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A Phase I and Biology Study of Gefitinib and Radiation in Children with Newly Diagnosed Brain Stem Gliomas or Supratentorial Malignant Gliomas

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Abstract

Purpose—To estimate the maximum tolerated dose (MTD); study the pharmacology of escalating doses of gefitinib combined with radiation therapy in patients ≤21 years with newly diagnosed intrinsic brainstem gliomas (BSG) and incompletely resected supratentorial malignant gliomas (STMG); and to investigate epidermal growth factor receptor (EGFR) amplification and expression in STMG.

Patients and methods—Three strata were identified: Stratum 1A - BSG; Stratum IB - incompletely resected STMG not receiving enzyme inducing anti-convulsant drugs (EIACD); and Stratum II - incompletely resected STMG receiving EIACD. Dose escalation using a modified 3 + 3 cohort design was performed in strata IA & II. The initial gefitinib dosage was 100mg/m^2/day

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Conflict of interest statement

Dr. Stewart received research funding from Astrazeneca for the conduct of the pharmacokinetic studies. Dr. Gururangan disclosed a consultant or advisory role with Boehringer Ingelheim Pharma. No other conflicts of interest have been declared.

commencing with radiation therapy and the dose-finding period extended until 2 weeks post-radiation. Pharmacokinetics (PK) and biology studies were performed in consenting patients.

Results—Of 23 eligible patients, 20 were evaluable for dose-finding. MTDs for strata IA and II were not established as accrual was halted due to four patients experiencing symptomatic intratumoral hemorrhage (ITH); 2 during and 2 post dose-finding. ITH was observed in 0 of 11 patients treated at 100mg/m^2/day, 1 of 10 at 250mg/m^2/day, and 3 of 12 at 375mg/m^2/day. Subsequently a second patient at 250mg/m^2/day experienced ITH. PK analysis showed the median gefitinib systemic exposure increased with dosage (p=0.04). EGFR was overexpressed in 5 of 11 STMG and amplified in 4 (36%) samples.

Conclusion—This trial provides clear evidence of *EGFR* amplification in a significant proportion of paediatric STMG and 250mg/m^2/day was selected for the Phase II trial.

Keywords

epidermal growth factor receptor; gefitinib; radiotherapy; brain stem neoplasms; supratentorial neoplasms; glioma

Introduction

Diffusely infiltrating brainstem gliomas (BSG) have been amongst the most therapeutically resistant paediatric brain tumors; 1- and 5-year progression-free survival (PFS) rates for these patients are less than 25% and 10%, respectively.[1, 2] A paucity of data exist to document the histology and biology of these tumors; in the limited instances where tissue has been obtained, histology has shown high-grade or infiltrating glioma.[3–7] More recent experience confirms the similarity of BSG to more accessible supratentorial malignant gliomas, a tumor setting where prognosis for children following incomplete resection is also quite poor with 1- and 5-year PFS rates of less than 50% and 20%, respectively.[8–10]

In view of these discouraging results, new therapeutic approaches are needed. Recent data demonstrate that a wide variety of tumors, including malignant gliomas, are driven to proliferate by aberrant activation of growth factor receptor-mediated signal transduction pathways.[11,12] Constitutive activation of these pathways also contributes to tumor resistance to conventional therapeutic agents.

Cell signaling via the EGFR (also known as ERBB1) has been implicated in the development of adult and paediatric high-grade gliomas.[13,14] EGFR amplification and over-expression affect 30%–50% of adult glioblastoma multiformes.[13–16] In paediatric high-grade gliomas, available data suggest that while EGFR amplification occurs with low frequency,[17,18] EGFR receptor over-expression is relatively common. Bredel *et al* reported elevated expression of this receptor in 81% of paediatric STMG, with over half demonstrating over-expression in >90% of tumor cells.[18] PBTC earlier showed that, EGFR protein is expressed to high levels and amplified in samples of childhood BSG.[19] These data suggest that the EGFR constitutes a promising therapeutic target for paediatric STMG and BSG.

