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ORIGINAL ARTICLE

A phase I/II trial of GW572016 (lapatinib) in recurrent glioblastoma multiforme: clinical outcomes, pharmacokinetics and molecular correlation

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Abstract
Purpose We undertook a phase I/II study of the EGFR/erbB2 inhibitor lapatinib in patients with recurrent glioblastoma multiforme (GBM) to determine response rate, pharmacokinetics (PK) and recommended dose in patients taking enzyme-inducing anti-epileptic drugs (EIAEDs) and to explore relationships of molecular genetics to outcome.
Methods Recurrent GBM patients taking EIAEDs were enrolled on the phase I portion (starting dose of lapatinib 1,000 mg po bid). In the absence of dose-limiting toxicity (DLT), escalation continued in cohorts of three patients. Patients not on EIAEDs enrolled in the phase II arm (lapatinib 750 mg bid po). Immunohistochemical and quantitative RT PCR studies were performed on tumor to determine PTEN and EGFRvIII status, respectively. Lapatinib PK was analyzed using HPLC with tandem mass spectrometry.
Results Phase II: Of 17 patients, 4 had stable disease and 13 progressed. Accrual ceased because of no responses. Phase I: Four patients received 1,000 mg bid and three, 1,500 mg bid. No DLT occurred, but escalation stopped because of lack of phase II efficacy. Lapatinib apparent oral clearance in patients taking EIAEDs was 106.9 L h^{-1} m^{-2} in comparison to 12.1 L h^{-1} m^{-2} in those not on EIAEDs. In 16 phase II patients, PTEN loss was seen in 6 and EGFRvIII expression in 4. No correlation was seen with outcome and molecular results.
Conclusions Lapatinib apparent oral clearance increased by approximately tenfold when given with EIAEDs. In this small sample, EGFRvIII expression and PTEN loss did not predict a favorable subtype. Overall, lapatinib did not show significant activity in GBM patients.

Keywords Lapatinib · Glioblastoma · Pharmacokinetics · Clinical trial

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Statement of clinical relevance

This study represents a combined phase I/II clinical trial in patients with recurrent glioblastoma multiforme using the EGFR/erbB2 tyrosine kinase inhibitor, lapatinib. While this study failed to show any appreciable benefit in this condition, there are several relevant results from this trial, important for human cancer research.

This study adds to the growing body of evidence that, in patients with glioblastoma multiforme, EGFR tyrosine kinase inhibitors are of little therapeutic benefit, especially as single agents. Our molecular genetic correlations, focusing on EGFRvIII mutation and PTEN expression, could not find a favorable subtype that might respond to lapatinib.

Importantly, we were able to perform pharmacokinetic analysis in patients taking hepatic enzyme-inducing anticonvulsants (EIAEDs). Lapatinib apparent oral clearance was substantially increased in these patients. Other cancer patients, especially those with brain metastases, may be placed on anticonvulsant medications. Our data clearly indicate such patients should not receive EIAEDs and lapatinib together until a proper effective dose can be identified.

Introduction

The standard of care for the initial management of glioblastoma multiforme (GBM) consists of concurrent chemoradiotherapy and adjuvant chemotherapy and has produced improved 2-year outcomes in this disease [1]. Nonetheless, at recurrence, there remains no effective salvage therapy and expected survival remains brief. New treatments are necessary to improve the outcomes in patients with recurrent GBM.

Most GBM tumor cells show alterations in epidermal growth factor receptor (EGFR)-related signaling pathways, including EGFR amplification as well as a constitutively active mutation of EGFR (EGFRvIII). As such, tyrosine kinases linked to EGFR are potential avenues for targeted therapy in this disease. Various trials with EFGR tyrosine kinase inhibitors, erlotinib and gefitinib have attempted to identify molecular subsets of GBM patients which are more likely to respond to these agents [2, 3]. The combination of EGFR overactivity and intact PTEN function seems to correlate best with response to these agents, but is a relatively uncommon finding in most GBM [2, 3].

GW572016 (lapatinib) is a novel tyrosine kinase inhibitor showing dual action on EGFR/ErbB-1 and Her-2/ErbB2 family of receptors [4]. While most preclinical trials with this agent focused on breast, colon and head and neck malignancies, it's similarity to other EGFR tyrosine kinase inhibitors and broader spectrum of activity made it an interesting agent to study in GBM with hopes of greater utility compared to the more selective EGFR tyrosine kinase inhibitors, gefitinib and erlotinib.

