Neuro-Oncology 15(4):490–496, 2013.
doi:10.1093/neuonc/nos322
Advance Access publication January 17, 2013

NABTT 0502: a phase II and pharmacokinetic study of erlotinib and sorafenib for patients with progressive or recurrent glioblastoma multiforme

David M. Peereboom, Manmeet S. Ahluwalia, Xiaobu Ye, Jeffrey G. Supko, Sarah L. Hilderbrand, Surasak Phuphanich, L. Burt Nabors, Myrna R. Rosenfeld, Tom Mikkelsen, and Stuart A. Grossman, for the New Approaches to Brain Tumor Therapy (NABTT) Consortium

Cleveland Clinic, Cleveland, Ohio (D.M.P., M.S.A.); Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins University, Baltimore, Maryland (X.Y., S.A.G.); Massachusetts General Hospital, Boston, Massachusetts (J.G.S., S.L.H.); Cedars-Sinai Medical Center, Los Angeles, California (S.P.); University of Alabama, Birmingham, Alabama (L.B.N.); Hospital Clinic/IDIBAPS, Barcelona, Spain (M.R.R.); Henry Ford Hospital, Detroit, Michigan (T.M.)

Background. The signal transduction pathways of epidermal growth factor receptor and Ras are both important in the growth of glioblastoma multiforme (GBM). We hypothesized that inhibition of both pathways would improve the survival time of patients with recurrent GBM.

Methods. Patients with recurrent/progressive GBM with 0–2 prior chemotherapy regimens received erlotinib 150 mg once daily and sorafenib 400 mg twice daily until progression. The primary endpoint was overall survival. Pharmacokinetic sampling was performed during cycle 1.

Results. The median overall survival was 5.7 months. Progression-free survival at 6 months was 14%. Toxicity was manageable. Clearance of erlotinib was markedly enhanced by sorafenib.

Conclusion. The study did not meet its objective of a 30% increase in overall survival time compared with historical controls. Erlotinib and sorafenib have significant pharmacokinetic interactions that may negatively impact the efficacy of the combination regimen.

Keywords: EGFR, erlotinib, pharmacokinetics, glioblastoma, Ras signaling, sorafenib, targeted therapy.

Received September 29, 2012; accepted November 20, 2012.

Corresponding Author: David M. Peereboom, MD, Burkhardt Brain Tumor and Neuro-Oncology Center, Neurological Institute, Cleveland Clinic, 9500 Euclid Ave, Cleveland, OH 44195 (peerebd@ccf.org).

Patients with glioblastoma multiforme (GBM), the most common malignant primary brain tumor, have a dismal outcome, with a median survival of ~15 months from diagnosis with standard therapy of surgery followed by radiation and temozolomide.[1] New agents are urgently needed to improve survival and quality of life for these patients. High-grade gliomas exhibit alterations in mitogenic signaling pathways such as that of epidermal growth factor receptor (EGFR).[2,3] Approximately 40% of GBM overexpress EGFR, and this marker correlates with an aggressive phenotype associated with poor outcome.[4,5] EGFR activation leads to a signal transduction cascade that enhances survival and infiltration of GBM cells in vitro.[6,7] Erlotinib interrupts this activation through inhibition of EGFR tyrosine kinase. Erlotinib was selected for clinical development based on its ability to inhibit EGFR tyrosine kinase and cell proliferation and induce cell cycle arrest and apoptosis and on its antitumor activity in a variety of in vitro and in vivo human tumor cell line models.[8] Erlotinib has modest single-agent activity against recurrent GBM with a 6-month progression-free survival (PFS6) of 11%–27%.[9,10]

Activation of the Ras signaling pathway in glioma leads to proliferation and tumor-associated angiogenesis.[11] Overexpression of astrocyte-specific activated Ras results in spontaneous glioma formation in mouse models.[12] Activation of the Ras signaling pathway thus appears to be important in the formation and progression of gliomas. Raf kinase is a critical enzyme in the Ras signaling cascade. As such, inhibition of Raf

kinase has the potential to inhibit this pathway and thereby inhibit glioma growth. Sorafenib is a Raf kinase inhibitor with oral bioavailability and moderate penetration of the CNS, as demonstrated by radiolabeling studies in rodents.[13]

Resistance of gliomas to EGFR-targeted agents results in part from multiple redundant pathways that may circumvent blockading of the EGFR pathway. Some GBM patients with EGFR overexpression do not respond to erlotinib,[9] suggesting that redundant signaling pathways might be responsible for resistance. Therefore, blockade of multiple pathways is likely necessary for optimal activity of targeted agents.

