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Phase I and Pharmacokinetic Studies of Erlotinib Administered Concurrently With Radiotherapy for Children, Adolescents, and Young Adults with High-Grade Glioma

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Abstract

STATEMENT OF TRANSLATIONAL RELEVANCE—Although chemotherapy has been used in most clinical trials for children with high-grade glioma, no drug regimen has so far stood out as particularly beneficial for these patients.

Based on pre-clinical and clinical experience in adults with similar tumors, we report for the first time the results of a Phase I study which used erlotinib during and after radiotherapy in children with high-grade glioma. We characterized the acute and chronic toxicities associated with erlotinib therapy. Within the context of a Phase I study, we also performed extensive pharmacokinetic and molecular studies.

We observed preliminary encouraging outcome in a subgroup of our patients, particularly those with anaplastic astrocytoma. Therefore, the current study established a platform for future clinical trials to incorporate erlotinib in the treatment of high-grade glioma in children.

Purpose—To estimate the maximum-tolerated dose (MTD) of erlotinib administered during and after radiotherapy (RT), and to describe the pharmacokinetics of erlotinib and its metabolite OSI-420 in patients between 3 and 25 years with newly diagnosed high-grade glioma who did not require enzyme-inducing anticonvulsants.

Experimental Design—Five dosage levels (70, 90, 120, 160, and 200mg/m² per day) were planned in this Phase I study. Dose-limiting toxicities (DLTs) were evaluated during first 8 weeks of therapy. Local RT (dose between 54 and 59.4 Gy) and erlotinib started preferentially on the same day. Erlotinib was administered once daily for a maximum of 3 years. Pharmacokinetic studies were obtained after first dose and on day 8 of therapy. Mutational analysis of *EGFR* kinase domain, *PIK3CA*, and *PTEN* was performed in tumor tissue.

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Results—Median age at diagnosis of 23 patients was 10.7 years (range, 3.7 to 22.5 years). MTD of erlotinib was 120mg/m² per day. Skin rash and diarrhea were generally well controlled with supportive care. DLTs were diarrhea (*n*=1), increase in serum lipase (*n*=1), and rash with pruritus (*n*=1). The pharmacokinetic parameters of erlotinib and OSI-420 in children were similar to those described in adults. However, there was no relationship between erlotinib dosage and drug exposure. No *EGFR* kinase domain mutations were observed. Two patients with glioblastoma harbored mutations in *PIK3CA* (*n*=1) or *PTEN* (*n*=1).

Conclusions—Although the MTD of erlotinib in children with newly diagnosed high-grade glioma was 120mg/m² per day, pharmacokinetic studies demonstrated wide inter-patient variability in drug exposure.

Keywords

children; erlotinib; glioma; high-grade; radiotherapy

INTRODUCTION

Whereas high-grade gliomas, in particular glioblastoma, represent more than 50% of all primary malignant brain tumors in adults, similar neoplasms arising outside the brainstem constitute only 10% of all primary brain tumors in children.¹ Like adults, the combination of maximum safe surgery, radiotherapy (RT), and standard chemotherapy has not improved the outcome of children older than 3 years with high-grade glioma (1).

Recently, the combination of RT and temozolomide followed by adjuvant temozolomide showed superior outcome compared to RT only in the treatment of adults with newly diagnosed glioblastoma (2). Several studies have used temozolomide in the treatment of children with high-grade glioma; however, the results so far have been disappointing (3–6).

New biologic agents which inhibit relevant cellular targets have been employed in the treatment of adults with high-grade glioma (7). Although high-grade gliomas in children are biologically distinct from those in adults (8–10), abnormalities in the epidermal growth factor receptor (EGFR) pathway are prevalent in both age groups (9,11,12). Although *EGFR* amplification is rare in pediatric high-grade glioma (9,11,13,14), these tumors commonly overexpress EGFR (9,11).

