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A Phase I/II Trial of Pazopanib in Combination with Lapatinib in Adult Patients with Relapsed Malignant Glioma

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Abstract

Purpose: Increased mitogenic signaling and angiogenesis, frequently facilitated by somatic activation of EGF receptor (EGFR; ErbB1) and/or loss of PTEN, and VEGF overexpression, respectively, drive malignant glioma growth. We hypothesized that patients with recurrent glioblastoma would exhibit differential antitumor benefit based on tumor PTEN/EGFRvIII status when treated with the antiangiogenic agent pazopanib and the ErbB inhibitor lapatinib.

Experimental Design: A phase II study evaluated the antitumor activity of pazopanib 400 mg/d plus lapatinib 1,000 mg/d in patients with grade 4 malignant glioma and known PTEN/EGFRvIII status not receiving enzyme-inducing anticonvulsants (EIAC). The phase II study used a two-stage Green–Dahlberg design for futility. An independent, parallel phase I component determined the maximum-tolerated regimen (MTR) of pazopanib and lapatinib in patients with grade 3/4 glioma receiving EIACs.

Results: The six-month progression-free survival (PFS) rates in phase II ($n = 41$) were 0% and 15% in the PTEN/EGFRvIII-positive and PTEN/EGFRvIII-negative cohorts, respectively, leading to early termination. Two patients (5%) had a partial response and 14 patients (34%) had stable disease lasting 8 or more weeks. In phase I ($n = 34$), the MTR was not reached. On the basis of pharmacokinetic and safety review, a regimen of pazopanib 600 mg plus lapatinib 1,000 mg, each twice daily, was considered safe. Concomitant EIACs reduced exposure to pazopanib and lapatinib.

Conclusions: The antitumor activity of this combination at the phase II dose tested was limited. Pharmacokinetic data indicated that exposure to lapatinib was subtherapeutic in the phase II evaluation. Evaluation of intratumoral drug delivery and activity may be essential for hypothesis-testing trials with targeted agents in malignant glioma. *Clin Cancer Res; 19(4); 900–8. ©2012 AACR.*

Introduction

Although molecular genetic abnormalities of glioblastoma (1–3) have been described and their associations with phenotypic features and prognosis have been reported (4–7), translation to improve therapeutic response has lagged, and outcomes remain poor. Specifically, median progression-free survival (PFS) and overall survival (OS) for patients with newly diagnosed glioblastoma are only 6.9 and 14.6 months, respectively, despite maximum safe resection and temozolomide chemoradiation (8). Following recurrence, salvage therapies have historically achieved 6-month PFS rates less than 10% and median survivals of 4 to 6 months (9–11).

Glioblastomas universally exhibit prolific and aberrant angiogenesis, mediated primarily by upregulated secretion of VEGF (12, 13). In addition, primary or *de novo* glioblastoma, which accounts for 90% to 95% of glioblastoma tumors, is characterized by dysregulated activation of PI3/Akt and RAS-MAPK cell signaling pathways, which in turn promotes tumor cell survival, proliferation, migration, and tumor-mediated angiogenesis (14). Aberrant activation of the EGF receptor (EGFR) occurs frequently and is associated with dysregulated cell signaling in glioblastoma (15). In contrast to VEGF/VEGFR inhibitors that have shown moderate single-agent antitumor activity in glioblastoma

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Translational Relevance

Previous studies showed the importance of ErbB [EGF receptor (EGFR) and HER2] signaling and the VEGF/VEGFR axis in driving glioblastoma progression. We hypothesized that concomitant inhibition of both pathways with pazopanib and lapatinib would be an effective and tolerable therapeutic approach, particularly among patients with ErbB/PTEN dysfunction. However, the investigational combination regimen evaluated herein did not show promising antitumor activity in the overall study population or in patients with either dysfunctional PTEN or EGFRvIII overexpression. Pharmacokinetic analyses indicated that dosing with lapatinib was subtherapeutic and that use of EIACs may decrease exposure to pazopanib. In addition, the assessment of PTEN and EGFRvIII was based on archival specimens. Future studies with targeted agents should consider prospective evaluation of target biomarkers, intratumoral drug delivery, and discontinuous or intermittent high-dose schedules to maximize the likelihood of target blockade.