We hypothesized that the inhibition of both EGFR and Ras signal transduction pathways would improve the survival of patients with recurrent GBM. A phase I trial of erlotinib and sorafenib in multiple tumor types found that the most frequent adverse events of all grades were fatigue, diarrhea, hypophosphatemia, and acneiform rash.[14] These adverse events were predominantly mild to moderate. The recommended phase II dosage of this combination was sorafenib 400 mg twice daily and erlotinib 150 mg daily. Pharmacokinetic analysis revealed no significant effect of erlotinib on the pharmacokinetic profile of sorafenib. Among 15 evaluable patients, 3 (20%) achieved a confirmed partial response, and 9 (60%) had stable disease as best response. A prior study showed that this regimen was well tolerated in patients with recurrent GBM; efficacy data are pending.[15] The hypothesis in this study was that inhibition of both EGFR and Ras pathways using the combination of erlotinib and sorafenib would produce a 30% improvement in overall survival in patients with recurrent or progressive GBM. The primary objective of this trial was to estimate the overall survival (OS) rate associated with this combined regimen in treating adult patients with recurrent GBM. The secondary objectives were to assess the toxicities, radiographic response rate, PFS6, and pharmacokinetics of this combination in this patient population. In addition, tumor and blood samples were submitted for the Molecular Targeted Combinations Correlative Study Initiative (MTC^2) for future studies to determine the relationship between tumor and blood biomarkers and clinical outcome of patients treated with the combination of targeted agents.

Methods

Patient Eligibility

Eligible patients were at least 18 years of age with measurable, histologically proven GBM that had progressed or recurred following radiation therapy and 0–2 prior chemotherapy regimens. Patients with previous low-grade glioma and subsequent biopsy-proven GBM that had progressed after radiotherapy and 0–2 prior chemotherapy regimens were eligible. Patients must have had tissue specimens available and agreed to have their blood and tissue blocks (or slides) submitted for the MTC^2. MRI or CT imaging was required within 2 weeks of starting therapy. Patients must have recovered from toxicity of prior therapy. The following time intervals from the completion of prior therapy must have elapsed prior to study entry: radiation, 3 months; cytotoxic chemotherapy, 3 weeks (6 wk for nitrosourea-containing chemotherapy); noncytotoxic FDA-approved agents (eg, thalidomide), 2 weeks; and investigational noncytotoxic agents, 3 weeks. Patients were required to have a Karnofsky performance status $\geq$60% and normal organ function as defined by an absolute neutrophil count $\geq 1500/mm^3$, platelet count $\geq 100\,000/mm^3$, hemoglobin >9 g/dL, creatinine ≤ 1.7 mg/dL, total bilirubin ≤ 1.5 mg/dL, transaminases ≤ 4 times above the upper limits of the institutional norm, and prothrombin time and partial prothrombin time no higher than the institutional norm. Patients were required to provide written informed consent, to have been maintained on a stable corticosteroid regimen from the time of their baseline scan until the start of treatment, and to have a Mini-Mental State Exam score of at least 15. Patients of child-bearing potential had to agree to use acceptable birth control methods.

Exclusion criteria included pregnancy, breast feeding, and concurrent malignancy, except curatively treated basal or squamous cell carcinoma of the skin or carcinoma in situ of the cervix and breast. Patients with prior malignancies were required to be disease free for at least 5 years. Additional exclusion criteria included serious concurrent infection or medical illness that would have jeopardized the ability of the patient to receive the treatment with reasonable safety; systolic blood pressure >140 mmHg or diastolic pressure >90 mmHg; prior therapy with erlotinib or sorafenib or any other agent targeting EGFR; known abnormalities of the cornea based on history (eg, dry eye syndrome, Sjogren's syndrome); congenital abnormality (eg, Fuch's dystrophy); abnormal slit-lamp examination using a vital dye (eg, fluorescein, Bengal-Rose); an abnormal corneal sensitivity test (Schirmer test or similar tear production test); therapy with cytochrome P450–inducing anticonvulsants; and combination antiretroviral therapy.

Treatment

Patients received erlotinib 150 mg once daily on an empty stomach and sorafenib 400 mg twice daily. They were instructed to take both drugs at the same time every morning, with the second sorafenib dose taken $\sim$12 h later. Treatment was on a continuous daily schedule with no breaks between each 28-day treatment cycle and continued until there was objective or clinical evidence of either disease progression or treatment-related, dose-limiting toxicity or the patient decided to discontinue treatment for any reason.

