

Phase I Trial, Pharmacokinetics, and Pharmacodynamics of Vandetanib and Dasatinib in Children with Newly Diagnosed Diffuse Intrinsic Pontine Glioma

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Abstract

Purpose: Testing of promising drug combinations is crucial in the treatment of diffuse intrinsic pontine glioma (DIPG). As the VEGF and platelet-derived growth factor (PDGF) pathways are critical in gliomas, we evaluated the safety, maximum tolerated dose (MTD), pharmacokinetics, and pharmacodynamics of vandetanib, a VEGFR-2 inhibitor, combined with dasatinib, a potent PDGFR inhibitor, during and after radiotherapy in children with newly diagnosed DIPG.

Experimental Design: Dasatinib was started concurrently with radiotherapy. Vandetanib was started 8 days later. We tested increasing doses of vandetanib (65 and 85 mg/m² once daily) and dasatinib (65 and 85 mg/m² twice daily). Dose-limiting toxicities were evaluated during the first 6 weeks of therapy. Plasma pharmacokinetics was obtained on days 8 and 42 ± 3 in all patients and concomitantly with cerebrospinal fluid (CSF) when possible. Inhibition of targets of dasatinib in peripheral blood mononuclear cells (PBMC) was evaluated.

Results: Twenty-five patients were treated. Treatment was well tolerated. The median duration of treatment was 184 days. Diarrhea was the most significant toxicity. Three patients experienced substantial myelosuppression. The steady-state plasma pharmacokinetics of vandetanib was comparable with previous studies. Although the plasma exposure to dasatinib decreased from days 8 to 42, it remained similar to adult studies. CSF to plasma exposure of vandetanib and dasatinib were approximately 2% in 2 patients. Phosphorylated 70S6K decreased during therapy in PBMCs.

Conclusions: The MTD of vandetanib and dasatinib in combination was 65 mg/m² for each drug. Other studies are underway to test dasatinib and other PDGFR inhibitors alone or in combination for this deadly cancer. *Clin Cancer Res*; 19(11); 3050–8. ©2013 AACR.

Introduction

Diffuse intrinsic pontine glioma (DIPG) is the deadliest central nervous system (CNS) cancer in childhood (1). The survival of children with DIPG has remained less than 10% despite multiple clinical trials that used radiotherapy, the mainstay of treatment, with or without chemotherapy (1). Children with newly diagnosed DIPG are ideal candidates

for experimental therapies because no chemotherapy regimen has shown benefit to date (1).

Histologically, glioblastoma accounts for most DIPGs (2). On the basis of the relevance of the VEGF pathway in glioblastoma (3), we conducted a phase I trial of vandetanib (Caprelsa, AstraZeneca), a small-molecule inhibitor of VEGF receptor 2, EGF receptor, and rearranged during transfection, administered during and after radiotherapy in children with newly diagnosed DIPG (4). Treatment with vandetanib was well tolerated by most patients. Although the combination of vandetanib and radiotherapy did not change the poor outcome for children with DIPG, some patients experienced longer survival (4).

The platelet-derived growth factor (PDGF) pathway and in particular its receptor A (PDGFRA) play key roles in gliomagenesis and in the formation of DIPGs (5, 6). *PDGFRA* is the most commonly amplified receptor tyrosine kinase (RTK) gene in DIPG (approximately 30% of cases; refs. 7–11). Mutations in *PDGFRA* occurred in 9% of DIPGs (11).

A phase I trial showed no benefit from combining imatinib mesylate (Gleevec, Novartis Pharmaceuticals), a

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Translational Relevance

Children with diffuse intrinsic pontine glioma (DIPG), the deadliest pediatric central nervous system cancer, are ideal candidates for novel therapies because local radiotherapy, the mainstay of treatment, provides only temporary improvement. There is strong evidence to support the role of the VEGF and PDGF pathways in the formation of DIPG. We reported the first clinical trial to use a rational combination of small-molecule inhibitors targeting those pathways in DIPG. By carrying out detailed studies, we have shown a potential negative pharmacokinetic interaction between vandetanib and dasatinib. Despite that, the exposure of our patients to both drugs was still comparable with that in studies using vandetanib and dasatinib as single agents. We also provided, for the first time, data about the cerebrospinal fluid exposure of vandetanib and dasatinib in 2 patients. Although this treatment did not change the poor outcome of children with DIPG, we believe that dasatinib and other PDGF receptor inhibitors warrant further testing in this disease.