Erlotinib (Tarceva[®], OSI Pharmaceuticals, Melville, NY; Roche, Basel, Switzerland; Genentech, South San Francisco, CA), a small-molecule inhibitor of EGFR, is also an antiangiogenic agent. Although EGFR inhibitors, and particularly erlotinib, have shown benefit when combined with RT in preclinical models of glioblastoma (15,16), to our knowledge these studies have not been performed in xenografts derived from pediatric patients. Several clinical trials demonstrated modest activity of erlotinib in the treatment of adults with recurrent high-grade glioma (17–19).

Therefore, we conducted this study to estimate the maximum-tolerated dose (MTD) and to characterize the pharmacokinetics of erlotinib administered concurrently with and after RT in pediatric patients with newly diagnosed high-grade glioma.

¹Central Brain Tumor Registry of the United States: 2004–2005 Statistical Report: Primary Brain Tumors in the United States Statistical Report, 1998–2002. <http://www.cbtrus.org/reports//2005-2006/2006report.pdf>

PATIENTS AND METHODS

Patients between 3 and 25 years of age with newly diagnosed high-grade glioma were eligible for this study. Additional eligibility criteria consisted of: (i) Interval between surgery and start of therapy ≤ 42 days; (ii) Performance score ≥ 40 ; (iii) Adequate hematologic (absolute neutrophil count $\geq 1,000/\mu\text{L}$, platelet count $\geq 100,000/\mu\text{L}$, and hemoglobin concentration $\geq 8\text{g/dL}$), renal (serum creatinine concentration < 2 times the institutional normal values for age), and hepatic (total bilirubin concentration < 1.5 times the institutional upper limit of normal, SGPT < 5 times the institutional upper limit of normal, and albumin $\geq 2\text{g/dL}$) function. Exclusion criteria consisted of: (i) Diagnosis of diffuse brainstem glioma; (ii) Diagnosis of secondary high-grade glioma; (iii) Presence of leptomeningeal metastatic disease; (iv) Use of enzyme-inducing anticonvulsants (EIACs); (v) Use of other concomitant anticancer therapy; (vi) Presence of other significant concomitant medical problems; (vii) Pregnant or lactating patients. Female patients of childbearing age and male patients of child-fathering potential had to agree to use safe contraceptive methods. For patients who were weaned off EIACs, at least 7 days were required between discontinuation of EIACs and start of erlotinib. Central histologic review of all cases was conducted by a neuro-pathologist (D.W.E).

Our institutional review board approved this protocol before initial patient enrollment and continuing approval was maintained throughout the study. Written informed consent for participation was obtained from patients or their legal guardians, and assents were obtained when appropriate.

Study Design and Treatment Plan

This single institution study followed a standard Phase I-study design. The dosage of erlotinib was increased by about 30% in each dosage level starting at 80% of the MTD in adults with solid tumors (20). Five dosage levels were planned (70, 90, 120, 160, and 200 mg/m^2 per day). A traditional 3+3 dose escalation scheme was used to estimate the MTD. MTD was defined as the highest dosage level in which no more than one of six assessable patients had experienced DLTs. The DLT-evaluation period comprised the first eight weeks of therapy. DLT was defined as any of the following toxicities attributable to erlotinib therapy: thrombocytopenia grade 3 and 4; neutropenia grade 4; or any grade 3 and 4 nonhematologic toxicity except for grade 3 diarrhea and grade 3 nausea and vomiting lasting ≤ 48 hours in patients not receiving optimal supportive therapy, grade 3 skin rash which did not affect normal daily activities, grade 3 fever or non-neutropenic infection, grade 3 seizures, grade 3 weight gain or loss, and grade 3 transaminase elevation that returned to grade 1 or baseline within 7 days. After enrollment of the first four patients on study, we also excluded as DLT grade 3 and 4 electrolyte abnormalities that resolved to $\leq$ grade 2 within 7 days. Toxicities were graded according to the Common Terminology Criteria for Adverse Events version 3.0.