Dose Modifications and Off-study Criteria

Toxicities were graded according to the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) version 3.0. Dose reductions

Table 1. Dose reductions for dose-limiting treatment toxicities

Dose Level	Dosage	
	Sorafenib	Erlotinib
1 (starting dose)	400 mg b.i.d.	150 mg q.d.
−1	200 mg b.i.d.	100 mg q.d.
−2	200 mg q.d.	75 mg q.d.

were required for any dose-limiting toxicity that occurred during a previous course of treatment. Doses were reduced according to the dose levels in Table 1. Dose reductions were made for each drug if toxicity could be attributed to that drug alone. Dose reductions for hematologic toxicities were based on blood counts obtained in preparation for the day of treatment. Patients in whom one agent was discontinued could continue to receive the other agent if, in the opinion of the treating physician and the NCI senior investigator, the patient could continue to benefit from treatment. Patients requiring dose reductions did not have the dose re-escalated with subsequent treatments. Subjects were withdrawn from the study if toxicities failed to recover to CTCAE grades 0–1 or baseline within 14 days or if they experienced drug-related adverse events requiring 3 dose reductions.

Response Evaluation

Neurological examinations and MRI/CT scans with volumetric analysis were used prior to every odd cycle of treatment to determine the response to therapy. The response had to be confirmed by MRI at least 4 weeks later to fulfill the definitions. Responses were determined by the modified Macdonald criteria.[16]

Pharmacokinetic Studies

Pharmacokinetic sampling was performed during the third week of cycle 1 to ensure that steady-state conditions for the repeated dosing schedule for both drugs had been reached.[17,18] Blood specimens (6 mL) were drawn from a peripheral arm vein and collected in tubes containing sodium heparin before initiation of treatment; immediately before dosing on day 15; at 0.5, 1, 2, 4, 6, and 8 h after taking the morning dose of both drugs; and before dosing on the following day. The samples were centrifuged (1100–1300 g, 4°C, 10 min) to afford plasma that was removed and kept frozen at −70°C until assayed. Actual dosing and sample collection times were recorded.

The concentrations of sorafenib and erlotinib were measured in plasma by 2 different analytical methods based upon liquid chromatography tandem mass spectrometry. Sorafenib was assayed as previously reported.[19] The analytical method for erlotinib was adapted from a published procedure, with minor modifications, as summarized in the Supplementary Materials.[20]

The steady-state minimum concentration (C_{min}^{ss}) of drug in plasma was calculated as the geometric mean of the assayed concentration of drug in the 2 samples collected before dosing on days 15 and 16. The steady-state maximum drug concentration (C_{max}^{ss}) was the sample with the highest assayed concentration. Plasma concentration-time curves for individual patients were analyzed by standard noncompartmental methods using WinNonlin Professional 5.0. The log-linear trapezoidal algorithm was used to estimate the area under the plasma concentration-time curve for the 12- or 24-h dosing intervals (AUC_{τ}^{ss}) for sorafenib or erlotinib, respectively. Apparent oral clearance (CL/F) was calculated as the dose given twice a day for sorafenib (400 mg) or once daily for erlotinib (150 mg) divided by AUC_{τ}^{ss}. Values of the pharmacokinetic parameters are reported as the geometric mean $\pm$ SD, with the SD estimated by the jackknife technique.[21–23]

Study Design and Statistical Considerations

This phase II trial was intended to detect a 30% increase in median time of survival (from 5 mo to 6.5 mo). The survival benchmark of 5 months was derived from the combined results of 3 prior phase II trials by the New Approaches to Brain Tumor Therapy (NABTT) Consortium in patients with recurrent GBM.[24–26] With a 1-sided test, the study had 80% power to detect this goal at an alpha level of 0.1. This goal required 49 deaths, for which 56 patients were required for accrual. Survival time was measured from the first day of the treatment to death. The probability of OS and PFS was estimated using the Kaplan–Meier method. An increase in median survival time of $<30\%$ was considered to be not promising enough clinically to pursue a randomized trial. Secondary endpoints of this trial included PFS6, radiographic response rate, pharmacokinetics, and toxicities.

Results

Patient Characteristics

Fifty-six patients were enrolled in the trial between January 2007 and October 2007, 55 of whom had died as of November 24, 2009. Patient characteristics are summarized in Table 2. The median age was 56 years (range, 31–78). The median KPS was 80 (range, 60–100). The median number of prior chemotherapy regimens was 1 (range, 1–2). The median time on study was 1.9 months. The most common reasons for coming off study included progressive disease (70%, $n = 39$), withdrawal of consent, (11%, $n = 6$), treatment delay >14 days (7%, $n = 4$), and toxicity (5%, $n = 3$).