Gefitinib (ZD1839, Iressa™, AstraZeneca), a low molecular weight synthetic molecule, is a potent and selective inhibitor of the EGFR tyrosine kinase that works by competing with adenosine triphosphate for its binding site, and blocking signal transduction pathways implicated in cancer cell proliferation, survival and other host-dependent processes thought to promote cancer growth.[20] At the time this clinical trial (PBTC-007) was initiated, gefitinib had demonstrated preclinical evidence of antitumor activity alone and in

combination with irradiation and had shown good antitumor activity in a wide range of human tumor xenografts after oral administration.

In both BSG and incompletely resected STMG, radiation therapy has demonstrated benefit.[21] Preclinical studies have demonstrated radiosensitization with concurrent exposure to EGFR specific inhibitory agents, providing further rationale for trials of upfront combinations of EGFR inhibitors and concurrent irradiation.[22]

In adult phase I studies, gefitinib was well tolerated after either intermittent or continuous dosing.[23–26] In these trials, dose-related toxicity was confined to the skin and gastrointestinal system; rarely, hepatic enzyme elevation occurred. Increasing intolerability was noted at daily doses of ≥600 mg. The combination of preclinical antitumor activity, known over-expression of the target pathway, and acceptable toxicity profile led us to study the agent in paediatric malignant gliomas.

The PBTC conducted a phase I trial of gefitinib in combination with radiation therapy in children with newly diagnosed BSG and incompletely resected STMG. The primary objectives were to define the safety of gefitinib administered orally once daily in combination with radiation therapy and to describe dose-limiting toxicities. Secondary objectives included characterizing the pharmacokinetic and pharmacogenetics of gefitinib in this patient population and to investigate *EGFR* expression and amplification in STMG.

Patients and Methods

Patient Eligibility

Patients ≥3 and ≤21 years of age with a newly diagnosed non-disseminated BSG or incompletely resected STMG were eligible. Other eligibility criteria included Karnofsky or Lansky performance score ≥50%, no prior chemotherapy (except corticosteroids) or radiotherapy, adequate bone marrow, renal, and hepatic function. Patients could not be pregnant, have an uncontrolled infection, or a history of deep venous or arterial thrombosis.

The institutional review boards (IRBs) of each participating PBTC institution approved the protocol before initial patient enrollment, and continuing approval was maintained throughout the study. Patients or their legal guardians gave written informed consent, and assent was obtained as appropriate at the time of enrollment.

Studies Before and During Treatment

A complete history, physical exam including detailed neurological exam and laboratory studies were obtained before treatment and periodically thereafter. Pretreatment evaluation included: CBC, electrolytes including magnesium, renal function tests (serum creatinine and BUN), liver function tests, fibrinogen, anticonvulsant levels in patients receiving enzyme-inducing anticonvulsant drugs (EIACD), and β-HCG in females of childbearing potential. MRI was obtained prior to therapy and at 8 week intervals during therapy.

Dosage, Drug Administration, and Treatment Plan

Gefitinib was provided in tablets that could be dissolved in water, as necessary. Patients received gefitinib once daily; a course was defined as 4 weeks of therapy. In the absence of disease progression or dose-limiting toxicity, treatment was continued for 13 courses (1 year).

Patients received local irradiation using conventional or conformal techniques; imaging and treatment plan were centrally reviewed (Quality Assurance Review Center, QARC). A total

dose of 55.8 Gy was given in 180 cGy daily fractions. Treatment was initiated within 4 weeks of diagnosis (BSG) or surgery (STMG).

Dose Escalation, Dose-Limiting Toxicity, and Maximum Tolerated Dose

The initial gefitinib dosage was 100mg/m^2/day commencing with radiation therapy. Dose escalation for patients in Strata IA and II were performed as shown in Table 1; patients registered in Stratum IB were assigned to the current dose level in Stratum IA.

The dose-finding interval extended from initiation through 2 weeks following completion of radiation therapy. A traditional 3+3 dose-finding algorithm was used to empirically estimate MTDs for strata IA and II. Cohorts of up to six patients could be enrolled at a dosage; the decision to escalate the dosage for the next cohort was made after three patients had completed the dose-finding interval. The MTD was defined as the highest dosage at which at least five of six patients did not experience DLT and the next higher dosage was unacceptably toxic.