(16–18), initial studies among patients with nonenriched recurrent glioblastoma treated with inhibitors of key PI3/Akt and RAS-MAPK pathway mediators have generated disappointing results (19–26).

Proposed strategies to improve outcome associated with targeted pathway inhibitors include rationally designed combinatorial regimens and accrual enrichment of patients predicted to have an increased likelihood of response based on tumor genetic characterization. Supported by preclinical studies showing that dual targeting of EGFR and VEGF leads to enhanced antitumor activity (27, 28), we hypothesized that inhibition of EGFR signaling using lapatinib (Tykerb; GlaxoSmithKline) administered with pazopanib (Votrient; GlaxoSmithKline), an oral multitargeted tyrosine kinase inhibitor of VEGF receptors (VEGFR)-1, VEGFR-2, VEGFR-3, platelet-derived growth factor receptors (PDGFR)-α/β, and c-kit, would be well tolerated and have antitumor activity among patients with recurrent glioblastoma. Furthermore, we stratified enrollment based on PTEN and EGFRvIII status of archival tumor specimens, as prior studies showed that these markers predict which patients with glioblastoma are more likely to respond to EGFR inhibitor therapy (29, 30). In addition, our study incorporated a phase I dose-escalation study to evaluate the impact of an enzyme-inducing anticonvulsant (EIAC) on escalating doses of lapatinib and pazopanib.

Patients and Methods

Patients

All eligible patients were at least 18 years of age with an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1 and adequate hematologic, renal, and hepatic function. Phase II study patients were additionally required to have grade 4 malignant glioma at first or second recurrence, no concurrent EIAC therapy, and sufficient archival tumor material for analysis of PTEN and EGFRvIII expression. Phase I patients were required to have a diagnosis of grade 3/4 malignant glioma at recurrence and to be receiving concurrent treatment with a CYP3A4 EIAC.

Patients were excluded if they had poorly controlled hypertension (≥140/90 mmHg), QTc prolongation (≥470 milliseconds), cerebrovascular accident in the previous 6 months, bleeding in the previous 6 weeks, venous or arterial thrombosis in the previous 3 months, a history of conditions affecting gastrointestinal absorption, or radiotherapy within the 12 weeks before initiating study treatment. Patients in phase II were excluded if they received more than 2 prior chemotherapy-containing treatment regimens and any VEGFR inhibitors or EIACs, although prior bevacizumab was allowed if it was more than 3 months since the last dose. All patients provided written informed consent in accordance with local Institutional Review Boards. The study was conducted in accordance with the Declaration of Helsinki.

Phase II study design and treatment

This was a multicenter, open-label, nonrandomized, phase II study (Clinicaltrials.gov registration: NCT00350727) among patients with recurrent malignant glioma not receiving EIAC (Supplementary Fig. S1). The study dose of pazopanib 400 mg once daily (q.d.) plus lapatinib 1,000 mg q.d. was based on results from a previous phase I study (VEG10006; NCT00158782; ref. 31). All patients received pazopanib 400 mg q.d. plus lapatinib 1,000 mg q.d. without food (1 hour before or 2 hours after a meal). On the basis of prior retrospective analyses of patients with recurrent glioblastoma treated with EGFR inhibitor therapy (29, 30), patients with either intact PTEN or EGFRvIII expression were stratified as biomarker-positive; those without PTEN and EGFRvIII expression were stratified as biomarker-negative.

The primary endpoints included 6-month PFS rate and safety in each stratum. A 2-stage Green–Dahlberg design was used wherein the second stage of the study was to proceed only if no more than 50% or 60% of patients in the biomarker-positive and biomarker-negative strata, respectively, had died or progressed after 2 months on study. The secondary endpoints were PFS and pharmacokinetics [area under the concentration-time curve to 24 hours postdose ($AUC_{(0-24)}$), maximum observed concentration (C_{max}), time to maximum concentration (t_{max}), concentration 24 hours postdose (C_{24})] of pazopanib and lapatinib in the absence of an EIAC. A translational objective was to identify predictive clinical markers.

Phase I study design and treatment

In the independent and parallel phase I study, the primary objective was to determine the safety, tolerability, and the maximum-tolerated regimen (MTR) of pazopanib combined with lapatinib in the presence of an EICA. A secondary objective was to assess the pharmacokinetics of pazopanib and lapatinib in the presence of an EIAC. The starting dose of pazopanib was 200 mg q.d., with escalation

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up to 600 mg twice daily (b.i.d.). The starting dose of lapatinib was 1,500 mg q.d., with escalation up to 1,000 mg b.i.d. Three to 6 patients were recruited at each dose level, and once the MTR was determined, up to 9 additional patients were to be enrolled in an expanded cohort (Supplementary Fig. S1).