modest PDGFRA and B inhibitor, with radiotherapy in children with newly diagnosed DIPG (12, 13). In another study, 4 patients with progressive brainstem gliomas whose tumors expressed PDGFRA were treated with imatinib mesylate; one of them experienced disease stabilization for 10 months (14). Because imatinib has limited penetration through the intact blood-brain barrier (15, 16), its usefulness in the treatment of patients with CNS tumors is questionable.

Dasatinib (Sprycel, BMS-354825, Bristol-Myers Squibb) is an oral inhibitor of multiple targets, including c-Kit, Src, and PDGFRA and B (17, 18). Dasatinib is a more potent PDGFR inhibitor than imatinib (17, 19). Several reports suggested that dasatinib may have better activity against CNS leukemic involvement than imatinib (20, 21). A phase I clinical trial yielded a recommended phase II dose of dasatinib in children with solid tumors that was higher than the standard adult doses (22, 23).

The development of promising drug combinations is critical for children with DIPG. There is evidence from preclinical studies that the combination of agents targeting the VEGF and PDGF pathways may be beneficial in high-grade gliomas (24–26). Therefore, we conducted this study to determine the safety, maximum-tolerated dose (MTD), pharmacokinetics, and pharmacodynamics of the combination of vandetanib and dasatinib administered during and after radiotherapy in children with newly diagnosed DIPG.

Patients and Methods

Patients between 18 months and 20 years old with newly diagnosed nonmetastatic DIPG or other brainstem high-grade gliomas were eligible for this study. Other eligibility criteria consisted of: (i) performance score 40 or more; (ii)

adequate hematologic [absolute neutrophil count $\geq 1,000/\mu\text{L}$, platelet count $\geq 100,000/\mu\text{L}$ (transfusion independent), and hemoglobin concentration ≥ 8 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 ≥ 2 g/dL) function; (iii) use of safe contraceptive methods for females of childbearing age and males of child fathering potential; and (iv) QTc interval in electrocardiogram < 450 msec. Exclusion criteria consisted of: (i) patients receiving other anticancer or experimental therapies; (ii) patients with other medical conditions that could not be adequately controlled or that would impair the evaluation of toxicities related to this therapy or alter drug metabolism or tolerance to treatment; (iii) use of enzyme-inducing anticonvulsants or other medications that could affect the function of CYP3A4, except for dexamethasone and fluconazole; (iv) patients with cardiac problems, including a history of arrhythmias and QTc interval prolongation; (v) use of other medications associated with significant risk of prolonging QTc interval; (vi) significant hypertension defined as blood pressure more than 95th percentile for age, height, and sex; and (vii) body surface area more than 1.8 m² for dosage levels in which the dose of dasatinib was 85 mg/m².

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' parents or legal guardians, and assents were obtained when appropriate.

Study design and treatment plan

This single-institution clinical trial followed a variation of the traditional phase I design. Unlike the traditional design, we required that 6 assessable patients be treated at each dosage level unless that dosage level was deemed too toxic. The starting dosage of vandetanib (65 mg/m²) corresponded to the lower dose used in combination trials in adults (100 mg per day; ref. 27). The starting dosage of dasatinib (65 mg/m²) was one level below the single-agent MTD in children (22). Figure 1 shows the planned dosage levels. Four dosage levels were initially planned, with escalating doses of vandetanib (65, 85, and 110 mg/m² once daily) and dasatinib (65 and 85 mg/m² per dose twice daily). The dose of dasatinib was not escalated beyond the single-agent MTD in children (85 mg/m²; ref. 22). Dosage level 2b was added during the study once dosage level 2a was found to be too toxic. Two lower dosage levels were also provided in case the starting dosage level was too toxic. MTD was defined as the highest dosage level in which no more than 1 of 6 assessable patients experienced dose-limiting toxicities (DLT). The DLT evaluation period comprised the first 6 weeks of therapy. DLTs consisted of the following toxicities attributable to study drugs: (i) grade 4 neutropenia; (ii) grade 3 or 4 thrombocytopenia; (iii) any grade 3 or 4