Efficacy

Overall survival.—Median OS was 5.7 months (95% confidence interval [CI]: 4.5–7.9 mo). This survival

was not significantly different from that of the NABTT database (median OS: 5.2 mo; 95% CI: 3.8–6.5 mo; $P = .1$, log-rank test), and the trial did not meet its primary objective.

A Cox regression model was used to estimate the hazard ratio (HR) of death compared with the historical control after adjusting for age, KPS, and surgical procedure over the completed trial follow-up period. There was a 15% reduction in the risk of death for patients on this trial compared with that of historical controls (HR: 0.85; 95% CI: 0.6–1.3; $P = .4$). Fifty-six patients in this study and 62 patients with the same histology in the NABTT historical database were used in the analyses.[24–26]

Progression-free survival.—Median PFS was 2.5 months (95% CI: 1.8–3.7 mo) in this study and 1.4 months (95% CI: 1.3–1.8 mo) in the historical control ($P = .01$, log-rank test). A Cox regression model was used to estimate the HR of progression compared with the historical control after adjusting for age, KPS, and extent of resection. There was a 35% reduction in hazard of disease progression for patients on this trial compared with historical controls (HR: 0.65; 95% CI: 0.4–0.99; $P = .045$). Eight of 56 patients (14%; 95% CI: 8%–28%) were alive with a PFS6 from the start of treatment.

Radiographic responses.—Fifty-one patients were evaluable for radiographic response. Three patients withdrew consent within 4 weeks of starting therapy before evaluation and did not have off-treatment imaging. One patient had a treatment delay >14 days and went off study without off-treatment imaging. One patient went off study after 2 weeks of therapy due to intercurrent illness. Best radiographic responses included partial response, 5% ($n = 3$, all unconfirmed); stable disease, 41% ($n = 23$), progressive disease, 45% ($n = 25$).

Toxicity.—Grades 3–4 toxicities that were felt to be possibly, probably, or definitely related to drug are listed in Table 3. The combination of erlotinib and sorafenib in this study was tolerated with toxicities comparable to those of the phase I combination study. No unexpected toxicities occurred given the known toxicities of each agent. No grade 5 toxicities occurred and no patient experienced pancreatitis. The 2 patients with elevated lipase, a toxicity common with sorafenib, remained asymptomatic and had reductions of lipase with dose reduction or discontinuation of sorafenib.

Pharmacokinetics.—Mean steady-state pharmacokinetic parameters for sorafenib and erlotinib are presented in Table 4 together with comparative data from previously reported clinical trials in which the same dosing regimens of both drugs were evaluated as monotherapies in patients with extraneural solid malignancies. Pharmacokinetic data for sorafenib were obtained from 48 patients, and data for erlotinib were available for 51 patients. Mean values of all parameters characterizing the steady-state pharmacokinetics of sorafenib given at a dosage of 400 mg twice a day were in excellent

Table 2. Patient characteristics

Characteristic ($N = 56$)	
Median age, y (range)	56 (31–78)
Sex, *n*	
Male	35 (63%)
KPS	
90–100	26 (46%)
60–80	30 (54%)

Table 3. Grade 3 or 4 events related[a] to sorafenib or erlotinib

	n	%
Fatigue	5	9
Lipase	4	7
Diarrhea	1	2
Nausea	1	2
Pain: extremity limb	1	2
Rash: hand-foot syndrome	1	2
AST	1	2
ALT	1	2

Abbreviations: AST, aspartate aminotransferase; ALT, alanine aminotransferase.
[a]Toxicities felt to be possibly, probably, or definitely related to drug.

Table 4. Steady-state pharmacokinetic parameters for sorafenib and erlotinib

Parameter	Sorafenib	Erlotinib
No. of patients	48	51
C_{min}^{ss}, μg/mL	4.6 ± 2.5[a] (4.3; 3.9–4.6)[b]	0.30 ± 0.34 (1.3; 0.86–1.5)[c]
C_{max}^{ss}, μg/mL	6.7 ± 3.5 (7.8; 4.3–1)	1.3 ± 0.48 (2.1; 1.7–2.5)
AUC_{τ}^{ss}, μg · h/mL	58 ± 29 (64; 40–89)	15 ± 9.0 (38; 26–44)
CL/F, L/h	6.9 ± 3.5 (6.3; 4.5–10)	10 ± 6.1 (3.9; 3.3–5.7)