Toxicities were graded according to the NCI Common Toxicity Criteria Version 2.0. DLTs were defined as follows: Grade 3 or 4 thrombocytopenia, grade 4 neutropenia, any grade 3 or 4 non-hematologic toxicity, any grade 2 non-hematologic toxicity persisting for more than 7 days and considered sufficiently significant or intolerable by patients to warrant treatment interruption and/or dose reduction, and any toxicity requiring interruption of radiation therapy for >5 consecutive days or 10 days total.

Patients who came off therapy during the DLT observation period for reasons other than toxicity or missed >7 days of gefitinib for reasons other than toxicity were replaced for purposes of estimating the MTD.

Pharmacokinetic Studies

Pharmacokinetic studies were performed in consenting patients. Serial blood samples for pharmacokinetic studies were collected on days 10, 11, or 12 of course 1 before gefitinib administration, and at 1, 2, 4, 6, 8, 12, and 24 hours after administration. Gefitinib concentrations were analyzed by isocratic reversed-phase high performance liquid chromatography with electrospray ionization mass spectrometric detection.[27] A one-compartment model was fitted to the gefitinib plasma concentrations using maximum likelihood estimation as implemented in ADAPT II.[28] The model parameters for each patient were used to simulate the plasma concentration-time profile, from which the area under the plasma concentration-time curve ($AUC_{\mathbf{0\text{-}24}}$) was calculated using the log-linear trapezoidal method.

EGFR amplification and expression

Diagnostic fixed tumour samples were obtained from consenting patients and analyzed by immunohistochemistry (IHC) to determine the expression of total EGFR, phospho-ERK1/2, and phospho-$S6^{S235/236}$. To remove observer bias, IHC staining of tumours was scored blind to treatment response using ImageJ software analysis that provides a measure of the mean percentage of immunopositivity detected in each ×200 field.[33] Dual-probe fluorescence in situ hybridization (FISH) was performed on paraffin-embedded sections with locus-specific probes for EGFR (RP11-148P17) and the 7q control probe (7q31.2, RP11-460J21 + CTB-133K23).

Results

Three of 23 patients enrolled in stratum IA and II were not evaluable for dose-finding: all withdrew without toxicity 14, 15, and 46 days after the start of gefitinib. Ten patients were enrolled in stratum IB. Patient characteristics are summarized in Table 2.

Estimation of MTD

Twenty patients were evaluable for dose-finding, including 17 in stratum IA, and 3 in stratum II (Table 1). Of the first six patients treated at 100mg/m^2/day in Stratum IA, one experienced grade 2 fibrinogen (hypofibrinogenemia) and another grade 3 lymphopenia; both DLTs, prompting a protocol amendment to exclude non-clinically significant hematologic toxicities as DLTs. Subsequently only one of five evaluable patients in stratum IA treated at the highest dosage investigated (375mg/m^2/day) experienced a DLT (grade 3 rash/desquamation). None of the three patients in stratum II treated at the highest dosage (100mg/m^2/day) evaluated experienced a DLT. While dose escalation was not based on Stratum IB, three of five patients at 375mg/m^2/day experienced DLTs (one grade 5 ITH, one grade 3 ITH & one grade 3 hypofibrinogenemia, dehydration and vomiting) during courses 1 and 2 (Table 1).

Although systemic toxicities were generally mild-moderate and reversible (Tables 2 and 3), there were four instances of symptomatic ITH noted among the 33 eligible patients prompting closure of the trial without having estimated MTDs in strata IA or II. Three patients experienced ITH among 12 treated at the 375mg/m^2/day dosage, one among 10 at the 250mg/m^2/day dosage and none among 11 at 100mg/m^2/day (Table 3). Subsequent to closure another patient treated at 250mg/m^2/day was retrospectively noted to have experienced an ITH post dose-finding. The difference in cumulative incidence functions of ITH among the three dosages investigated was not statistically significant (p=0.20). No relationship to concomitant dexamethasone administration or evidence of thrombocytopenia or coagulation abnormality was noted. Three instances of ITH occurred in patients with STMG and two in patients with BSG. In one patient with a BSG, ITH was associated with progressive tumour, and the death of a patient with a GBM was attributed to ITH.