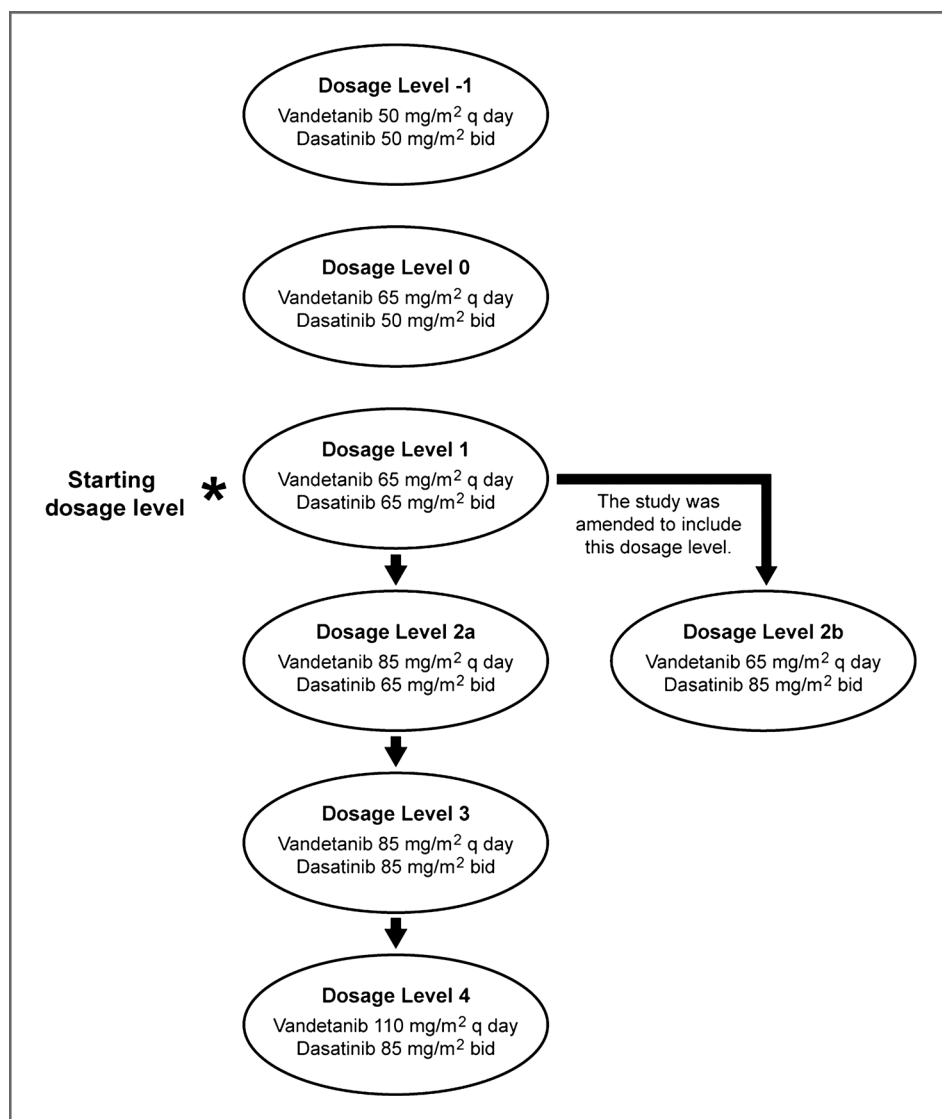


Figure 1. Scheme of dose escalation. q day, every day; bid, twice a day.

nonhematologic toxicity except for grade 3 weight change, grade 3 hypertension, grade 3 elevation in transaminases that returned to baseline or \leq grade 1 within 7 days, and grade 3 or 4 electrolyte abnormalities that returned to \leq grade 2 within 7 days; (iv) grade 1 QTc interval prolongation associated with ventricular tachycardia or torsade de pointes or grade 1 and 2 QTc interval prolongation associated with rhythm abnormalities in a 24-hour Holter; (v) any grade 2 nonhematologic toxicity lasting more than 7 days and causing significant clinical repercussion. Toxicities were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 3.0.