Erlotinib and local RT started on the same day except for three patients who were weaned off EIACs before the start of erlotinib. In the latter cases, erlotinib therapy was started within 6 days from beginning of RT. Three-dimensional conformal RT was delivered as 1.8-Gy fractions to a total dose of 59.4 Gy. The treatment volume encompassed the tumor bed, the surrounding residual T2-weighted signal abnormality, a 2-cm margin to account for microscopic disease, and a 0.3 to 0.5-cm margin to account for uncertainty in immobilization and patient's positioning. Whole brain RT was administered at a dose of 54 Gy for patients with $> 70\%$ of the brain volume involved by tumor. Erlotinib was administered as 25-, 100-, and 150-mg tablets 1 hour before or 2 hours after meals once daily during and after RT. Maximum planned treatment duration was 3 years. The day 2 dose of erlotinib was held in patients undergoing pharmacokinetic studies. For patients not receiving erlotinib at the MTD, the drug dose could be increased once to the next dosage level if therapy had been well tolerated during first 6 cycles of therapy.

Radiologic evaluation consisting of brain and spine MRI were done within 2 weeks before enrollment on study. Brain MRI was repeated 8 weeks after start of therapy, and every 12 weeks thereafter. Complete and partial responses were defined as complete disappearance and $\geq 50\%$ reduction of tumor as measured by bi-dimensional measurements, respectively. Stable or improved neurologic findings for at least 6 weeks on a stable or decreasing dose of corticosteroids were required in both instances. Progressive disease was defined as worsening neurologic findings attributed to tumor progression, and/or increase of $> 25\%$ in bi-dimensional tumor measurements, and/or appearance of tumor in previously uninvolved areas and/or need of increasing doses of corticosteroid to maintain stable neurologic exam. Stable disease consisted of all other situations not defined above.

Pharmacokinetic Studies

Blood samples for optional pharmacokinetic studies were obtained in consenting patients. Two milliliters of blood were collected in heparinized tubes before and at 1, 2, 4, 8, 24 (± 2), 30 (± 4), and 48 (± 4) hours after first dose of erlotinib, and before and at 1, 2, 4, 8, and 24 (± 2) hours after dose on day 8. Once collected, blood samples were centrifuged and the plasma was stored at -80°C until analysis. Pharmacokinetic analysis of erlotinib and its primary metabolite OSI-420 was performed by high-performance liquid chromatography with tandem mass spectrometry (21). The lower limits of quantification for erlotinib and OSI-420 were 10 ng/ml and 1 ng/ml, respectively. The inter-assay precision during validation was $< 7\%$ for both erlotinib and OSI-420 at all concentrations. Pharmacokinetic parameters consisting of maximum plasma concentration (C_{max}), area under concentration-time curve during dosing intervals ($\text{AUC}_{0 \rightarrow 24}$ and $\text{AUC}_{0 \rightarrow 48}$), and time to maximum plasma concentration (T_{max}) after day 1 and 8 of therapy were calculated using standard noncompartmental methods from the plasma concentration-time profiles of erlotinib and OSI-420.

Molecular and Immunohistochemical Studies

DNA was extracted from formalin-fixed, paraffin-embedded tissue using standard techniques. The entire *PTEN* coding sequence (exons 1 to 9), exons 1, 9, and 20 of *PIK3CA*, which contain hotspots for mutations, and exons 17 to 24 of *EGFR*, which encode its kinase domain, were evaluated using exon-specific polymerase-chain reaction (PCR) amplification with previously described primers (22–24). PCR products were purified from agarose gels using QIAEX II Purification kit (Qiagen, Valencia, CA) and direct DNA sequencing was performed using Applied Biosystem 3730XL DNA Analyzers. All potential mutations were verified by re-amplification from genomic DNA and direct sequencing of the mutated exon.