For the twenty patients with BSG (Stratum IA), the 1-year survival and PFS rates were 48% (SE 11%) and 16.1% (SE 7.4%), respectively. For the 13 patients with STMG, the 1-year survival and PFS rates were 28.8% (SE 12.2%) and 15.4% (SE 8.2%), respectively.

Pharmacokinetics

Serial samples for gefitinib pharmacokinetic studies were collected from 22 patients during week 2 of course one – none of whom were receiving EIACD. As summarized in Table 4, the median gefitinib AUC value increased with increasing dosage (p=0.04). At the recommended phase II dosage (250mg/m^2/day), the median peak gefitinib plasma concentration was 0.83 μg/ml (range 0.45 to 1.36 μg/ml); observed at a median of 4.2 hr (range 2 to 6 hr) after drug administration. The median gefitinib half-life for all patients studied was 11.1 hr (range 1.8 to 41.3 hr). The median apparent oral clearance for all patients studied was 15.7 L/hr/m^2 (range 1.8 to 34.0 L/hr/m^2). The effect of dexamethasone administration on gefitinib apparent oral clearance was studied in seven patients who had pharmacokinetic studies conducted while on a stable dexamethasone dosage and later after discontinuing dexamethasone: no statistically significant difference was noted (paired t-test; p=0.15). In our limited analyses no evidence of a relationship between gefitinib pharmacokinetics and toxicity was observed.

Tumour EGFR amplification and expression

Intense membrane EGFR immunoreactivity was evident in tumour material from 5 of 11 patients with STMG (IB n=8, II n=3). Three of the eleven cases displayed high-level EGFR amplification (Figure 1); one additional case displayed low-level gain in the context of polyploidy. Each of these four cases displayed intense membrane-associated EGFR immunoreactivity in contrast to only one tumour sample that was diploid for the EGFR locus (p=0.01, Fisher's Exact test).

Discussion

This study provides clear evidence that *EGFR* is amplified and highly-expressed in a significant proportion of paediatric STMG. The PBTC reported previously that one-half of high-grade intrinsic BSG express elevated levels of EGFR in the context of gene amplification.[19] EGFR overexpression was also reported in paediatric STMG although only a handful of tumours displayed an increase in gene copy number.[15–17] Here, we identified intense membrane EGFR immunoreactivity in 5 of 11 (45%) STMG; of which three (27%) and one (9%) contained high-level and low-level gene amplification, respectively. A very recent publication by Zarghoon et al[38] based on whole-genome profiling techniques suggest that platelet-derived growth factor receptor α and Poly polymerase as potential therapeutic targets in BSG. They also noted EGFR immunopositivity in 7 of their 11 (64%) cases and low copy number gain in one. Together these data indicate that EGFR is likely to play an important role in the biology of paediatric as well as adult STMG and justifies a phase II evaluation of gefitinib in newly diagnosed paediatric patients with these tumours.

The pharmacokinetics of gefitinib are similar to those previously reported for children with refractory solid tumours receiving gefitinib as a single agent.[34] The median apparent oral gefitinib clearance in that study was 14.8 L/hr/m^2 compared with 15.7 L/hr/m^2 for this trial. A wide variation was noted in our gefitinib apparent oral clearance, similar to the wide interpatient variability noted in the previous paediatric study (4.8 to 24.8 L/hr/m^2).[34] Although the maximum gefitinib plasma concentration and area under the concentration-time curve generally increased with gefitinib dosage, marked inter-patient variability within each dosage level was noted. Recent reports have suggested that gefitinib undergoes preferential distribution from the blood into brain tumour tissue.[35,36] Thus, at the dosages tolerated and gefitinib exposures achieved in the present study it is likely that tumour concentrations approached or exceeded the concentration (2 μmol/l) necessary for inhibition of wild-type EGFR.[35]

Due to concern that the observed intratumoural hemorrhages could be related to the study drug, accrual to the Phase I component of the study was halted before MTDs could be estimated. Because of the apparently higher incidence of ITH at 375mg/m^2, treating patients at this or higher gefitinib dosages in the efficacy phase of the trial would be unacceptable, leading us to recommend studying the 250mg/m^2 dosage level in the phase II trial for patients newly diagnosed with a BSG. This is in contrast to the MTD of 400 mg/m^2/day established for paediatric solid tumours.[34]

A subsequent retrospective review of newly diagnosed BSG treated at a single institution with radiation therapy with or without concurrent cytotoxic agents showed symptomatic ITH in 9 of 48 patients within 12 months of diagnosis.[37] Results which are similar to symptomatic ITH occurring in 2 of 20 patients with BSG and 3 of 13 patients with STMG in our trial.