[a]Mean ± SD of the parameter for patients evaluated in NABTT 0502.
[b]Mean values (median; range) from previously reported single-agent clinical trials of sorafenib[14,17,19,36–38] given at the same dose and schedule as NABTT 0502.
[c] [b]Mean values (median; range) from previously reported single-agent clinical trials of erlotinib[41,42,52,53] given at the same dose and schedule as NABTT 0502.

agreement with historical data from single-agent clinical trials. In contrast, mean values of the C_{min}^{ss}, C_{max}^{ss}, and AUC_{τ}^{ss} of erlotinib were all well below the lower range of mean values reported for the 150 mg once a day dosing regimen in single-agent studies of the drug. These findings suggest that the pharmacokinetics of sorafenib were unaffected by erlotinib, whereas the clearance of erlotinib was markedly enhanced by sorafenib when the 2 agents were given concurrently.

Discussion

In this study, the combination of erlotinib and sorafenib in patients with recurrent GBM was intended to inhibit the EGFR and Ras signal transduction pathways, both of which are relevant to the growth of GBM. Activity was modest. Although PFS and PFS6 did compare favorably with those of historical controls in the NABTT database, the goal of a 30% increase in median time of survival compared with the NABTT database was not met.

Several factors may explain the modest activity of the combination of erlotinib and sorafenib in this trial. Although the use of a combination of targeted therapies is attractive in the treatment of cancer in general and of GBM in particular, gliomas have alternative compensatory pathways that maintain the aggressive growth phenotype even in the presence of EGFR inhibition.[27] Other receptor tyrosine kinases, such as platelet derived growth factor receptor, insulin growth factor 1 receptor, and c-Met, can be concurrently upregulated in GBM, resulting in compensation for decreased signaling by EGFR.[28–30] Furthermore, penetration of the blood–brain barrier may be insufficient for activity of these agents against GBM.[31] Although erlotinib has modest CNS penetration, more recent data suggest that sorafenib is a substrate for blood–brain barrier efflux pumps.[31,32]

In addition, the degree of EGFR inhibition in brain tumors is variable. EGFR tyrosine kinase inhibitors show inconsistent effects on phosphorylation and downstream signaling. It is possible that the tissue concentrations of erlotinib failed to reduce phosphorylated EGFR, suggesting a "molecular underdosing."[33] Resistance to EGFR inhibition can occur through desensitizing mutations in the kinase itself or through the activation of alternate oncogenic pathways.[34] Finally, EGFR inhibitors have efficacy limited to certain populations, such as those whose tumors express the EGFR variant III mutant receptor with wild-type phosphatase and tensin homolog (PTEN).[35]

The steady-state pharmacokinetics of sorafenib, when given concurrently with erlotinib, were in excellent agreement with single-agent clinical trials of the drug.[14,17,19,36–38] The apparent absence of an effect of erlotinib on the pharmacokinetic behavior of sorafenib in patients with primary brain tumors is consistent with the findings of 2 prior clinical trials of this combination.[14,39] In contrast, the mean clearance (CL/F) of erlotinib was found to be 2.6-fold greater than the median value for 5 clinical trials of single-agent erlotinib in patients with extraneural solid tumors.[40–42] The greater CL/F of erlotinib when given together with sorafenib was consistent with 2 other clinical trials in which this combination was evaluated.[14,39] The data from these trials were not available before this trial was completed. The interaction has potential clinical relevance, as lower plasma levels of erlotinib when given together with sorafenib were associated with a worse outcome in non–small cell lung cancer patients.[39]

Hepatic metabolism, mediated primarily by cytochrome (CY)P3A4 with a secondary contribution from CYP1A2, represents a major pathway of elimination for erlotinib.[18] The pharmacokinetics of erlotinib are readily altered by agents that modulate CYP3A4 activity. In particular, a 2-fold increase in the AUC of erlotinib resulted when it was administered together with the potent CYP3A4 inhibitor ketoconazole.[43] Alternatively, the mean CL/F of erlotinib was 2-fold greater in glioma patients who received enzyme-inducing antiseizure drugs compared with those who did not.[10,44–47] With regard to presystemic effects, the oral bioavailability of erlotinib is diminished as the acidity of the stomach is neutralized.[48]

Mechanisms that could potentially explain the basis for this apparent drug interaction are not obvious. Sorafenib does not induce either CYP1A2 or CYP3A4 in vitro.[49] Sorafenib also diminishes systemic exposure to oral gefitinib, and it was hypothesized that the interaction may result from CYP3A4 activation.[50] The possibility that sorafenib somehow diminishes the extent to which erlotinib is absorbed from the gastrointestinal tract when the 2 agents are orally administered together cannot be discounted. The pharmacokinetics of erlotinib are linear when the drug is given orally at the range of doses that have been evaluated.[18] In contrast, sorafenib exhibits saturable absorption at dosages >400 mg b.i.d., with no further increase in plasma concentrations at higher doses, although the mechanism responsible for this effect is unknown.[38]