Because lapatinib undergoes hepatic metabolism through CYP3A/4 pathways, it was important to perform a combined phase I/II trial with this agent, enrolling those patients on enzyme-inducing antiepileptic drugs (EIAEDs) in a phase I dose-escalating design. Establishing the pharmacokinetic behavior of the drug in patients on EIAEDs in comparison to those not taking such agents was felt to be relevant not only for the GBM population, but also for patients with other malignancies that might be taking EIAEDs or corticosteroids.

Additionally, it was important in this trial to look at molecular correlates of treatment outcome, especially EGFRvIII and PTEN status. This report presents the clinical outcome and pharmacokinetic data of a phase I/II trial of lapatinib in GBM in first relapse and endeavors to correlate this with molecular genetic changes.

Methods

Patient selection

Patients were eligible for study enrollment if they were $\geq$18 years of age with a histologically-confirmed diagnosis of GBM in first relapse following either radiotherapy (RT) alone or RT with concurrent/adjuvant chemotherapy. Patients were required to be at least 6 weeks since their last RT and/or chemotherapy treatment and at least 2 weeks since surgery for recurrent tumor. Residual disease measuring at least 1 $\times$ 1 cm on contrast-enhanced CT or MRI was required. Eligible patients had an ECOG status of 0–2 and adequate cardiac, renal, liver and hematologic function [absolute neutrophil count $\geq 1.5 \times 10^9\ L^{-1}$, platelet count $\geq 100 \times 10^9\ L^{-1}$, serum creatinine $<1.5 \times$ upper limit of normal (ULN), AST and ALT $\leq 2.5 \times$ ULN, serum total bilirubin <ULN, and LVEF $\geq$50%]. Patients were excluded for other underlying significant medical problems or pregnancy.

All centres were required to have research ethics board (REB) approval and patients were enrolled only after signing an REB-approved consent form. Patients also consented to collection of paraffin-embedded tissue sections from their diagnostic specimens for molecular correlative studies.

Trial design

Patients receiving EIAEDs

Patients on EIAEDs were assigned to the phase I arm of the trial. This was an open label dose escalation trial starting at a dose of lapatinib (supplied from the NCI/Division of Cancer Treatment and Diagnosis) of 1,000 mg bid orally daily. Further escalation was performed after 3 patients were successfully enrolled and treated with no emerging dose-limiting toxicities (DLT). The dose escalation schedule was shown in Table 1.

If DLT was seen in 1/3 patients, 3 further patients would be enrolled at that dose level. If further DLT became evident, that dose level would become the maximum administered dose (MAD). If no further DLT was seen then the trial would continue to the next dose level. If 2/3 patients developed DLT, then that dose would be considered the MAD. The recommended phase II dose would be one dose level below the MAD.

Table 1 Dose escalation schedule for the phase I study

Dose level	Dose of GW572016 given orally, twice daily (total daily dose)	Minimum number of patients
−1	750 mg bid (1,500 total)	–
1 (starting)	1,000 mg bid (2,000 mg total)	3
2	1,500 mg bid (3,000 mg total)	3
3	2,000 mg bid (4,000 mg total)	3
4	2,500 mg bid (5,000 mg total)	3

Toxicity was evaluated using the NCI common terminology criteria for adverse events (CTCAE), version 3.0. Dose-limiting toxicity was defined as one or more of the following events occurring during cycle 1 of therapy: absolute neutrophil count $<0.5 \times 10^9\ L^{-1}$, febrile neutropenia with absolute neutrophil count $<1.0 \times 10^9\ L^{-1}$, platelet count $< \times 10^9\ L^{-1}$, or bleeding felt to be due to thrombocytopenia, diarrhea ≥Grade 3 despite anti-diarrheal agents, rash ≥Grade 3, other Grade 3 effects felt to be related to treatment, or missing 7 days of therapy due to toxicity.

Patients in the phase I trial arm were allowed to continue on lapatinib in the absence of tumor progression or unacceptable toxic effects.

Patients not on EIAEDs

Eligible patients not receiving EIAEDs were enrolled in the phase II portion of the trial and were treated with lapatinib 750 mg bid orally daily. Additionally, once the recommended phase II dose was identified for patients on EIAEDs, these patients could then be entered in the phase II portion of the trials to be treated with the dose recommended. Toxicities were again monitored using the CTCAE version 3.0. Up to two dose reductions were permitted for significant toxicity (500 mg twice daily and 500 mg once daily).

All patients were monitored with clinical evaluations every 4 weeks. Complete blood counts and biochemistry studies were performed weekly for the first 8 weeks then biweekly thereafter. MRI or CT head and echocardiogram/MUGA scan were performed every 8 weeks.