Unstained slides were available for immunohistochemistry in 21 cases. Immunohistochemistry was performed for PTEN, phosphorylated AKT (p-AKT) and S6 (p-S6; #9559, 1:100; #9271, 1:50 and #2211, 1:100, respectively; Cell Signaling, Danvers, MA) (25). Endothelial cells served as an internal positive control for PTEN. Brain tissue sections from *Pten* conditional knockout or control mice also served as positive and negative controls, respectively. Tumor lesions were considered positive if greater than 25% cells were immunoreactive. Immunoreactivity was graded as strongly positive (++), weakly positive (+), or negative (–).

Statistical Analysis

Progression-free (PFS) and overall survival (OS) were estimated according to the method of Kaplan and Meier (26). Standard error estimates were calculated by the method of Peto et al. (27). PFS reflected the interval between diagnosis and clinical and/or radiologic tumor progression, or death. OS was defined as the interval between diagnosis and death. Patients not experiencing an event were censored at their last follow-up date.

RESULTS

Twenty-three patients were enrolled on study between March 2005 and June 2007. The patients' characteristics before start of therapy are shown in Table 1. Three patients were older than 18 years at diagnosis. We retrospectively found that one patient had undergone placement of carmustine-loaded wafers before start of RT and erlotinib therapy at another institution; this patient was considered assessable for DLT evaluation. One patient harbored an anaplastic astrocytoma which originated in the brainstem and exophytically extended towards the left cerebellopontine angle.

Toxicities

Of three patients initially enrolled on the 70mg/m² dosage level, one patient experienced grade 3 hypokalemia which was easily corrected with oral potassium supplementation. The protocol was then opened for three additional patients at the same dosage level. A second patient enrolled on the 70mg/m² dosage level also developed easily reversible grade 3 hypokalemia and hypophosphatemia. Following the amendment to exclude as DLTs grade 3 and 4 electrolyte abnormalities that resolved to ≤ grade 2 within 7 days, none of three additional patients enrolled on the 70mg/m² dosage level experienced DLTs. Likewise, all three patients treated at the 90mg/m² dosage level tolerated therapy well. Erlotinib dosage was increased to 120mg/m² and one patient experienced grade 3 diarrhea despite optimal supportive care. None of five other assessable patients treated at this dosage level experienced DLTs. One patient at the 120mg/m² dosage level was not assessable for toxicity because of early tumor progression. Of three patients enrolled on the 160mg/m² dosage level, one patient experienced DLT consisting of grade 3 increase in serum lipase concomitant with episodes of abdominal pain but without other findings compatible with pancreatitis. Of three additional patients enrolled at the same dosage level, one patient experienced grade 3 intolerable skin rash and pruritus. Therefore, 120mg/m² per day was established as the MTD for our patients. Table 2 provides a summary of significant toxicities during the DLT-evaluation period.

Chronic therapy with erlotinib has been well tolerated. Treatment duration in our patients ranged from 6 weeks to 26 months. Eighteen and 12 patients have received treatment for at least 6 and 12 months, respectively. One patient with left thalamic glioblastoma developed a second neoplasm (rhabdomyosarcoma) outside the RT field within 6 months of start of therapy. One patient with a thalamic tumor died of intratumoral hemorrhage at the time of radiologic progression and following ventricular decompression due to hydrocephalus. Although the hemorrhage was definitely related to the surgical procedure, it could be possibly associated with erlotinib therapy. Other chronic toxicities included grade 1/2 indirect hyperbilirubinemia, grade 1–3 hypokalemia and hypophosphatemia, and grade 1–3 lymphopenia.

Pharmacokinetic Studies

The results of the pharmacokinetic parameters, which were obtained in 17 patients, are summarized in Table 3. Although the calculated dose of erlotinib was rounded to the nearest 25 mg, the actual dosage administered to patients was within 12% of the prescribed dosage in all but one patient. The latter patient received erlotinib at the lowest dosage level and the actual dosage was 19% higher than the calculated dose. The AUC_{0→48} (after first dose) and AUC_{0→24} (day 8 of therapy) of erlotinib did not increase with increasing dosage (Figure 1). No correlation was identified between AUC_{0→48} of erlotinib and OSI-420 normalized to dosage and patient age at diagnosis.