The MTR was defined as the highest combined dose of pazopanib and lapatinib at which no more than 1 of 6 patients experienced dose-limiting toxicity (DLT) within the first 28 days of treatment (see Supplementary Methods for details).

Study assessments

All patients who received at least 1 dose of study drugs were assessed for safety. A complete medical history, physical examination, vital signs, laboratory tests, ECOG performance status, electrocardiograms, and cardiac assessments (multigated acquisition scan or echocardiogram) were conducted throughout the study and within 28 days after the last pazopanib and/or lapatinib dose. Blood pressure was monitored at scheduled clinic visits. Adverse events were continuously monitored using the National Cancer Institute Common Toxicity Criteria for Adverse Events version 3.0. Disease assessments were conducted at baseline, at weeks 4 and 8, and every 8 weeks thereafter by gadolinium-enhanced MRI scans. Response was evaluated according to the Macdonald and colleagues' criteria (32). PFS was estimated using Kaplan–Meier curves.

Pharmacokinetic assessments

In the phase I study, blood samples were collected for determination of pazopanib and lapatinib concentrations on day 15, within 1 hour before dosing and at 1, 2, 4, 6, 8, and 24 hours after dosing. In the phase II study, blood samples were collected for determination of pazopanib and lapatinib concentrations on day 1 of cycle 2, within 1 hour before dosing and at 0.25, 0.5, 1, 1.5, 2, 4, 6, 8, and 24 hours after dosing.

Noncompartmental analysis of concentration–time data was conducted using noncompartmental Model 200 (for extravascular administration) of WinNonlin Professional Edition version 5.2 (Pharsight Corporation) to calculate the pharmacokinetic parameters, including $AUC_{(0-24)}$, C_{max}, and t_{max}.

Immunohistochemistry

These protocols were developed and standardized by the Image Cytometry Laboratory, Duke University Medical Center (Durham, North Carolina; see Supplementary Methods for details). The tests were validated under Good Laboratory Practices standards devised by the College of American Pathology (Northfield, IL), and the laboratory is certified under the Clinical Laboratory Improvements Amendments of 1988. Five micrometer tissue sections were derived from formalin-fixed, paraffin-embedded, archival tumor tissue obtained at original diagnosis or at recurrence.

The intensity of cytoplasmic/membranous staining detected by immunohistochemistry was scored on a scale of 0 to 4+ and the distribution was defined as the percentage of cells with any level of expression. Immunohistochemistry staining was defined as "positive" for tumors expressing 2 to 4+ intensity in at least 25% of tumor cells and as "negative" for tumors expressing either 0 to 1+ staining in any percentage of tumor cells or 2 to 4+ intensity in less than 25% of tumor cells (33).

Results

Patient characteristics

There were 41 patients in the phase II study, including 19 biomarker-positive and 22 biomarker-negative patients, and 34 patients in the phase I dose-escalation and expansion cohorts (Table 1). The majority of patients were male (72%) and had grade 4 malignant glioma (87%). Patients enrolled in phase I were younger (median age, 48 years) than those in phase II (median age, 55 years).

Phase II trial data

Exposure and safety. The median duration of exposure to pazopanib and lapatinib was 56 days (range, 18–1,037 days). Forty patients (98%) experienced at least 1 adverse event, and 35 patients (85%) experienced treatment-related adverse events. The most frequently reported treatment-related adverse events included diarrhea (56%), fatigue (34%), hypertension (24%), and rash (24%; Table 2). A total of 11 patients (27%) experienced a total of 33 interruptions in pazopanib and/or lapatinib dosing because of adverse events. Dose reductions because of adverse events occurred in 4 patients with grade 1/2 events, including rash (1), diarrhea and increased serum bilirubin (1), nausea and vomiting (1), and confusional state (1). Five patients (12%) permanently discontinued study therapy because of adverse events.