Response criteria were based on clinical and radiographic criteria. Radiographic response was based on evaluation of contrast-enhanced lesion(s) seen on MRI/CT. Definite improvement was defined as an estimated 50% or more decrease in the size of the enhancing lesion(s) on the CT/MRI scan as calculated by the product of cross-sectional diameters. Equivocal/no change was defined as <50% decrease and <25% increase in the size of the enhancing lesion(s) on the CT/MRI scan as calculated by the product of cross-sectional diameters. Definite progression was defined as an estimated 25% or more increase in the size of the enhancing lesion(s) on the CT/MRI scan as calculated by the product of cross-sectional diameters, or the appearance of new lesions. Clinical response was defined using Levin criteria [5]. While corticosteroids were allowed for the treatment of cerebral edema, a stable dosage was strongly encouraged prior to imaging assessment and patients could not be defined as clinically improved while on an increasing dose of corticosteroids.

A partial response was determined by the presence of definite improvement on imaging with either an improved or equivocal clinical status while on a stable or decreasing corticosteroid dose. Stable disease was defined by an equivocal imaging result and improved or equivocal clinical status while on a stable or decreasing corticosteroid dose. Disease progression was defined by a worsening radiographic result and equivocal or worsening clinical status while on a stable or increasing dose of corticosteroids. Patients remained on treatment in the absence of disease progression and serious drug-related toxicity.

Pharmacokinetics

Blood samples (2 ml) for pharmacokinetic studies of lapatinib, were collected on day 15 of cycle 1 in all phase I patients and in those phase II patients who consented to these additional blood samples. Patients were advised to take lapatinib on an empty stomach (either 1 h before or 1 h after meals). Serial samples were collected in heparinized tubes before and 1, 2, 4, and 6 to 10 h after lapatinib administration, plasma separated, and stored for future analysis using high performance liquid chromatography with tandem mass spectrometry [6]. The lower limit of quantitation for this method was 15 ng ml^{-1}, the within-day and between-day precision was <8%, and accuracy was between 102 and 105%.

Using a non-compartmental analysis, we estimated the maximum plasma concentration (C_{max}) and the time for the maximum concentration (T_{max}) for each patient. Pharmacokinetic population parameters were estimated using nonlinear mixed effects modeling method implemented in NONMEM version V (GloboMax LLC, Ellicot City, MD, USA). The first-order conditional estimation method (FOCE) with INTERACTION was implemented [7]. A one-compartment model with first-order elimination was used as the base model (ADVAN 2 subroutine). The pharmacokinetic parameters estimated included apparent oral clearance (CL/F), apparent volume of distribution (V/F), and the absorption rate constant (*k*a). The distribution of the parameters was assumed log-normal and thus, inter-subject (IIV) was modeled using an exponential error model. The intra-individual variability or residual error was evaluated using a mixed proportional and additive error model.

Diagnostic graphs and additional statistical analyses were completed with R (R-project, version. 2.4.1).

Patient-specific characteristics such as sex, age, concomitant administration of dexamethasone, and EIAED co-administration were evaluated for their significance in the model to explain the inter-individual variability observed in lapatinib apparent oral clearance. A covariate was considered significant if its addition to the structural model reduced the objective function value (OFV) at least 3.84 units ($P < 0.05$, based on the χ^2 test for the difference in the log-likelihood between two hierarchical models that differ by one degree of freedom), and θ_p was significantly different than zero, $P < 0.05$, i.e., $1.96 \times \text{SE} \times (\theta_p) < \theta_p$.

After estimation of the model parameters for each patient, lapatinib concentrations up to 12 h after drug administration were simulated using pharmacokinetic parameters for that patient. The area under the concentration–time curve to 12 h ($AUC_{0\rightarrow12}$) was calculated from these simulated data using the log-linear trapezoidal method using ADAPT II [8].

Pharmacogenomic studies

In consenting patients, whole blood was collected prior to treatment. Genomic DNA was extracted using standard molecular procedures, and 10 ng of DNA from each patient was used for genotyping. PCR-RFLP techniques were used to genotype patients for the CYP3A4*1 [9]. CYP3A5*3 polymorphisms were also analyzed using PCR-RFLP techniques as previously described [10]. ABCB1 polymorphisms in exon 21 (G2677T/A) and exon 26 (C3435T) were genotyped by PCR amplification followed by sequencing [10]. The ABCG2 polymorphisms were genotyped as described in ref. [11] with the following modifications: 10 ng of genomic DNA was used as template, and annealing for the exon 2 and 5 PCR reactions was at 55°C. Amplification product (4 μl) was sequenced using the forward PCR primer.