Radiotherapy and dasatinib were started on the same day. Vandetanib was started on day 9 of therapy. Three-dimensional conformal radiotherapy was delivered as 1.8-Gy fractions once daily, 5 days a week, for a cumulative dose of 54 Gy. One patient received 55.8 Gy because of a 2-day

interruption in radiotherapy. The treatment volume encompassed the entire tumor defined by the combination of T1, T2-weighted, and FLAIR sequences, 2 cm (transverse and caudal) and 3 cm (superior) margins to account for microscopic disease, and a 0.3 to 0.5 cm margin to account for uncertainty in immobilization and patients' positioning. The superior margin was increased from 2 to 3 cm based on our preliminary results of the pattern of progression of patients with DIPG (28). One patient who had extensive tumor extension in both thalami required whole-brain radiotherapy at a dose of 54 Gy.

Dasatinib was administered with or without food as 20-, 50-, or 70-mg tablets. The tablets of dasatinib could be cut in half or crushed to ease administration. When applicable, an interval of at least 4 hours was recommended between the administration of dasatinib and histamine receptor 2 antagonists. Vandetanib was administered with or without food as 50-mg tablets or as a solution (10 mg/mL). The

tablets of vandetanib could not be cut or crushed. The actual dose of both medications was calculated on the basis of the body surface area before the start of therapy and was rounded to the nearest 10 mg. Changes in dose based on variations of body surface area were allowed after 6 months of therapy. The choice of vandetanib formulation for each patient was determined by the ability to provide actual doses rounded to the nearest 10 mg. Treatment was divided into 28-day courses. The maximum planned treatment duration was 2 years from the start of radiotherapy. Once the MTD had been established, we treated 10 additional patients at that dosage level to obtain additional information about the toxicity of this treatment regimen, as well as pharmacokinetic, and pharmacodynamic data.

Patients' evaluation consisted of clinical assessment, complete blood counts, chemistry panel, and urinalysis weekly during the first 6 weeks of therapy, at week 8, and monthly thereafter. An electrocardiogram was carried out weekly during the first 6 weeks of therapy, at weeks 8 and 12, and every 8 weeks thereafter. An echocardiogram was obtained before the start of therapy, at the completion of radiotherapy, and every 6 months thereafter during treatment. A brain MRI was obtained before the start of therapy, at the completion of radiotherapy, and every 8 to 10 weeks thereafter for the duration of therapy. An MRI of the knee was obtained at baseline, after 4 courses of therapy, and if possible at the discontinuation of therapy to evaluate potential treatment toxicity, particularly VEGF receptor 2 inhibition, on the cartilaginous growth plates of growing bones. Serial assessment of quality of life of all patients was obtained during and after radiotherapy; these results will be provided in a separate manuscript.

Pharmacokinetic studies

Blood (3 mL) was collected for mandatory pharmacokinetic studies before and at 1, 2, 4, 8 (± 2), and 24 (± 6) hours after the morning dose of the study drugs on days 8 and 42 (± 3) of therapy. The evening dose of dasatinib was withheld on both days. The start of vandetanib administration was delayed until day 9 to allow for pharmacokinetic analysis of dasatinib at a steady state with and without concomitant vandetanib. Multiple optional, serial collections of concurrent cerebrospinal fluid (CSF) and plasma samples were carried out when possible. Samples were collected in heparinized tubes, mixed, and centrifuged, and the plasma and CSF were stored at -80°C until analysis.

Vandetanib was measured in plasma and CSF by high-performance liquid chromatography with tandem mass spectrometric detection (29). Details about the method used for the analysis of dasatinib as well as the pharmacokinetic modeling for both drugs are provided in the supplementary data.

Pharmacodynamic studies

Details about the serial analysis of plasma angiogenic factors and measurement of the effects of dasatinib on targets in peripheral blood mononuclear cells (PBMC) were provided in the supplementary data.

FISH

FISH analysis of *PDGFRA* (4q12) and *MET* (7q31) was conducted in all available tumor samples as previously described (9).