Molecular and Immunohistochemical Studies

One *PTEN* (R130*) and one *PIK3CA* (H1047R) mutation were identified in patients with glioblastoma. No *EGFR* kinase domain mutations were identified.

Immunohistochemical analysis showed that the tumor containing a *PIK3CA* mutation expressed normal levels of PTEN, while the tumor containing a truncating *PTEN* mutation showed loss of PTEN protein in tumor cells, while normal and reactive cells expressed PTEN (Figure 2). Both tumors showed elevated levels of p-AKT and p-S6, indicating activation of downstream effectors in the phosphatidylinositol 3-kinase (PI3K)/AKT pathway (Figure 2). However, elevated levels of p-AKT (19 of 19) and p-S6 (15 of 20) were also detected in tumors lacking mutations in *PIK3CA* or *PTEN*.

Outcome

No objective radiologic responses to therapy were observed. Disease stabilization was documented in 16 patients. Four patients have remained without evidence of disease after undergoing gross tumor resection at a median of 2.15 years (range, 1.5 to 2.2 years). Sixteen patients have already experienced tumor progression (Table 4).

The 1-year PFS and OS for all patients were $56\% \pm 10\%$ and $78\% \pm 9\%$, respectively. The 2-year PFS and OS for all patients were $35\% \pm 12\%$ and $48\% \pm 12\%$, respectively. The 1-year PFS and OS for patients with glioblastoma were $33\% \pm 12\%$ and $67\% \pm 13\%$, respectively. The 1-year PFS and OS for patients with anaplastic astrocytoma were $75\% \pm 14\%$ and $86\% \pm 12\%$, respectively.

DISCUSSION

We have shown that erlotinib administered during and after RT at a dosage of $120\text{mg}/\text{m}^2$ per day is well tolerated by pediatric patients with newly diagnosed high-grade glioma. The MTD reached in the current study is higher than the established MTD of erlotinib for children (28) and adults with recurrent solid tumors, (20) and equivalent to the MTD established for adults with recurrent high-grade glioma who did not receive EIACs (17). Smoking or use of EIACs influence the pharmacokinetics of erlotinib by increasing its metabolism, and hence the tolerance to therapy (17,29). None of our patients smoked or required EIACs during therapy. Unlike previous studies, we arbitrarily mandated a shorter interval of 7 days between discontinuation of EIACs and start of erlotinib. Of 9 patients who were weaned off EIACs before start of therapy; five discontinued EIACs less than 10 days before start of erlotinib. However, the DLT-evaluation period of 8 weeks in the current study was much longer than previous studies (17,20,28). Therefore, we considered negligible the impact of previous use of EIACs on the evaluation of toxicities in the current study. We can not elaborate on the influence of corticosteroids on the exposure to erlotinib since only one of five patients who received dexamethasone during initial pharmacokinetic studies went on to have repeat evaluation 2 weeks after discontinuation of corticosteroids; the clearance of erlotinib remained stable in this patient.

The side effects of erlotinib observed in our patients were very similar to those reported in adults (17,20). Hypokalemia during erlotinib therapy had been previously described; (30) however, hypophosphatemia had only been reported when erlotinib therapy was combined with other medications (31). The mechanism of electrolyte abnormalities associated with erlotinib therapy is not clear. Like other pediatric Phase I studies, we excluded electrolyte abnormalities as DLTs based on the lack of clinical significance of these toxicities and with the hope of reaching higher drug concentrations of erlotinib in the central nervous system with the use of higher dosage levels (21). We also removed tolerable grade III skin rash as a DLT because of the reported better outcome of patients with other cancers who experienced this toxicity during erlotinib therapy (32).