EGFRvIII and PTEN analysis

All patients submitted tumor tissue blocks as part of enrollment on the trial. The tissue was analyzed for molecular genetic changes thought to be important in predicting response to EGFR tyrosine kinase inhibitors. The Applied Molecular Profiling Laboratory (Princess Margaret Hospital, Toronto, ON) received a total of 23 formalin-fixed paraffin-embedded glioblastoma blocks and sections for analysis of EGFRvIII mutation and PTEN immunohistochemistry (IHC).

EGFRvIII mutation was evaluated by quantitative real-time PCR. RNA was extracted with organic solvents (trizol, phenol, and chloroform). The quality and quantity of extracted RNA was assessed by electrophoresis and spectrophotometry. Total RNA was reverse transcribed using TaqMan Reverse Transcription Reagents kit and MultiScribe (Applied Biosystems). PCR primers were designed to amplify exons 4 and 9 of the EGFR gene, and the amplicons generated were 99 bp. Real-time PCR was performed using Platinum SYBR Green qPCR SuperMix-UDG (Invitrogen) on 7900HT platform (Applied Biosystems). A positive result was considered when the expression of exon 4 was less than or equal to 25% compared to expression of exon 9.

For PTEN IHC, paraffin tissue sections were used. Microwave antigen retrieval was carried out under pressure at 120°C for 10 min in 10 mM citrate buffer, pH 6.0. The primary antibody PTEN (Cell Signaling Technology, Danvers, MA, Cat no: 9559) was applied at 1:200 dilution overnight inside a moist chamber. Subsequent incubation with a biotinylated anti-rabbit IgG and detection used a streptavidin biotin system (Signet Pathology Systems/Covance, Princeton, NJ). Immunoreactivity was visualized after 5 min incubation with the Nova Red substrate chromogen (Vector Laboratories, Burlingame, CA). Slides were counterstained in Mayer's haematoxylin. Positive IHC was considered to represent present PTEN activity, whereas absent staining was interpreted as absent PTEN activity (Fig. 1). There were several cases of questionably positive staining (i.e. some cells staining positive but not others) and while these were classified as equivocal, they were considered as positive for evaluation of the relationship between clinical outcome and the PTEN expression because not all protein was absent as assessed by IHC.

Statistical considerations

The phase I portion of this study proceeded as a fixed dose escalation trial to determine the MAD as described above. All patients were evaluated for response, but only those treated at the recommended phase II dose for patients on EIAEDs were to be considered as part of the phase II sample size.

The phase II portion of this study was designed to evaluate the efficacy of lapatinib in the treatment of measurably recurrent GBM. A multinomial design was employed using both objective response and early progression as the endpoints of interest. Early progression was defined as documented progression as outlined above occurring on or before the first 8-week evaluation of therapy. Interest in further evaluation of this agent would occur if the true response rate was $\geq$20% (H_a for response = 0.20) or if the true early progression rate was 40% or less (H_a for progression = 0.40). There would be no interest in evaluating this agent, if the true response rate was $\leq$5% (H_0 for response = 0.05) and if the early progression rate was 60% or more (H_0 for progression = 0.60).

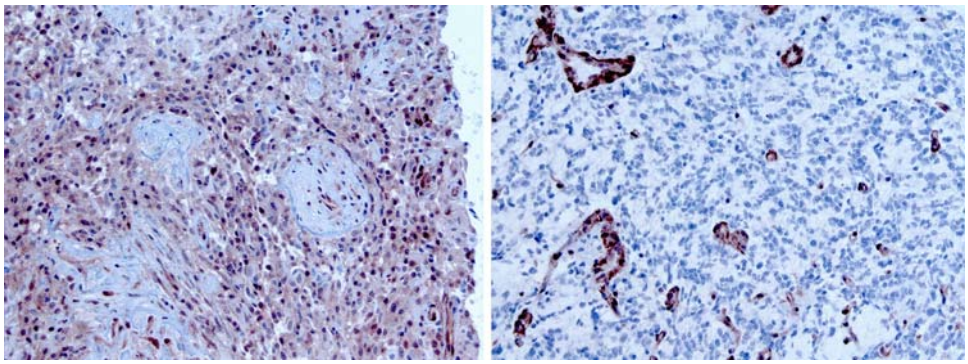

Fig. 1 PTEN Immunohistochemistry (IHC). Glioblastoma tumor samples showing positive (*left*) and negative (*right*) IHC for PTEN. The limited positive staining seen on the right represents PTEN presence in endothelial cells

In the first stage of accrual to the phase II study, 15 patients were to be enrolled and evaluated for response. Entry would be stopped if no responses were seen and if 10 or more patients had early progression. If one or more responses was seen or fewer than 10 early progressions occurred, a second phase of accrual would enter 15 more evaluable patients (30 total). Further investigation of lapatinib would be considered if 4 or more responses occurred or 13 or less early progressions were seen in the final sample of 30 patients.