Our current knowledge of the tumour- or radiation-related ITH, as discussed above, suggests the apparent dose-related incidence associated with gefitinib may have been spurious.

However given data available at the time, it was reasonable to stop the trial early to ensure patient safety. The pharmacokinetics of gefitinib indicate that the 250mg/m^2/day dosing selected for the phase II trial will achieve tumour concentrations necessary for inhibition of wild-type EGFR, which our data suggest will be amplified or highly-expressed in a significant proportion of high-grade gliomas.

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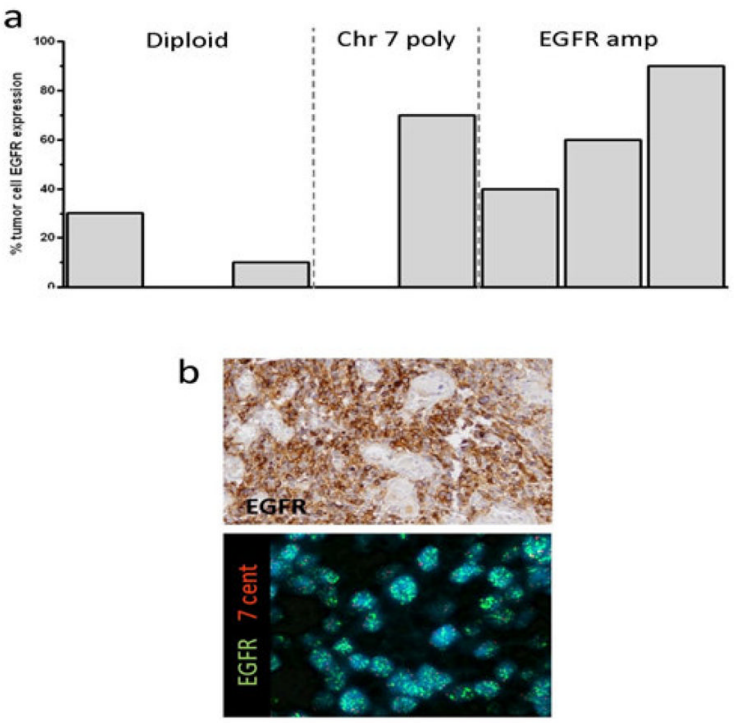

FIGURE 1.

a. Graph summarizing percent of tumour cells expressing epidermal growth factor receptor (EGFR) according to *EGFR* copy number status. **b.** Top panel: EGFR immunohistochemistry showing intense membrane staining in the sample in which 90% of cells express EGFR. Bottom panel: FISH analysis confirming high-level *EGFR* amplification in this same tumour.

Table 1

Dose-Limiting Toxicities and Intratumoral Hemorrhage Summaries

Stratum	Dose	No. of Patients Entered	No. of Evaluable Patients	No. of Patients with DLT	Observed DLTs Courses 1 and 2	Observed CNS Hemorrhage Courses 3+
IA[*] (BSG)	100	6	6	2[^]	Gr-2 Fibrinogen Gr-3 Lymphopenia	
	250	7	6	0		Gr-4
	375	7	5	1	Gr-3 Rash/desquamation	Gr-4
IB[§] (STMG)	100	2	2	0		
	250	3	3	0		Gr-4
	375	5	5	3	Gr-3 CNS hemorrhage Gr-5 CNS hemorrhage Gr-3 Dehydration Gr-3 Fibrinogen Gr-3 Vomiting	
II (STMG + EIACD)	100	3	3	0		

[*] No patients in this cohort received enzyme-inducing anticonvulsants.