We were also able to assess the long-term side effects of erlotinib therapy in children since more than three-fourths of our patients have received therapy for at least 6 months. No

significant long-term toxicities possibly associated with erlotinib therapy have been observed except for a second neoplasm and a fatal episode of intratumoral hemorrhage in the context of tumor progression. The short interval from start of therapy and the diagnosis of second neoplasm and its occurrence outside the RT fields suggest a reason unrelated to therapy; however, no evidence in history, physical exam, or molecular analysis of tumor samples supported a predisposition to develop a second cancer in this patient.

The results of our pharmacokinetic studies obtained at steady-state, the inter-patient variability in the pharmacokinetic parameters of erlotinib, and the ratio of erlotinib to OSI-420 exposure were similar to previously published data in adults (17,20). In the study of Prados et al., patients not requiring EIACs who received erlotinib at a dose of 200mg per day had mean C_{max} , T_{max} , and AUC_{0-24} of 1.44 $\mu\text{g/ml}$, 1.8 h, and 21.2 $\mu\text{g}\cdot\text{h/ml}$, respectively (17). We reported very similar results in patients who received erlotinib at 120mg/m² per day, which is equivalent to an adult dose slightly above 200mg. In the current study, no association was observed between the dosage of erlotinib and exposure. Likewise, Prados et al. did not observe a relationship between the dose of erlotinib and exposure in patients treated with erlotinib alone (17).

Several studies have evaluated the relationship between molecular characteristics of high-grade glioma in adults and response to treatment with small-molecule EGFR inhibitors (33–38). Haas-Kogan et al. showed that patients with glioblastoma whose tumors expressed high levels of EGFR and low levels of p-AKT responded better to erlotinib therapy (34). In another study, co-expression of EGFRvIII and PTEN was associated with better response of patients with high-grade glioma to EGFR inhibitors (36). Unlike non-small cell lung cancer, (22,39) *EGFR* kinase domain mutations are rarely found in high-grade gliomas in adults (33–38). Likewise, *EGFR* kinase domain mutations were not observed in our patients. Since the activity level of the PI3K/AKT pathway seems to influence the tumor response to EGFR inhibitors, (34) we performed an exploratory evaluation of two key regulatory genes (*PIK3CA* and *PTEN*) and three immunohistochemical markers (PTEN, p-AKT and p-S6) of this pathway. Similar to previous studies, (9,13,40,41) we demonstrated mutations in either one of these genes in a subset of our patients with glioblastoma. Immunohistochemistry showed strong expression of p-AKT and p-S6 in the tumors bearing mutation in *PIK3CA* or *PTEN*, consistent with activation of the PI3K pathway. However, similar evidence of PI3K pathway activation was observed in the majority of tumors lacking detectable mutations in *PIK3CA* or *PTEN*, suggesting that the pathway is engaged by alternative mechanisms in this cohort of pediatric high-grade glioma. Despite our detailed molecular and immunohistochemical analysis in the context of a Phase I study, no correlation was found between markers of PI3K/AKT pathway activation and response to the current therapy.

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Figure 1.

Erlotinib exposure after first dose (area under concentration-time curve from 0 to 48 hours; Figure 1A) and on day 8 of therapy (area under concentration-time curve from 0 to 24 hours; Figure 1B)

Figure 2.

Representative PTEN, phosphorylated AKT and S6 immunostaining of high grade gliomas. The tumor containing a *PIK3CA* mutation (A–C) expressed high levels of PTEN (A), and showed evidence of PI3K pathway activation by strong immunoreactivity for both phosphorylated AKT (B) and S6 (C). The tumor cells harboring PTEN mutation (D–F) showed loss of PTEN expression in tumor cells, with normal expression in surrounding normal cells (D). Consistent with an absence of functional PTEN, tumor cells were positive for phosphorylated AKT (E) and S6 (F).