Results of PTEN expression and EGFRvIII mutational analysis were explored for relationship to clinical outcome only in patients enrolled in the phase II portion of the trial, to ensure that any observations were based on outcomes from patients receiving full therapeutic doses of lapatinib. No formal statistical analysis was planned for the small sample size foreseen.

Results

Patients

The characteristics and demographics of the patients enrolled in this trial are summarized in Table 2. Seven patients on EIAEDs were entered into the phase I trial (4 in the first dose level and 3 in the second dose level). Accrual to the phase I arm was stopped prior to DLT being reached because of the results of the phase II arm (see below). The EIAEDs were carbamazepine in 1 patient and phenytoin in 6 patients.

The phase II portion of the trial enrolled 17 patients (see Table 2 for patient characteristics). Five phase II patients and three phase I patients were taking corticosteroids at baseline.

Table 2 Patient characteristics

	Phase I	Phase II
No. of patients	7	17
Median age (range) years	52 (30–64)	59 (31–72)
Gender		
F	2	5
M	5	12
ECOG status		
0	2	6
1	3	9
2	2	2
Prior treatment		
Radiotherapy	7	17
Chemotherapy	7	15
Median time from diagnosis (range) mos	7.6 (4.7–36.9)	12.8 (6.2–21.8)
Concomitant medications		
EIAEDs	7	0
Corticosteroids	3	5

Outcomes

Phase I study

Four patients were enrolled in the first dose level (1,000 mg bid) as one patient experienced a Grade 3 neutropenia which spontaneously recovered and it was felt prudent to enroll an additional patient to this cohort before proceeding to the next dose level. Three patients were then enrolled to the second dose level (1,500 mg bid). No DLT was seen at this dose level: the worst non-hematological toxicity was Grade I diarrhea. The study ceased further enrollment as the phase II portion was completed without evidence of efficacy and it was not felt to be appropriate to obtain a recommended phase II dose of inactive therapy in this population. All seven patients were evaluable for best treatment response and only one patient showed stable disease, with six others showing early progression.

Phase II study

Seventeen patients were evaluated in the phase II portion of the study. A total of 76 4-week cycles of lapatinib were administered. The median number of cycles received by patients in this group was 2 (range 1–18). No patients

showed objective radiographic response to lapatinib. Four patients demonstrated stable disease whereas 13 patients showed early progression. Given the lack of objective responses and the 76% early progression rate, further enrollment into this trial was stopped as per the multinomial design.

Toxicity

Lapatinib was a well tolerated agent in this study. Among the seven phase I patients, no attributable Grade 3 non-hematologic toxicities were seen. One Grade 3 neutropenia and one Grade 3 lymphopenia was seen at the first dose level (1,000 mg bid) and two Grade 2 lymphopenias were seen at the second dose level (1,500 mg bid). Among the 17 phase II patients, the only significant non-hematologic toxicity was one episode of Grade 3 diarrhea. Hematologic adverse events were minimal, except for one patient with Grade 3 lymphopenia. Rash ($n = 7$) and diarrhea ($n = 8$) were the most common adverse effects of treatment. Other common non-hematologic complaints included heartburn ($n = 5$), fatigue ($n = 4$), pruritis ($n = 3$), nausea ($n = 3$) and headache ($n = 4$). No cases of cardiotoxicity manifested by impaired LVEF were identified.

Pharmacokinetics

Six of 22 patients did not participate in the pharmacokinetic study due to withdrawal of consent, patient preference or progressive disease. In the 16 remaining patients, a total of 80 samples were analyzed for lapatinib pharmacokinetics.

The C_{max}, T_{max}, and $AUC_{0\rightarrow12}$ values are presented in Table 3. The three patients included in the 1,000-mg dose level were receiving concomitant EIAEDs during the lapatinib pharmacokinetic study. Moreover, as shown in Fig. 2a, a relation between lapatinib AUC and actual dosage received could not be established primarily due to the effect of co-administration of EIAEDs with lapatinib. Similarly, no relation was observed between lapatinib AUC and dose level in this study population as depicted in Fig. 2b.