This study was limited by its use of an historical database as a benchmark against which OS and PFS were compared. In addition, there were no data on EGFR variant III, PTEN, methyl guanine DNA methyl transferase, or other mutations within the tumors. The impact of these markers upon the efficacy of combination targeted-agent regimens is unknown. The radiographic responses were measured according to the modified Macdonald criteria, as the RANO (Response Assessment in Neuro-Oncology) criteria had not been established at the time of this trial.[51]

In conclusion, this trial demonstrated modest activity for the combination of erlotinib and sorafenib in patients with recurrent glioblastoma. Although PFS compared favorably with historical controls within the NABTT Consortium, the study did not reach its primary endpoint. Pharmacokinetic studies demonstrated a significant and potentially clinically important increase in the clearance of erlotinib by sorafenib. Further study of combination targeted agents would benefit from the selection of patients with molecular markers that predict response to therapy.

Supplementary Material

Supplementary material is available at *Neuro-Oncology Journal* online (http://neuro-oncology.oxfordjournals.org/).

Conflict of interest statement. None declared.

Funding

This work was supported by the National Cancer Institute (grant no. CA-62475).

References

1. Stupp R, Mason WP, van den Bent MJ, et al. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. *N Engl J Med*. 2005;352:987–996.
2. Libermann TA, Nusbaum HR, Razon N, et al. Amplification, enhanced expression and possible rearrangement of EGF receptor gene in primary human brain tumours of glial origin. *Nature*. 1985;313:144–147.
3. Libermann TA, Nusbaum HR, Razon N, et al. Amplification and overexpression of the EGF receptor gene in primary human glioblastomas. *J Cell Sci Suppl*. 1985;3:161–172.
4. Tang P, Steck PA, Yung WK. The autocrine loop of TGF-alpha/EGFR and brain tumors. *J Neurooncol*. 1997;35:303–314.
5. Schlegel J, Merdes A, Stumm G, et al. Amplification of the epidermal-growth-factor-receptor gene correlates with different growth behaviour in human glioblastoma. *Int J Cancer*. 1994;56:72–77.
6. Lund-Johansen M, Bjerkvig R, Humphrey PA, Bigner SH, Bigner DD, Laerum OD. Effect of epidermal growth factor on glioma cell growth, migration, and invasion in vitro. *Cancer Res*. 1990;50:6039–6044.
7. Halatsch ME, Gehrke E, Borhani FA, et al. EGFR but not PDGFR-beta expression correlates to the antiproliferative effect of growth factor withdrawal in glioblastoma multiforme cell lines. *Anticancer Res*. 2003;23:2315–2320.
8. Grunwald V, Hidalgo M. Development of the epidermal growth factor receptor inhibitor Tarceva (OSI-774). *Adv Exp Med Biol*. 2003;532:235–246.
9. Vogelbaum MA, Peereboom D, Stevens G, Barnett GH, Brewer C. Response rate to single agent therapy with the EGFR tyrosine kinase inhibitor erlotinib in recurrent blioblastoma multiforme: results of a phase II study. Proceedings of the Ninth Meeting of the Society for Neuro-Oncology, 384. Abstract TA-359; 2004.
10. Prados MD, Lamborn KR, Chang S, et al. Phase 1 study of erlotinib HCl alone and combined with temozolomide in patients with stable or recurrent malignant glioma. *Neuro Oncol*. 2006;8:67–78.
11. Guha A, Feldkamp MM, Lau N, Boss G, Pawson A. Proliferation of human malignant astrocytomas is dependent on Ras activation. *Oncogene*. 1997;15:2755–2765.
12. Ding H, Roncari L, Shannon P, et al. Astrocyte-specific expression of activated p21-Ras results in malignant astrocytoma formation in a transgenic mouse model of human gliomas. *Cancer Res*. 2001;61:3826–3836.