Clinical activity. One patient in each biomarker strata achieved a partial response based on investigator assessment (Table 3); of note, these responses were maintained for 7.4 and 3.7 months. The 6-month PFS rates were 0% and 15% in the biomarker-positive and biomarker-negative strata, respectively. Median PFS was similar between the 2 groups: 56 days [95% confidence interval (CI), 45–113) in the biomarker-negative stratum and 62 days (95% CI, 56–90) in the biomarker-positive stratum (Fig. 1). The study was stopped for lack of efficacy.

Pharmacokinetics. Analysis of data from 22 patients in the phase II study showed that the mean pazopanib C_{24} was higher than the therapeutic target concentration of 15 μg/mL (Table 4). However, the mean lapatinib C_{max} (1.3 μg/mL) was lower than the value (2.43 μg/mL) that has been associated with therapeutic benefit (34). The pharmacokinetics of either agent did not differ between the biomarker strata (data not shown).

Parallel phase I trial data

Overall, 6 dosing levels were evaluated (Table 5) and the median duration of exposure to pazopanib and lapatinib was 56 days (range, 1–830 days). With the exception of the initial dosing cohort (pazopanib 200 mg q.d. plus lapatinib

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Table 1. Patient baseline characteristics and disposition

		Phase II	
	Phase I[a] (*N* = 34)	**Biomarker-positive (*n* = 19)**	**Biomarker-negative (*n* = 22)**
Median age, y (range)	48 (26, 70)	56 (42, 73)	50 (20, 76)
Sex, *n* (%)			
Male	23 (68)	14 (74)	17 (77)
Female	11 (32)	5 (26)	5 (23)
ECOG performance status, *n* (%)			
0	5 (15)	8 (42)	5 (23)
1	28 (82)	11 (58)	16 (73)
2	1 (3)[b]	0	1 (5)
Histology at screening, *n* (%)			
Grade 3	8 (24)	0	0
Grade 4	24 (71)	19 (100)	22 (100)
Missing	2 (6)	0	0
Biomarkers, *n* (%)			
$EGFRvIII^{-}/PTEN^{+}$	ND	8 (42)	NA
$EGFRvIII^{+}/PTEN^{-}$	ND	7 (37)	NA
$EGFRvIII^{+}/PTEN^{+}$	ND	4 (21)	NA
pAKT status, *n* (%)			
Positive	ND	10 (53)	19 (86)
Negative	ND	9 (47)	3 (14)
MAPK status, *n* (%)			
Positive	ND	14 (74)	14 (64)
Negative	ND	5 (26)	6 (27)
Missing/unknown	ND	0	2 (9)
Months since diagnosis, median (interquartile range)	33.5 (14.8–57.6)	10.2 (7.3–18.4)	11.9 (8.1–19.1)
Prior therapy, *n* (%)			
Chemotherapy	34 (100)	17 (89)	22 (100)
Radiotherapy	33 (97)	18 (95)	22 (100)
Biologics	10 (29)	4 (21)	7 (32)
Immunotherapy	1 (3)	1 (5)	1 (5)
Best response to most recent prior chemotherapy, *n* (%)			
CR	1 (3)	0	0
PR	1 (3)	0	1 (5)
SD	11 (32)	6 (32)	10 (45)
PD	19 (55)	11 (58)	11 (50)
Unknown/missing	2 (6)	2 (11)	0
Concomitant EIAC, *n* (%)			
Any EIAC	34 (100)	0	0
Phenytoin	24 (71)	0[c]	0
Carbamazepine	11 (32)	0	0
Phenobarbital	2 (6)	0	0
Primidone	1 (3)	0	0

Abbreviations: CR, complete response; MAPK, mitogen-activated protein kinase; NA, not applicable; ND, not determined; PD, progressive disease; PR, partial response; SD, stable disease.

[a]Phase I cohorts included: pazopanib (PAZ) 200 mg + lapatinib (LAP) 1,500 mg q.d., PAZ 800 mg + LAP 1,500 mg q.d., PAZ 800 mg q.d. + LAP 500 mg b.i.d., PAZ 800 mg q.d. + LAP 750 mg b.i.d., PAZ 800 mg q.d. + LAP 1,000 mg b.i.d., PAZ 600 mg b.i.d. + LAP 1,000 mg b.i.d.

[b]One patient had a baseline ECOG of 2 that improved to 1 at screening; therefore, this was not considered a protocol violation.

[c]One patient received phenytoin 300 mg after initiating study treatment.