Table 1
Patients Characteristics at the Start of Therapy

Characteristics		No. of Patients(<i>n</i> =23)	%
Age at diagnosis (years)	Median	10.7	
	Range	3.7–22.5	
Sex	Male	13	57
	Female	10	43
Diagnosis	Glioblastoma	12	52
	Anaplastic Astrocytoma	8	35
	Other High-Grade Gliomas [¶]	3	13
Location	Cerebral Hemispheres	15	65
	Thalamus	5	22
	Cerebellum	2	9
	Other [§]	1	4
Performance Status	90–100	11	48
	70–80	5	22
	50–60	7	30
Degree of Resection	Gross/Near Total	11	48
	Subtotal	6	26
	Partial/Biopsy	6	26

[¶], anaplastic oligoastrocytoma (*n* =2) and anaplastic ganglioglioma (*n* =1)

[§], This patient had an anaplastic astrocytoma originating from the lateral brainstem and extending towards the cerebellopontine angle

Table 2
Summary of Toxicities During the Dose-Limiting Toxicity-Evaluation Period

Range of Actual Dosage (mg/m ²)	70mg/m ² (n=7) §				90mg/m ² (n=3)				120mg/m ² (n=7) ‡				160mg/m ² (n=6)			
	68-83				85-87.5				107-128				151.5-167			
Toxicity (Grade)	1	2	3	4	1	2	1	2	1	2	3	4	1	2	3	4
Lymphopenia	1	3	1 ¶	2 ¶	1	2	2	2	2	2	1 ¶	1 ¶	-	-	3 ¶	1 ¶
Rash	1	5	-	-	1	2	5	2	5	2	-	-	1	2	1 †	-
Diarrhea	2	-	-	-	3	-	5	-	5	-	1 †	-	4	-	-	-
Nausea	5	-	-	-	2	-	2	1	2	1	-	-	3	-	-	-
Vomiting	3	1	-	-	1	-	-	2	-	2	-	-	2	-	-	-
Hypokalemia	1	-	2 ¶	-	-	-	1	-	1	-	1 ¶	-	2	-	-	-
Hypophosphatemia	2	1	1 ¶	-	-	1	-	-	-	-	-	-	1	1	-	-
Indirect hyperbilirubinemia	-	-	-	-	1	-	2	-	2	-	-	-	-	2	-	-
Mucositis	-	-	-	-	1	2	1	1	1	1	-	-	-	1	-	-
Fatigue	2	1	-	-	-	-	1	-	1	-	-	-	2	-	-	-
Anorexia	2	-	-	-	-	1	1	1	1	1	-	-	1	2	-	-
Elevation of SGPT	2	-	-	-	2	-	3	-	3	-	-	-	1	-	-	-
Infection without neutropenia	-	3	1 ¶	-	1	-	1	-	1	4	-	-	1	3	-	-
Increase of serum lipase	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1 †	-

§ Four patients were treated before and three patients after the study was amended to exclude grade 3 and 4 electrolyte abnormalities that resolved to at least grade 2 within 7 days

¶ One patient was not assessable for toxicity because of early tumor progression

† dose-limiting toxicity

‡ These toxicities were not considered dose-limiting toxicities according to the study criteria

