Platinum TaqDNA Polymerase (Invitrogen, California, USA) was used according to the manufacturer's instructions. PCR amplicons were purified using QIAquick PCR Purification Kit PCR (QIAGEN Inc. Valencia, California, USA). Sequencing was performed in forward and reverse directions using the same primers as those used for the second round PCR, according to standard techniques. Colon cancer line SW48, in which all 28 promoter sites are methylated was used as a positive control.

## Results

### Patient characteristics

Twenty-eight patients were enrolled between December 2003 and December 2005. One patient did not receive treatment and was excluded from all efficacy and safety analyses. Among the 27 evaluable patients, the median age was 52 years and the median Karnofsky performance status (KPS) was 90 (Table 1). Eight patients had gross total resection, eight had subtotal resection, and 11 had biopsy only. RPA grouping was as follows: group III–11 patients; group IV–7; and group V–9 [24]. EGFR was amplified in 9 patients, not amplified in 17 patients, and not determined in 1 patient.

### Efficacy

Twenty of the 27 patients (74%) completed the first 42 day treatment period during which chemotherapy was given concurrently with radiation, with no dose modifications. Three patients had treatment delayed, and four patients discontinued treatment with erlotinib and/or temozolomide due to toxicity. Thirteen patients (48%) continued on treatment post radiation and received a median of five cycles of therapy (range 1–18+).

Twenty-two (81%) patients had progressive disease and 4 (15%) patients came off study for adverse events. Eighteen patients (67%) have died. The 6-month progression free survival was 30%, and the median progression-free survival was 2.8 months. The median overall survival was 8.6 months. Five patients remain on study with a median follow-up of 16 months (range 9.7–21.5 months).

There was a trend but no statistical correlation between efficacy and EGFR amplification. The overall survival of

**Table 1** Patient demographics

Characteristic	
Age, years (median, range)	52 (18–74)
Sex (n, %)	
Men	17 (63)
Women	10 (37)
Karnofsky performance status (n, %)	
90–100	18 (67)
60–80	9 (33)
Extent of surgery (n, %)	
Gross total resection	8 (30)
Subtotal resection	8 (30)
Biopsy only	11 (40)
EGFR status (n = 26) (n, %)	
Amplified	9 (35)
Negative	17 (65)

**Table 2** Treatment-related adverse events

Adverse event (27 patients)	Grade (n)			
	1–2	3	4	5
Neutropenia with fever	0	1	0	2
Sepsis without neutropenia	0	0	0	1
Pneumocystis pneumonia	0	0	0	1
Neutropenia without fever	4	4	0	0
Thrombocytopenia	4	4	4	0
Lymphopenia	2	15	0	0
Anemia	6	3	1	0
ALT increase	10	2	0	0
Fatigue	23	2	0	0

those with and without EGFR amplification was 11 months and 8 months, respectively ( $P = 0.13$ ). The failure free survival of those with and without EGFR amplification was 5.9 months and 2.6 months, respectively ( $P = 0.16$ ).

### Toxicity

The maximum dose of erlotinib reached before the occurrence of grade 2 rash was 50 mg/day in one patient, 100 mg/day in five patients, and 150 mg/day in 21 patients. Four deaths occurred during the study, three of which were definitely related to treatment. Three deaths occurred in the last four patients accrued to the trial with termination of the trial after enrolling 27 of the 30 planned patients. One patient died of pneumocystis carinii pneumonia (PCP), confirmed by bronchoscopy, despite prophylaxis with pentamidine. Two patients died of refractory bone marrow aplasia and one died of non-neutropenic sepsis. Treatment-related Grade 3 or 4 toxicities included thrombocytopenia, anemia, lymphopenia, fatigue, and febrile neutropenia (Table 2).

### MGMT promoter methylation analysis

Adequate paraffin-embedded tissue was available for 10 patients. After appropriate processing and digestion, suitable genomic DNA for analysis was obtained in four patients. Each of the four patients in whom adequate DNA was available for bisulfite methylation analysis showed methylation of at least one of 28 potential *MGMT* promoter sites (range, 1–17 sites methylated; average of seven sites; median value five sites).

## Discussion

The goal of this study was to estimate the PFS and OS in patients with newly diagnosed GBM treated with erlotinib

and RT-temozolomide. The primary objective of 6-month PFS of 75% was not met. In this study the 6-month PFS was 30%, the median PFS was 2.8 months, and the median OS was 8.6 months. These results are substantially worse than the median PFS of 6.9 months and the OS of 14.6 months for patients who receive RT with concurrent temozolomide [2]. The short PFS might raise the question as to whether patients were taken off study prematurely for pseudo-progression [25]. Cases in question were reviewed by a multidisciplinary brain tumor board. At least one patient had a biopsy performed which confirmed progression. The short overall survival of patients in this study also speaks against pseudo-progression as a significant event for patients in this trial.

Although inhibition of EGFR with receptor tyrosine kinase inhibitors, such as erlotinib or gefitinib showed promise when this study was designed, these agents have subsequently shown only moderate activity as single agents in patients with GBM [17, 26–29]. In one trial, 10 of 24 (42%) patients with recurrent or progressive GBM receiving erlotinib had a partial response or stable disease with a median time to progression of about 4.5 months [17]. Analogous results were observed with EGFR antagonists in patients with non-small cell lung cancer in that second and third line therapy with single agent gefitinib or erlotinib produced survival benefits while these agents combined with standard cytotoxic regimens as first-line therapy for metastatic disease showed no benefit [30–32].