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Table 2. Summary of treatment-related adverse events in 10% or more of patients[a]

	Phase I patients, *n* (%)[b] (*N* = 34)		Phase II patients, *n* (%; *N* = 41)	
	All grades	Grade 3/4	All grades	Grade 3/4
Diarrhea	19 (56)	1 (3)	23 (56)	2 (5)
Fatigue	9 (26)	2 (6)	14 (34)	1 (2)
Hypertension	9 (26)	0	10 (24)	1 (2)
Nausea	8 (24)	1 (3)	7 (17)	1 (2)
Elevated ALT	6 (18)	2 (6)	4 (10)	1 (2)
Thrombocytopenia	5 (15)	2 (6)	3	0
Neutropenia	4 (12)	3 (9)	1	0
Elevated AST	4 (12)	1 (3)	3	2 (5)
Rash	4 (12)	0	10 (24)	1 (2)
Lymphopenia	NR	NR	8 (20)	5 (12)
Hyperbilirubinemia	NR	NR	5 (12)	0
Vomiting	NR	NR	5 (12)	1 (2)
Dermatitis acneiform	NR	NR	6 (15)	0
Elevated amylase	NR	NR	4 (10)	0
Elevated lipase	NR	NR	4 (10)	3 (7)
Leukopenia	NR	NR	4 (10)	0

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; NR, not reported.

[a]Per National Cancer Institute Common Toxicity Criteria for Adverse Events version 3.0 criteria for toxicity grading.

[b]Phase I cohorts included: pazopanib (PAZ) 200 mg + lapatinib (LAP) 1,500 mg q.d., PAZ 800 mg + LAP 1,500 mg q.d., PAZ 800 mg q.d. + LAP 500 mg b.i.d., PAZ 800 mg q.d. + LAP 750 mg b.i.d., PAZ 800 mg q.d. + LAP 1,000 mg b.i.d., PAZ 600 mg b.i.d. + LAP 1,000 mg b.i.d.

1,500 mg q.d.), 1 patient in each of the other 5 cohorts experienced a DLT. Treatment-related adverse events (Table 2) occurred in 28 patients (82%), were primarily grades 1 or 2, and most frequently included diarrhea (56%), hypertension (26%), fatigue (26%), nausea (24%), thrombocytopenia (15%), and neutropenia (12%). Four patients (12%) permanently discontinued study therapy due to adverse events.

Table 3. Tumor response based on investigator-assessed best response

		Patients, n (%)				
Dose Level, mg	*n*	Complete	Partial	Stable (≥8 wk)	Progressive (stable <8 wk)	Progressive
Phase I (concomitant EIAC)						
PAZ 200 + LAP 1,500	4	0	1 (25)	1 (25)	0	2 (50)
PAZ 800 + LAP 1,500	6	0	0	2 (33)	4 (67)	0
PAZ 800 + LAP 500[a]	5	0	0	1 (20)	2 (40)	2 (40)
PAZ 800 + LAP 750[a,b]	7	0	1 (14)	1 (14)	2 (29)	2 (29)
PAZ 800 + LAP 1,000[a]	6	0	1 (17)	1 (17)	2 (33)	2 (33)
PAZ 600[a] + LAP 1,000[a,b]	6	0	0	4 (67)	1 (17)	0
Phase II (no EIAC)						
PAZ 400 + LAP 1,000[c]	41	0	2 (5)	14 (34)	16 (39)	9 (22)
Biomarker-positive[d]	19	0	1 (5)	7 (37)	7 (37)	4 (21)
Biomarker-negative[d]	22	0	1 (5)	7 (32)	9 (41)	5 (23)

Abbreviations: LAP, lapatinib; PAZ, pazopanib.

[a]Administered twice daily.

[b]One patient in this cohort was not evaluable because of missing postbaseline tumor assessment.

[c]All patients in phase II.

[d]Positive/negative for PTEN or EGFRvIII or both.