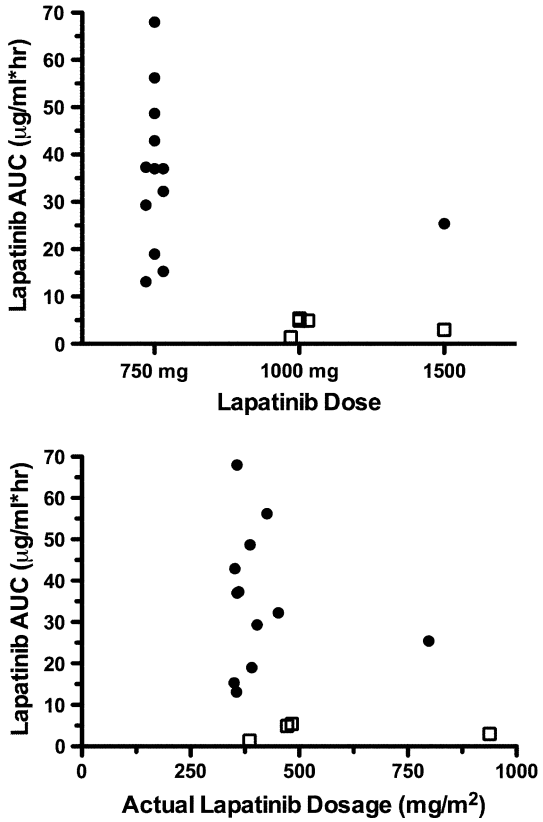

Fig. 2 Lapatinib $AUC_{0\rightarrow12}$ relationship to dose level (**a**) and actual dosage (**b**)

Table 3 Lapatinib pharmacokinetic data

Lapatinib dose level	750 mg ($n = 11$)	1,000 mg[a] ($n = 3$)	1,500 mg[a] ($n = 2$)
C_{max} (μg ml^{-1})	4.36 (1.74–8.47)	0.82[a] (0.28–1.04)	2.61 and 0.79
T_{max} (h)	4.0 (2.0–5.3)	2.0 (1.9–4.0)	6 and 2
$AUC_{0\rightarrow12}$ (μg h ml^{-1})	37.0 (13.1–68.0)	4.9 (1.4–5.4)	25.4 and 3.0

Lapatinib was administered twice daily. Data is expressed as median (range)

n number of patients, C_{max} maximum plasma concentration, T_{max} time for maximum concentration, $AUC_{0\rightarrow12}$ area under concentration–time curve for 12 h

[a] Five out of five patients were on EIAEDS

Based on visual inspection of the lapatinib concentration–time data, a one-compartment model with first-order elimination adequately fitted the lapatinib plasma concentration–time data. The population pharmacokinetic parameters obtained for the structural model were: CL/F = 23.1 (8.3) L h^{-1} m^{-2}; V/F = 98.2 (23.9) L m^{-2}, *k*a = 0.111 (0.017) h^{-1}. A large inter-individual variability in the parameter estimates of the base model was observed accounting for 127 and 56.8% for apparent clearance and volume, respectively.

The inter-individual variability could not be estimated for the absorption rate constant (*k*a). Thus, we empirically fixed this variability at 25% (CV%). Also, the proportional component of the residual variability was fixed at a value corresponding to the lower limit of quantitation of the assay.

In the univariate analysis, the following covariates were analyzed for their significance in the model: age (as a categorical variable, i.e., age less than or equal to 30 years old or greater than 30 years), sex, and concomitant drug administration (i.e., dexamethasone or EIAEDs). Graphical plots of empirical Bayesian estimates of apparent oral clearance

Table 4 Lapatinib pharmacokinetic parameter estimates for final model

Model	Mean (SE)	IIV (SE)
Final model[a]		
CL/F (L h^{-1} m^{-2})[a]	12.1 (3.2)	0.367 (0.150)
Vd/F (L m^{-2})	124.0 (52.3)	0.522 (0.256)
ka (h^{-1})	0.124 (0.039)	
Residual variability		
ε_{prop}	0.017 (0.004)	

EIAEDs = 1 or 0 when patient is on and off EIAEDs, respectively. Data is shown as mean parameter estimates (SE)

CL/F apparent oral clearance, *Vd/F* apparent volume of distribution, *Ka* absorption rate constant

[a] The final model corresponds to: CL/F(L $h^{-1}m^{-2}$) = 12.1 + 94.8 (EIADS usage)

versus age, sex or concomitant administration of dexamethasone showed no apparent relationship. Moreover, these covariates were not statistically significant under the univariate analysis ($P > 0.05$) using NONMEM.

The concomitant administration of EIAEDs was related to lapatinib apparent oral clearance ($\Delta OFV = -19.0$, $P < 0.0001$). When concomitant administration of EIAEDs was considered in the model as related to lapatinib apparent oral clearance, it explained 77.5% of the inter-individual variability in apparent oral clearance while improving the model. The population pharmacokinetic parameter estimates for the final model are presented in Table 4.