[^] Upon observing these two DLTs, the protocol was amended and the DLT definition was changed.

[§] Dose-escalation was not based on DLTs observed in stratum IB

Abbreviations: DLT=Dose-limiting Toxicity, BSG=Brain stem gliomas, STMG=Supertentorial Malignant Gliomas, EIACD=Enzyme Inducing Anti-convulsant Drugs, Gr=grade

TABLE 2

Patient demographics

		Numbers of Patients		
		Stratum IA (BSG)	**Stratum IB (STMG)**	**Stratum II (STMG + EIACDs)**
Gender	Male	6	8	1
	Female	14	2	2
Ethnicity	Unknown	.	1	.
	Hispanic or Latino	5	1	.
	Non-Hispanic	15	8	3
Diagnosis	AA	2*	.	1
	BSG	18	.	.
	GBM	.	9	1
	MG	.	.	1
	MOA	.	1	.
Age at Study Entry: Median (min, max)		7.4 (3.4, 15.1)	13.5 (3.1, 20.9)	12.8 (10.4, 15.2)

* Two BSG patients were biopsied

Abbreviations: AA=Anaplastic Astrocytoma, BSG=Brain Stem Gliomas, GBM=Glioblastoma Multiforme, MG=Malignant Gliomas, MOA=Mixed Oligoastrocytoma, STMG=Supertentorial Malignant Gliomas, EIACD=Enzyme Inducing Anti-Convulsant Drugs

Table 3

Non dose-limiting toxicities (≥ Grade 2) attributed to therapy and observed in 4 or more (> 10%) of the 30 evaluable patients

	Stratum IA (BSG)					Stratum IB (STMG)					Stratum II (STMG + EIACDs)	
	Crs-1 and 2		Crs-3+			Crs-1 and 2		Crs-3+			Crs-1 and 2	Crs-3+
Grade	**2**	**3**	**2**	**3**	**4**	**2**	**3**	**2**	**3**	**4**	**2**	**2**
Lymphopenia	2	6	3			5	2					
Diarrhea patients without colostomy	3		3	1				1				
Rash/desquamation	3		2			2					1	
Leukocytes (total WBC)	6							1				
Vomiting	3					1		1	2			
Fatigue (lethargy, malaise, asthenia)	2		3	1				1				
SGPT (ALT) (serum glutamic pyruvic transminase)			3					1			1	1
Neutrophils/granulocytes (ANC/AGC)	3		1			1						
Headache	2					1						1
Fibrinogen	1		2	1								
Nausea	1					2						
CNS hemorrhage/bleeding					2					1		

Abbreviations: BSG=Brain stem gliomas, STMG=Supertentorial Malignant Gliomas, EIACD=Enzyme Inducing Anti-convulsant Drugs, Crs=course

Table 4

Summary* of gefitinib pharmacokinetic parameters in relation to dosage

	100 mg/m^2/d	250 mg/m^2/d	375 mg/m^2/d
No. of patients	8	6	8
Actual dosage (mg/m^2/d)	98.1 (86.2–104.2)	252/6 (244.8–261.6)	379.9 (367.6–410.9)
C_{max}† (μg/mL)	0.44 (0.28–0.71)	0.83 (0.45–1.36)	1.89 (0.93–2.42)
T_{max}† (h)	4.9 (1.2–6.2)	4.2 (2.0–6.0)	4.2 (2.3–6.4)
$t_{1/2}$ (h)	9.9 (1.8–19.9)	17.6 (4.8–41.3)	10.4 (3.8–39.2)
Cl/F (L/h/m^2)	12.8 (1.8–21.8)	20.9 (12.6–33.9)	15.0 (5.8–25.8)
AUC_{0-24} (μg/mL*h)	5.3 (3.7–11.8)	11.8 (4.3–16.7)	25.3 (18.4–35.3)

*Values are median and range.

†Observed C_{max} and T_{max}.

Abbreviations: $C_{max,}$=maximum concentration; $T_{max,}$=time of maximum gefitinib concentration; Cl/F=apparent oral clearance; AUC_{0-24}=area under the concentration-time curve from 0 to 24 hours.