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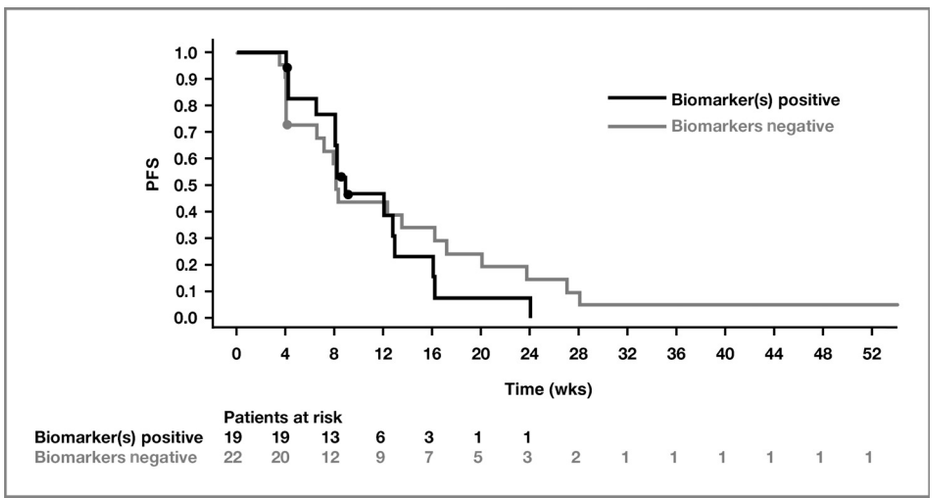

Figure 1. PFS in patients with recurrent glioblastoma receiving pazopanib in combination with lapatinib in the phase II study. One patient in the biomarker-negative group had PFS of 125.3 weeks.

Pharmacokinetic analyses from 30 phase I patients revealed that overall, mean C_{max} and $AUC_{(0-24)}$ increased in a dose-proportional manner (Table 4). Median and geometric mean pazopanib C_{max} values generally were similar across cohorts that received pazopanib 800 mg q.d.. Median and geometric mean pazopanib concentrations assessed at 24 hours were lower than the targeted therapeutic exposure (15 μg/mL) in these cohorts. Mean lapatinib C_{max} values in these cohorts were lower than the value (2.43 μg/mL) associated with therapeutic benefit (34).

The median pazopanib C_{24} was approximately 4-fold higher in the pazopanib 600 mg b.i.d. plus lapatinib 1,000 mg b.i.d. expansion cohort (43.8 μg/mL) than in the pazopanib 800 mg q.d. plus lapatinib 1,000 mg b.i.d. dose-escalation cohort (10.9 μg/mL), and was above the target therapeutic concentration (15 μg/mL). However, the geometric means of lapatinib C_{max} after 1,000 mg b.i.d. were similar in the expansion and dose-escalation cohorts (~0.74 μg/mL), both of which were below the targeted C_{max} of 2.43 μg/mL.

Table 4. Summary of pazopanib and lapatinib pharmacokinetic parameters

Dose level, mg	*n/n*	AUC_{0-24}, μg.h/mL[b]	C_{max}, μg/mL[b]	C_{24}, μg/mL[b]	t_{max}, h[c]
PAZ 200/LAP 1,500	4/4	107 (52, 155)[c]/3.3 (2.2, 5.3)[c]	10.6 (4.2, 12.4)[c]/0.5 (0.3, 0.6)[c]	1.4 (0.9, 3.0)[c]/0.03 (0.02, 0.04)[c]	2.0 (1.8, 4.0)/2.9 (2.0, 4.0)
PAZ 800/LAP 500[a]	4/3	635 (100, 704)[c,d]/NR	36.0 (12.2, 55.2)[c]/0.2 (0.1, 0.6)[c]	6.6 (0.7, 13.7)[c,d]/0.08 (0.04, 0.11)[c]	4.0 (2.0, 6.0)/4.0 (2.0, 6.0)
PAZ 800/LAP 1,500	6/5	449 (336, 601)[e]/6.9 (3.9, 12.4)	33.6 (26.7, 42.4)/0.7 (0.4, 1.2)	8.7 (4.6, 16.4)[e]/0.1 (0.04, 0.26)	3.2 (1.0, 8.0)/2.0 (1.1, 8.0)
PAZ 800/LAP 750[a]	6/6	457 (197, 1,063)/NR	39.3 (21.8, 70.7)/0.7 (0.3, 1.6)	8.9 (2.9, 27.2)/0.33 (0.12, 0.93)	4.0 (2.0, 8.0)/2.0 (0, 4.0)
PAZ 800/LAP 1,000[a]	5/6	331 (157, 697)/9.9 (3.3, 29.2)	27.3 (15.4, 48.3)/0.6 (0.2, 1.4)	7.4 (2.0, 27.6)/0.38 (0.11, 1.32)	3.9 (1.0, 8.0)/4.0 (1.0, 23.7)
PAZ 600[a]/LAP 1,000[a]	4/5	NR	43.8 (38.9, 56.9)[c]/0.7 (0.4, 1.2)	43.0 (26.1, 47.2)[c,d]/0.36 (0.14, 0.89)	5.0 (2.1, 23.7)/2.1 (2.0, 2.4)
PAZ 400/LAP 1,000	22/22	615 (512, 739)[f]/17.6 (12.6, 24.8)[g]	37.5 (33.3, 42.3)/1.3 (1.0, 1.7)	19.6 (15.1, 25.6)[g]/0.36 (0.24, 0.53)[g]	3.0 (0, 8.0)/4.0 (2.0, 8.0)