13. Carter C, Wessler C, Gellert J, Lathia C, Radtke M, Rossberg W, Schwartz B (eds). Bayer Pharmaceuticals Corporation, Investigator's Brochure Sorafenib/Raf Kinase Inhibitor. Version No.5. Date: 09 September 2004.
14. Duran I, Hotte SJ, Hirte H, et al. Phase I targeted combination trial of sorafenib and erlotinib in patients with advanced solid tumors. *Clin Cancer Res*. 2007;13:4849–4857.
15. Prados MD, Gilbert M, Kuhn J, et al. Phase I/II study of sorafenib and erlotinib for patients with recurrent glioblastoma (NABTC 05-02). *J Clin Oncol*. 2009;27:88s.
16. Macdonald DR, Cascino TL, Schold SC, Jr, Cairncross JG. Response criteria for phase II studies of supratentorial malignant glioma. *J Clin Oncol*. 1990;8:1277–1280.
17. Awada A, Hendlisz A, Gil T, et al. Phase I safety and pharmacokinetics of BAY 43-9006 administered for 21 days on/7 days off in patients with advanced, refractory solid tumours. *Br J Cancer*. 2005;92:1855–1861.
18. Scheffler M, Di Gion P, Doroshyenko O, Wolf J, Fuhr U. Clinical pharmacokinetics of tyrosine kinase inhibitors: focus on 4-anilinoquinazolines. *Clin Pharmacokinet*. 2011;50:371–403.
19. Nabors LB, Supko JG, Rosenfeld M, et al. Phase I trial of sorafenib in patients with recurrent or progressive malignant glioma. *Neuro Oncol*. 2011;13:1324–1330.
20. Zhao M, He P, Rudek MA, Hidalgo M, Baker SD. Specific method for determination of OSI-774 and its metabolite OSI-420 in human plasma by using liquid chromatography-tandem mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci*. 2003;793:413–420.
21. Lacey LF, Keene ON, Pritchard JF, Bye A. Common noncompartmental pharmacokinetic variables: are they normally or log-normally distributed? *J Biopharm Stat*. 1997;7:171–178.
22. Miller R. The Jacknife—a review. *Biometrika*. 1974;61:1.
23. Mizuta E, Tsubotani A. Preparation of mean drug concentration—time curves in plasma. A study on the frequency distribution of pharmacokinetic parameters. *Chem Pharm Bull (Tokyo)*. 1985;33:1620–1632.
24. Batchelor TT, Gilbert MR, Supko JG, et al. Phase 2 study of weekly irinotecan in adults with recurrent malignant glioma: final report of NABTT 97-11. *Neuro Oncol*. 2004;6:21–27.
25. Grossman SA, Alavi JB, Supko JG, et al. Efficacy and toxicity of the antisense oligonucleotide aprinocarsen directed against protein kinase C-alpha delivered as a 21-day continuous intravenous infusion in patients with recurrent high-grade astrocytomas. *Neuro Oncol*. 2005;7:32–40.
26. Grossman SA, Phuphanich S, Lesser G, et al. Toxicity, efficacy, and pharmacology of suramin in adults with recurrent high-grade gliomas. *J Clin Oncol*. 2001;19:3260–3266.
27. Mukasa A, Wykosky J, Ligon KL, Chin L, Cavenee WK, Furnari F. Mutant EGFR is required for maintenance of glioma growth in vivo, and its ablation leads to escape from receptor dependence. *Proc Natl Acad Sci U S A*. 2010;107:2616–2621.
28. Chakravarti A, Loeffler JS, Dyson NJ. Insulin-like growth factor receptor I mediates resistance to anti-epidermal growth factor receptor therapy in primary human glioblastoma cells through continued activation of phosphoinositide 3-kinase signaling. *Cancer Res*. 2002;62:200–207.
29. Huang PH, Mukasa A, Bonavia R, et al. Quantitative analysis of EGFRvIII cellular signaling networks reveals a combinatorial therapeutic strategy for glioblastoma. *Proc Natl Acad Sci U S A*. 2007;104:12867–12872.
30. Stommel JM, Kimmelman AC, Ying H, et al. Coactivation of receptor tyrosine kinases affects the response of tumor cells to targeted therapies. *Science*. 2007;318:287–290.