Pharmacogenomics

Thirteen single nucleotide polymorphisms were analyzed in four genes of putative relevance for lapatinib absorption and disposition. No statistically significant associations with lapatinib pharmacokinetic parameters were observed in a total of nine patients with variant alleles in CYP3A5*3, ABCB1, or ABCG2 ($P > 0.05$). The lapatinib apparent oral clearance for the CYP3A4*1A/*1B heterozygous individual was 7.4 L h^{-1} m^{-2} compared with the lapatinib apparent oral clearance for CYP3A4*1A/*1A wild-type carriers, which was 21.0 (SD ± 15.0) L h^{-1} m^{-2}.

EGFRvIII and PTEN analysis

Sixteen of the 17 phase II patients had EGFRvIII mutation analysis and PTEN IHC studies. Results are shown in Table 5. Of the four patients with stable disease, one demonstrated an EGFRvIII mutation. In that instance, the PTEN IHC activity was equivocally positive. The three other patients who demonstrated stable disease with lapatinib did not have EGFRvIII detected and one had loss of PTEN activity with the remaining two patients showing equivocally positive PTEN activity. Of the 12 patients with early progression, 4 had non-interpretable results from the EGFRvIII PCR analysis (2 inconclusive and 2 failed tests). Of the 8 remaining samples, 3 showed an EGFRvIII mutation and of those, one patient clearly showed intact PTEN activity and another showed equivocally positive PTEN activity. While the numbers are too small for any degree of certainty, we could not detect a relationship between combined EGFRvIII mutation/intact PTEN activity and tumor outcome (SD or PD) in response to lapatinib.

Table 5 Correlative molecular analysis of patients in the phase II study (n = 16 of 17 patients)

Patient no.	Treatment response	EGFRvIII mutation	PTEN activity
1	SD (7.0 mos)	Neg	Absent
2	SD (5.5 mos)	Neg	Equivocal
3	SD (12.6 mos)	Neg	Equivocal
4	SD (10.1 mos)	Pos	Equivocal
5	PD	Pos	Absent
6	PD	N/A	Absent
7	PD	Neg	Present
8	PD	Inc	Absent
9	PD	Neg	Present
10	PD	Inc	Present
11	PD	N/A	Equivocal
12	PD	Neg	Equivocal
13	PD	Pos	Present
14	PD	Neg	Absent
15	PD	Neg	Absent
16	PD	Pos	Equivocal

SD stable disease, *PD* progressive disease, EGFRvIII *Pos* mutation present, *Neg* mutation not detected, *Inc* inconclusive, considered Neg for analysis, *N/A* failed test, *PTEN present* normal PTEN staining, *Absent* no detectable PTEN staining, *Equivocal* slight staining or only some cells staining for PTEN, considered present for analysis

Discussion

Tyrosine kinase inhibitors have received considerable interest in oncology as an increasing number of molecular targets of intracellular signaling are identified. The ErbB family of receptors has received considerable attention. This group includes EGFR/ErB-1 and Her-2/ErbB-2 receptors which are important in several tumor types including breast, lung and glial tumors. EGFR pathway inhibitors have shown clinical utility in the treatment of lung cancer [12] and Her-2 receptor blockade has shown benefit in subsets of patients with breast cancer [13–15].

In GBM, EGFR tyrosine kinase inhibitors have met with limited success. EGFR is frequently overexpressed or

mutated in glioblastoma tumor cells (40–90%). Despite this, response rates to EGFR tyrosine kinases such as gefitinib and erlotinib, are typically low (0–15%) [16, 17]. Haas-Kogan et al. [3] have demonstrated tumor shrinkage in glioma patients on erlotinib correlates with both an overexpression of EGFR and non-phosphorylation of PKB/Akt (a downstream kinase in the EGFR signaling pathway). Melinghoff et al. [2] found that their patients responded to erlotinib or gefitinib if the tumors expressed EGFRvIII (a constitutively active mutation of EGFR) and had intact PTEN activity. PTEN is a phosphatase which acts to remove the activation link between EGFR signaling and downstream activation of the PI3K signaling pathway. Therefore, according to both these studies, for the EGFR tyrosine kinase inhibitors to be active, there must be evidence of "oncogene addiction" where overactivity of EGFR signaling leads directly to activation of the PI3K/Akt pathway since PTEN is intact. Accordingly, GBM cells with absent PTEN do not have a functioning activation pathway between EGFR and PI3K/Akt and would be resistant to inhibitors of EGFR. Unfortunately, most GBMs have mutant PTEN which likely accounts for much of the limited utility seen with EGFR tyrosine kinase inhibitors to date.