Abbreviations: LAP, lapatinib; NR, not reported; PAZ, pazopanib.

[a]Administered twice daily.

[b]Data presented as geometric mean (95% CI), except where indicated.

[c]Data presented as median (range).

[d]$n = 3$.

[e]$n = 5$.

[f]$n = 18$.

[g]$n = 19$.

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Table 5. Summary of DLT reported in phase I

	Dose level, mg	Patients enrolled, *n*	Patients with DLTs, *n*	DLTs
Cohort 1	PAZ 200 + LAP 1,500	4	0	—
Cohort 2	PAZ 800 + LAP 1,500	6	1	Grade 3 elevated ALT
Cohort 3	PAZ 800 + LAP 500[a]	5	1	Grade 3 elevated ALT
Cohort 4	PAZ 800 + LAP 750[a]	7	1	Grade 3 pancreatitis, thrombocytopenia, and seizure
Cohort 5	PAZ 800 + LAP 1,000[a]	6	1	Grade 3 thrombocytopenia, fatigue, and diarrhea; grade 4 confusion
Cohort 6 (expansion cohort)	PAZ 600[a] + LAP 1,000[a]	6	1	Grade 2 thrombocytopenia and grade 3 neutropenia

Abbreviations: ALT, alanine aminotransferase; LAP, lapatinib; PAZ, pazopanib.
[a]Administered twice daily.

On the basis of safety and pharmacokinetic review, pazopanib 600 mg b.i.d. plus lapatinib 1,000 mg b.i.d. (cohort 6) was considered safe and tolerable.

All patients in the phase I study had measurable disease at baseline. Three patients achieved a partial response lasting 6.5, 2.4, and more than 7.6 months: 1 patient each in the pazopanib 200 mg q.d. plus lapatinib 1,500 mg q.d., pazopanib 800 mg q.d. plus lapatinib 750 mg b.i.d., and pazopanib 800 mg q.d. plus lapatinib 1,000 mg b.i.d. cohorts (Table 3 and Supplementary Fig. S2).

Discussion

The rationale for this study was based on 2 emerging strategies designed to enhance the antitumor activity of targeted signaling inhibitors in oncology. First, we used a combinatorial regimen predicted to generate at least additive antitumor activity (35). Indeed, preclinical studies have confirmed enhanced glioblastoma inhibition following dual VEGF/EGFR-targeted therapy (27, 28). Second, we stratified patients based on tumor PTEN and EGFRvIII status because these markers have been previously associated with response to EGFR inhibitor therapy (29, 30). Despite these considerations, the combination of pazopanib plus lapatinib exhibited limited antitumor activity in the current study. Of note, disappointing activity was also observed in a recent study in which the addition of the EGFR tyrosine kinase inhibitor erlotinib to bevacizumab yielded antitumor activity no better than previously noted with bevacizumab alone (36). Furthermore, activity in the current study did not differ among patients stratified by tumor EGFRvIII or PTEN status. In fact, the antitumor activity observed was similar to that achieved with either pazopanib or lapatinib monotherapy in patients with recurrent glioblastoma (37, 38).

Although previous clinical data suggested that combining pazopanib and lapatinib did not alter exposure of either drug at the dose level used in the phase II component (i.e., pazopanib 400 mg plus lapatinib 1,000 mg; ref. 31), the plasma levels of lapatinib in this study were subtherapeutic compared with those defined in earlier studies [steady state geometric mean (95% CI) C_{max} of 2.43 μg/mL (1.57, 3.77) and AUC of 36.2 μg.h/mL (23.4, 56); ref. 39]. Clearly, the subtherapeutic levels of lapatinib likely contributed to the poor outcome, and dosing strategies to achieve better intratumoral lapatinib concentrations in future studies with glioma patients may include pulsatile, intermittent schedules at higher dose level.