Table 3

Summary of Pharmacokinetic Parameters of Erlotinib and OSI-420

Dosage Level (mg/m ²)	AFTER FIRST DOSE					DAY 8 OF THERAPY				
	Erlotinib					Erlotinib				
	C _{max} (mg/ml)	T _{max} (h)	AUC _{0→∞} (mg·h/ml)	C _{max} (mg/ml)	AUC _{0→∞} (mg·h/ml)	C _{max} (mg/ml)	T _{max} (h)	AUC _{0→∞} (mg·h/ml)	C _{max} (mg/ml)	AUC _{0→∞} (mg·h/ml)
70 (n =7)	1.3 (0.94–2.2)	4 (2.1–8.2)	34.9 (23.1–52.6)	0.13 (0.05–0.2)	2.1 (1.2–3.3)	1.8 (1.7–2.8)	2.1 (1–4.1)	28.8 (21.8–30.9)	0.25 (0.2–0.6)	3.3 (2–6.9)
90 (n =2)	1.6 (1.3–1.8)	3.1 (2–4.1)	23.9 (14.4–33.3)	0.16 (0.12–0.2)	1.9 (1.4–2.3)	1.3	2.6 (1.2–4)	21.2 (18.1–24.2)	0.17	2.1 (1.7–2.4)
120 (n =5)	1.2 (0.5–2)	2.2 (1–4)	27.8 (18.9–28.8)	0.14 (0.1–0.3)	2.5 (1.7–2.7)	1.4 (1–3)	1.75 (1.3–4)	23.1 (11.8–51.2)	0.3 (0.1–0.5)	2.8 (1.9–5.9)
160 (n =3)	1.8 (1.3–3)	2.2 (2–2.5)	37.1 (33.1–50.6)	0.32 (0.26–0.33)	4.2 (4.1–5.2)	2 (1.3–2.4)	2.2 (1–4)	35.5 (23.7–51)		4.3 (3.8–6.1)

Note: Values are provided as median with range in parenthesis

Abbreviations: C_{max}, maximal concentration; T_{max}, time for maximal concentration; AUC_{0→∞}, area under concentration-time curve from zero to infinity

Table 4

Details About Treatment and Outcome for all Patients

Patient Number	Dosage Level(mg/m2)	Age at Diagnosis/Gender	Histologic Diagnosis	Tumor Location	Surgical Resection	PFS (years)
1	70	7.5/M	Anaplastic Astrocytoma	Cerebellum	Biopsy	0.7
2	70	10/M	Glioblastoma	Multifocal	PR	2.2
3	70	8.2/F	Glioblastoma	Thalamus	STR	0.5
4	70	6.3/F	Glioblastoma	Thalamus	NTR	0.7
5	70	9.9/F	Glioblastoma	Gliomatosis Cerebri	PR	0.9
6	70	10.4/F	Anaplastic Astrocytoma	Gliomatosis Cerebri	Biopsy	1.9
7	70	14/M	Glioblastoma	Temporal Lobe	NTR	1.3
8	90	22.5/M	Anaplastic Oligoastrocytoma	Temporal Lobe	GTR	1.9
9	90	11.8/M	Anaplastic Ganglioglioma	Frontal Lobe	GTR	2.2 +
10	90	18.1/F	Anaplastic Astrocytoma	Temporal Lobe	GTR	2.2 +
11	120	13.1/M	Glioblastoma	Temporal Lobe	GTR	2.1 +
12	120	14.4/F	Glioblastoma	Frontal Lobe	NTR	0.6
13	120	3.7/F	Glioblastoma	Frontal Lobe	NTR	0.2
14	120	7.2/M	Glioblastoma	Bi-thalamic	Biopsy	0.4
15	120	13.7/M	Glioblastoma	Frontal Lobe	STR	0.4
16	120	6.2/M	Anaplastic Oligoastrocytoma	Parietal Lobe	GTR	1.5 +
17	120	19/M	Anaplastic Astrocytoma	Frontal Lobe	STR	1.5 +
18	160	13.2/F	Anaplastic Astrocytoma	Brainstem	NTR	1.3 +
19	160	14.8/M	Glioblastoma	Frontal Lobe	NTR	0.6
20	160	10.7/F	Glioblastoma	Thalamus	STR	0.2
21	160	8/F	Anaplastic Astrocytoma [‡]	Thalamus	Biopsy	1.2
22	160	14.3/M	Anaplastic Astrocytoma [‡]	Frontal Lobe	STR	1 +
23	160	7.4/M	Anaplastic Astrocytoma [‡]	Cerebellum	STR	0.9

Abbreviations: PFS, progression-free survival; PR, partial resection; STR, subtotal resection; NTR, near-total resection; GTR, gross total resection

[‡]Histologic diagnosis was changed to glioblastoma at second operation