31. Agarwal S, Sane R, Ohlfest JR, Elmquist WF. The role of the breast cancer resistance protein (ABCG2) in the distribution of sorafenib to the brain. *J Pharmacol Exp Ther*. 2011;336:223–233.
32. Rogers LR, LoRusso P, Nadler P, Malik G, Shields A, Kaelin W. Erlotinib therapy for central nervous system hemangioblastomatosis associated with von Hippel-Lindau disease: a case report. *J Neurooncol*. 2011;101:307–310.
33. Lassman AB, Rossi MR, Raizer JJ, et al. 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.
34. Camp ER, Summy J, Bauer TW, Liu W, Gallick GE, Ellis LM. Molecular mechanisms of resistance to therapies targeting the epidermal growth factor receptor. *Clin Cancer Res*. 2005;11:397–405.
35. Mellinghoff IK, Wang MY, Vivanco I, et al. Molecular determinants of the response of glioblastomas to EGFR kinase inhibitors. *N Engl J Med*. 2005;353:2012–2024.
36. Clark JW, Eder JP, Ryan D, Lathia C, Lenz HJ. Safety and pharmacokinetics of the dual action Raf kinase and vascular endothelial growth factor receptor inhibitor, BAY 43-9006, in patients with advanced, refractory solid tumors. *Clin Cancer Res*. 2005;11:5472–5480.
37. Moore M, Hirte HW, Siu L, et al. Phase I study to determine the safety and pharmacokinetics of the novel Raf kinase and VEGFR inhibitor BAY 43-9006, administered for 28 days on/7 days off in patients with advanced, refractory solid tumors. *Ann Oncol*. 2005;16:1688–1694.
38. Strumberg D, Richly H, Hilger RA, et al. Phase I clinical and pharmacokinetic study of the novel Raf kinase and vascular endothelial growth factor receptor inhibitor BAY 43-9006 in patients with advanced refractory solid tumors. *J Clin Oncol*. 2005;23:965–972.
39. Lind JS, Dingemans AM, Groen HJ, et al. A multicenter phase II study of erlotinib and sorafenib in chemotherapy-naive patients with advanced non–small cell lung cancer. *Clin Cancer Res*. 2010;16:3078–3087.
40. Hidalgo M, Siu LL, Nemunaitis J, et al. Phase I and pharmacologic study of OSI-774, an epidermal growth factor receptor tyrosine kinase inhibitor, in patients with advanced solid malignancies. *J Clin Oncol*. 2001;19:3267–3279.
41. Lu JF, Eppler SM, Wolf J, et al. Clinical pharmacokinetics of erlotinib in patients with solid tumors and exposure-safety relationship in patients with non–small cell lung cancer. *Clin Pharmacol Ther*. 2006;80:136–145.
42. Rudin CM, Liu W, Desai A, et al. Pharmacogenomic and pharmacokinetic determinants of erlotinib toxicity. *J Clin Oncol*. 2008;26:1119–1127.
43. Rakhit A, Pantze MP, Fettner S, et al. The effects of CYP3A4 inhibition on erlotinib pharmacokinetics: computer-based simulation (SimCYP) predicts in vivo metabolic inhibition. *Eur J Clin Pharmacol*. 2008;64:31–41.
44. Raizer JJ, Abrey LE, Lassman AB, et al. A phase II trial of erlotinib in patients with recurrent malignant gliomas and nonprogressive glioblastoma multiforme postradiation therapy. *Neuro Oncol*. 2010;12:95–103.
45. Raizer JJ, Abrey LE, Lassman AB, et al. A phase I trial of erlotinib in patients with nonprogressive glioblastoma multiforme postradiation therapy, and recurrent malignant gliomas and meningiomas. *Neuro Oncol*. 2010;12:87–94.
46. Sathornsumetee S, Desjardins A, Vredenburgh JJ, et al. Phase II trial of bevacizumab and erlotinib in patients with recurrent malignant glioma. *Neuro Oncol*. 2010;12:1300–1310.
47. van den Bent MJ, Brandes AA, Rampling R, et al. 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.
48. Ter Heine R, Fanggiday JC, Lankheet NA, et al. Erlotinib and pantoprazole: a relevant interaction or not? *Br J Clin Pharmacol*. 2010;70:908–911.
49. Kane RC, Farrell AT, Saber H, et al. Sorafenib for the treatment of advanced renal cell carcinoma. *Clin Cancer Res*. 2006;12:7271–7278.
50. Adjei AA, Molina JR, Mandrekar SJ, et al. Phase I trial of sorafenib in combination with gefitinib in patients with refractory or recurrent non-small cell lung cancer. *Clin Cancer Res*. 2007;13:2684–2691.
51. 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;28:1963–1972.
52. Tan AR, Yang X, Hewitt SM, et al. Evaluation of biologic end points and pharmacokinetics in patients with metastatic breast cancer after treatment with erlotinib, an epidermal growth factor receptor tyrosine kinase inhibitor. *J Clin Oncol*. 2004;22:3080–3090.
53. Calvo E, Malik SN, Siu LL, et al. Assessment of erlotinib pharmacodynamics in tumors and skin of patients with head and neck cancer. *Ann Oncol*. 2007;18:761–767.