However, it is also possible that the underlying rationale for biomarker stratification used in the current study may be erroneous. Indeed, the observation of association of PTEN with response to EGFR tyrosine kinase inhibitor therapy among patients with recurrent glioblastoma is derived from a retrospective analysis (30), and prospective studies have failed to confirm an association with between PTEN, EGFR, or EGFRvIII status and response to EGFR-targeted therapy (23, 24, 26, 37, 40). Furthermore, biomarker characterization in the current study was conducted on archival tumor material obtained primarily at original diagnosis, and therefore may not accurately reflect tumor characteristics at recurrence.

Additional factors that may have contributed to the low activity of these agents include the overall complexity of aberrant cell signaling in malignant glioma (3, 41, 42), deficiency of critical tumor suppressor molecules such as PTEN (43), and activation of alternative pathway mediators (44–46). Finally, although the antitumor activity of EGFR-inhibiting agents may be enhanced by combination with VEGFR-targeting agents in some glioblastoma models (28), it is also possible that anti-VEGF therapy may have diminished lapatinib delivery to the tumor microenvironment (47).

Antiepileptic drugs are commonly used in patients with primary and metastatic brain tumors and may modulate cytochrome function, thereby potentially altering exposure to drugs metabolized by these enzymes (including pazopanib and lapatinib). Of note, both pazopanib and lapatinib exposures in this study were affected by coadministration of EIACs. Specifically, C_{max}, $AUC_{(0-24)}$, and C_{24} were higher for both agents among patients treated in the phase II

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portion of the study (no EIACs) compared with patients treated at comparable dose levels in the phase I aspect of the study (on EIACs). These findings are consistent with those previously reported for lapatinib (37); however, our results are the first to document a negative impact of EIACs on pazopanib exposure in patients with cancer. Pharmacokinetic analyses revealed that patients in both the phase II and phase I components (pazopanib 600 mg b.i.d. cohort) achieved therapeutic levels of pazopanib C_{24}. In contrast, mean therapeutic plasma concentrations of lapatinib were not achieved among patients treated on either the phase II or parallel phase I components of the study. More recently defined agents such as levetiracetam or lacosamide, which are not metabolized by cytochrome P450 enzymes, may be attractive alternatives for patients with central nervous system tumors who require antiepileptic therapy.

In conclusion, although our study confirmed that pazopanib plus lapatinib is well tolerated among patients with recurrent malignant glioma, minimal evidence of antitumor activity was observed. These findings underscore the importance of incorporating pharmacokinetic and interaction analyses into early clinical development of agents for patients with primary central nervous system tumors. Furthermore, it is imperative that future clinical trials continue to strive to identify predictive biomarkers of response to molecular-targeted therapeutics, with the goal of optimizing the delivery of precision medicines for patients with glioblastoma. Although our study indicates that pazopanib plus lapatinib is not sufficiently active with the doses and schedule tested in patients with recurrent glioblastoma, VEGF/VEGFR, and ErbB/EGFR remain important mediators of glioblastoma pathophysiology, and thus remain attractive therapeutic targets for clinical development.

Disclosure of Potential Conflicts of Interest

P.Y. Wen is a consultant/advisory board member of Novartis, Merck, and Stemline. T. Mikkelsen is a consultant/advisory board member of Roche Genentech and Merck. J. Raizer has honoraria from Speakers Bureau of Genentech and is a consultant/advisory board member of Genentech and Novartis. A.B. Suttle has ownership interest (including patents) in GlaxoSmithKline. B. Ma is employed as a Statistician in GlaxoSmithKline. D.A. Reardon is a consultant/advisory board member for Genentech/Roche, EMD Serono, Novartis, Abbott Pharmaceuticals and Abgenix, Inc., and is a speaker's bureau member for Genentech. No potential conflicts of interest were disclosed by the other authors.

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Clinical Cancer Research

A Phase I/II Trial of Pazopanib in Combination with Lapatinib in Adult Patients with Relapsed Malignant Glioma

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