There are several proposed mechanisms for the poor efficacy of single agent erlotinib and the antagonism of erlotinib in combination with chemotherapy. Although activation of EGFR is important for the activation of phosphatidylinositol 3-kinase (PI3K) and Akt, numerous receptor kinases may be activated by GBM cells and treatment with a single tyrosine kinase inhibitor, such as erlotinib, is not sufficient to decrease cell signaling and anchorage-independent growth [33]. One potential mechanism for antagonism is that EGFR inhibitors, such as erlotinib, may cause cell cycle arrest thereby making cells less sensitive to the cell cycle dependent effects of radiation therapy and temozolomide. In vitro and preclinical studies have shown schedule dependent interactions between the EGFR inhibitors and chemotherapy. The tyrosine kinase inhibitor gefitinib significantly improved the antitumor activity of paclitaxel in a human mammary carcinoma xenograft model, but only when gefitinib was given on a pulsatile schedule, not with continuous dosing [34]. Similarly, the cytotoxicity of erlotinib with pemetrexed in NSCLC cells was synergistic when administered together or with pemetrexed followed by erlotinib, however there was antagonistic activity when erlotinib was administered prior to pemetrexed in erlotinib-sensitive cells [35]. The combination of erlotinib and bortezomib

had additive cytotoxicity in the H358 bronchioalveolar cell line, but there was schedule dependence with the additive effect only when the two agents were used together or bortezomib was added first [36]. The combination of erlotinib followed by bortezomib had little activity. The EGFR receptor inhibitors cause G1 cell cycle arrest while temozolomide causes cell cycle arrest in G2-M [37]. Erlotinib and gefitinib may prevent cells from progressing beyond G1 and may therefore compromise the activity of other cell cycle-specific agents, such as temozolomide.

At the time this trial was designed, the prognostic or predictive value of MGMT promoter methylation and the presence of PTEN and EGFRvIII expression and of phosphorylated PKB/Akt were not known [2, 27, 38, 39]. Therefore these assays were not included in the design of this trial. The tissue available after the trial was complete was analysed for MGMT promoter methylation status. Of the 10 patients for whom adequate amounts of tissue were available for analysis, in only four patients could suitable, full or near-full length genomic DNA be obtained to allow analysis of the MGMT promoter. In each of these four, at least one MGMT promoter site was methylated. It is unclear that these results affected response to therapy in these patients since the effect of methylation of one of more sites of the MGMT promoter on post-transcriptional, translational and post-translational levels of the protein remains uncertain. This small cohort of patients may have included a high proportion of patients whose tumors had an unmethylated MGMT promoter. The poor response to erlotinib could have resulted from a high proportion of patients whose tumors lacked EGFRvIII or had wild type PTEN [26]. In addition the poor outcomes could not be explained by an adverse grouping of patients in poor prognosis RPA groups. Finally, during the conduct of this trial, no concurrent trials at our institution selected out better prognosis patients in a manner that would have left predominantly poor prognosis patients available for this trial.

This trial had an unacceptably high number of deaths prompting early closure of the study. Three deaths were related to treatment: two patients developed refractory bone marrow aplasia and one developed PCP. The latter patient had no evidence of erlotinib-induced fibrosis [40]. It is conceivable that prophylactic trimethoprim/sulfamethoxazole may have prevented this case of PCP, although no cases of PCP related to temozolomide have occurred at our institution. Erlotinib did not appear to exacerbate the toxicities of concurrent temozolomide and RT nor did the addition of this agent appear to cause the increased rate of death on this trial.

The combination of erlotinib with RT and temozolomide using dose escalation to a pharmacodynamic endpoint was feasible in patients with newly diagnosed GBM. The

secondary objectives of testing the feasibility of pharmacodynamic dose escalation and evaluating the toxicity of this regimen were met as the patients in this trial reached erlotinib dosing to the predetermined grade 2 rash. Since all patients developed a rash and most reached grade 2, correlation with efficacy was not possible.

In summary, this trial of the combination of RT, temozolomide and erlotinib for patients with newly diagnosed GBM was not efficacious and had an unacceptably high death rate. The lack of efficacy may have resulted from concurrent use of a cytostatic agent, which may cause cell cycle arrest, with the cytotoxic agents. Erlotinib-induced cytostatic cell cycle arrest may reduce or eliminate the sensitivity of GBM cells to radiation and temozolomide. The above theory is contrasted by two recent studies which have shown more encouraging results using the regimen in this trial [41, 42]. These trials showed median survivals of 84 weeks and 15 months, respectively. The latter study, however, did not appear to benefit patients when compared to EORTC 26981 [2]. The design of these trials was similar to the current study. The study by Prados had two cohorts according to the use or non-use of EIAEDs. In the non-EIAED arm, patients received erlotinib 100 mg/day during radiation therapy with no dose escalation during that phase. In the current study, patients received a lower dose, 50 mg/day, for the first two weeks of the study and then escalated to 100–150 mg/day. Thus the dosing was very similar to the non-EIAED arm of that study. In the study by Brown, patients started RT on erlotinib alone for the first week before receiving full dose combination therapy. While these regimens have some differences, it is doubtful that these can explain the wide disparity in outcome between the three studies. Future studies with these agents administered on a different schedule, in combination with other tyrosine kinase inhibitors, or in patients selected for factors, such as MGMT methylation or EGFRvIII mutation, may lead to improved efficacy.

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