Statistical analysis

Progression-free survival (PFS) was the interval between the start of therapy and disease progression or death, whichever occurred first. Overall survival (OS) was defined as the interval from the start of therapy to death. The distribution of OS was estimated using the Kaplan–Meier method. The Wilcoxon-signed rank sum test and the sign test were used to analyze paired results of pharmacokinetic and pharmacodynamic studies. Spearman correlations were used to assess the association between selected pharmacokinetic and pharmacodynamic parameters. We used a significance threshold of 0.05 without adjustment for multiple testing in the latter analysis due to the exploratory nature of these studies.

Longitudinal changes in the levels of plasma angiogenic markers and their association with dosage level were explored via the mixed-effects models. Cox proportional hazards models were used to examine the association between the level of plasma angiogenic factors and PFS or OS. The serial levels of plasma angiogenic factors were treated as time-varying covariates in these models.

Results

Twenty-five patients were enrolled on the study between October 2009 and April 2011. Table 1 shows the patients' characteristics.

Toxicities

A summary of toxicities for all patients during and after the DLT evaluation period is provided in Tables 2 and 3, respectively.

One of 6 patients treated at dosage level 1 experienced a DLT (grade 3 increase in amylase). Two of 3 patients

Table 1. Patient characteristics

Characteristics	Patients (n = 25)	(%)
Median age (range)	5.8 years (2.3–17.2)	
Gender		
Male	12	48
Female	13	52
Diagnosis		
DIPG	24	96
Medullary GBM	1	4
Histological diagnosis		
Biopsy	GBM (n = 2)	8
Autopsy ^a	GBM (n = 12)	48

Abbreviation: GBM, glioblastoma.

^aTwelve patients had no histologic confirmation. One patient had histologic confirmation at diagnosis and at autopsy.

Table 2. Summary of toxicities attributable to the study drugs during the first 6 weeks of therapy

Toxicities	Dosage level					
	1 (n = 16)		2a (n = 3)		2b (n = 6)	
	Grades 1/2	Grade 3	Grades 1/2	Grade 3	Grades 1/2	Grade 3
Neutropenia	3	1	1	1	1	0
Thrombocytopenia	6	0	1	1 ^a	2	0
Anemia	4	2	1	0	1	0
Hypoalbuminemia	10	1 ^a	2	0	5	0
Proteinuria	13	0	3	0	4	0
Hypophosphatemia	11	1	3	0	4	1
Hypokalemia	6	1	2	0	0	3
Increase in transaminases	11	0	2	0	5	0
Increase in amylase ± lipase	0	1 ^a	0	1 ^a	0	0
Anorexia	4	0	2	0	1	0
Fatigue	7	0	1	0	0	0
Hypertension ^b	9	0	3	0	0	0
Prolonged QT _c interval	2	0	1	0	3	0
Vomiting	10	0	2	0	4	0
Diarrhea	6	0	3	0	2	1 ^a

^aDose-limiting toxicity.

^bRestricted to grade 2 or higher.

enrolled at dosage level 2a experienced DLTs consisting of a grade 3 increase in amylase and lipase in one and grade 3 thrombocytopenia in the other. None of the first 3 patients treated at dosage level 2b had DLTs. However, all 3 experienced significant toxicities attributable to study drugs within 1 month of completion of the DLT evaluation period. Those toxicities consisted of grade 2 intolerable diarrhea and gastrointestinal hemorrhage in one; grade 4 thrombocytopenia, grade 3 diarrhea, and grade 3 colitis in another; and grade 4 neutropenia and grade 3 neutropenic fever in the third. Three additional patients had already started therapy at dosage level 2b before those serious toxicities were seen; one of them experienced a DLT (grade 3 diarrhea). Therefore, we deemed dosage level 2b too toxic. Of the 2 remaining patients at dosage level 2b, the dose of study drugs was electively reduced to dosage level 1 after 4 weeks of therapy in one patient, and the second patient withdrew consent for study participation after 8 days of dasatinib; neither of these patients had serious toxicities attributable to study drugs.

Ten additional patients were treated at the MTD (dosage level 1). One of the 10 experienced a DLT (grade 3 hypoalbuminemia).