Lapatinib is a dual action tyrosine kinase inhibitor having activity both at Her-2/ErbB-2 receptors and EGFR. There have been several trials of this agent in breast cancer revealing clinical benefit (combined complete response, partial response and stable disease rates) of 14–22% [18–20]. Those patients most likely to respond showed overexpression of Her-2/ErbB-2 and negative estrogen and progesterone receptor status [21]. In these trials, the drug was well tolerated with rash, diarrhea and nausea being the chief side effects. Instances of cardiac dysfunction (a known risk in Her-2/ErbB-2 inhibitors) was low with lapatinib (1.3%) [22]. Because of this broader spectrum of tyrosine kinase inhibition and good side effect profile, it was felt that a trial of this agent in GBM was indicated despite the fact that gliomas rarely show Her-2/ErbB-2 changes.

This trial was designed as a combined phase I/II study after it was realized that lapatinib was metabolized through CYP3A/4 pathways. Many patients with brain tumors are treated with enzyme-inducing anti-epileptic drugs (EIAEDs) such as phenytoin and carbamazepine, which induce the CYP3A/4 metabolic pathway and can lead to accelerated metabolism of chemotherapeutic agents. Therefore, patients on EIAEDs were enrolled in a separate phase I trial of dose escalation, while those patients not on EIAEDs participated in the phase II trial.

We were unable to demonstrate any benefit of lapatinib in the 17 patients enrolled in the phase II arm of this trial. Only four patients demonstrated stable disease and 13 patients showed early progression, thus, even in the absence of response, there was no signal of activity in terms of delay in progression in meaningful numbers of patients. Because of these results, as per protocol, the phase II portion of the trial was closed to accrual. At that time, seven patients had been enrolled into the phase I arm of the trial and although a recommended dose had not been identified, the lack of efficacy of the agent made the pursuit of a recommended dose in this population no longer relevant. In both EIAED and non-EIAED patients, we were able to confirm that lapatinib was a well tolerated agent. No Grade 4 toxicities were seen and no cardiac toxicity was identified.

There are several possible reasons for the lack of efficacy in this tumor group. Lapatinib may perform better in cells that have both ErB-1 and ErbB-2 expression rather than isolated ErbB-1 overexpression. In fact, lapatinib may bind only to inactive/intermediate form of EGFR and constitutively active EGFR (i.e., EGFRvIII mutation) may not be inhibited at all [23]. Although we were not able to directly assess this in our study population, it is also possible that lapatinib penetration past the blood–tumor barrier is suboptimal. Lapatinib is fairly large, polar, protein-bound molecule and would be expected to have limited penetration into the CNS.

Additionally, after the publication of the Melinghoff et al. study [2], we wondered if our sample population may have included patients who did not show EGFRvIII mutation and intact PTEN activity in their tumors. To test this, we performed molecular correlative studies to examine this possibility by evaluating EGFRvIII analysis by real-time PCR and PTEN analysis by immunohistochemistry. We found that 3/16 patients had evidence of the EGFRvIII mutation and had positive or equivocally positive PTEN. Two of those patients showed early progression and one showed stable disease. Although the numbers were small and our correlative studies were limited by a lack of frozen tissue, we were unable to demonstrate that the presence of EGFRvIII mutation and intact PTEN activity were associated with any trends in the clinical outcomes to lapatinib.

Extensive pharmacokinetic data were obtained in this study. The mean population estimate for the apparent oral lapatinib clearance was 12.1 $L\,h^{-1}\,m^{-2}$. The only patient-related covariate that was significantly related to lapatinib apparent oral clearance was co-administration of EIAEDs. However, the mean population estimate of lapatinib apparent oral clearance in patients receiving EIAEDs was 106.9 $L\,h^{-1}\,m^{-2}$, or approximately tenfold greater than those that were not receiving EIAEDs. Although the lapatinib pharmacokinetic parameters observed in this study (e.g., T_{max}, C_{max}, AUC) for those patients not on EIAEDs were similar to those values reported in the literature, no data are available for comparison for patients on EIAEDs. However, based upon the very high lapatinib apparent oral clearance and subsequent low lapatinib systemic exposure observed in these patients, co-administration of lapatinib

and enzyme-inducing anticonvulsants should be avoided pending further phase I studies. This information is important as lapatinib has shown some activity in treatment of brain metastases in breast cancer [24]. For those patients with brain metastases that will require anti-epileptic drugs, avoidance of enzyme-inducing anticonvulsants will be important unless lapatinib is used at a much higher dosage.

Conclusion

This phase I/II trial of lapatinib in patients with relapsed GBM failed to show significant activity of this agent. Despite the presence of EGFRvIII mutation and intact PTEN immunohistochemistry in three patients, none responded to lapatinib. Lapatinib is metabolized by CYP3A/4 pathways and its apparent oral clearance was significantly impacted by EIAEDs. Patients on lapatinib should be advised to avoid EIAEDs pending further dose-finding studies.

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