No changes in cartilaginous growth plates were detected by either knee x-ray or MRI. Osteonecrosis was seen by knee MRI in 2 patients 4 and 8 months after the start of therapy; both had received dexamethasone. Further details about cartilaginous and bony effects of this therapy were previously reported (30).

Two patients aged 2.3 and 2.6 years at diagnosis developed radiation necrosis outside the tumor involving the thalamus, temporal lobe, basal ganglia, and cerebellum 7.3

and 5.4 months after the start of radiotherapy, respectively. Both required corticosteroids to control their symptoms for 1 and 3 months, respectively. Clinical and radiologic improvement of radiation-induced abnormalities occurred within 4 months. A third patient aged 3.6 years at diagnosis was found to have radiation necrosis in the left thalamus 19.4 months after the start of radiotherapy and concomitant with tumor progression. We attributed the occurrence of radiation necrosis outside the brainstem to the larger radiotherapy fields used in this study. None of these patients received whole-brain radiotherapy.

The median duration of treatment was 184 days (range, 8–819 days). Treatment for up to 3 years was allowed for 2 patients who had completed 2 years of therapy. Six patients required dose reductions of dasatinib ($n = 2$), vandetanib ($n = 1$), or both ($n = 3$) after the completion of the DLT evaluation period because of toxicities. Five of them had dose reductions due to grade 2/3 diarrhea with or without rectal bleeding. Four patients required a second dose reduction because of recurrent toxicities.

Pharmacokinetic studies

Steady-state pharmacokinetic studies following single-agent dasatinib (day 8) and during drug combination were obtained in 25 and 24 patients, respectively. A summary of our results is shown in Table 4. When all dosage levels were combined, the apparent oral clearance of dasatinib increased by approximately 35% between days 8 and 42 (median, 1,985 vs. 2,653 mL/min/m²; $P = .025$). The mean maximum concentration (C_{max}) of dasatinib decreased between days 8 and 42 for the 65 and 85 mg/m² dosage levels, but the half-life was unchanged. This suggests that

Table 3. Summary of toxicities attributable to the study drugs after the first 6 weeks of therapy

Toxicities	Dosage level										
	0 (n = 2)			1 (n = 18)			2a (n = 1)		2b (n = 3)		
	Grades 1/2	Grade 3	Grade 4	Grades 1/2	Grade 3	Grade 4	Grades 1/2	Grade 3	Grades 1/2	Grade 3	Grade 4
Neutropenia	0	1	0	2	2	0	0	0	0	1	1 ^a
Thrombocytopenia	1	0	0	3	0	0	1	0	0	0	1 ^a
Anemia	1	1	0	3	0	1	1	0	1	0	1
Hypoalbuminemia	0	1	0	13	2	0	1	0	1	2	0
Proteinuria	1	0	0	13	1	0	1	0	1	0	0
Hypophosphatemia	0	1	0	8	1	0	1	0	0	0	0
Hypokalemia	1	0	1	4	2	0	0	1	0	1	0
Increase in transaminases	1	0	0	12	0	0	0	0	1	0	0
Fatigue	1	0	0	6	1	0	0	0	0	0	0
Hypertension ^b	0	0	0	3	2	0	0	0	0	0	0
Prolonged QT _c interval	0	0	0	7	1	0	0	0	0	0	0
Vomiting	1	0	0	11	1	0	1	0	0	0	0
Diarrhea	1	0	0	11	2	0	0	1	2 ^{a,c}	1 ^a	0
Colitis	1	0	0	0	0	0	0	0	0	1 ^a	0
GI hemorrhage	0	0	0	1	2	0	0	0	0	1 ^a	0
Infection	0	1	0	3	2	0	1	0	0	1 ^a	1

NOTE: Dosage of study drugs was reduced in 6 patients during the DLT-evaluation period because of DLTs (n = 5) or electively (n = 1). Only 24 patients continued therapy beyond the DLT-evaluation period as consent was withdrawn after 8 days of therapy in one patient. Abbreviation: GI, gastrointestinal.

^aClinically significant toxicities which occurred within 1 month of completion of the DLT-evaluation period.

^bRestricted to grade 2 or higher.

^cOne patient had a grade 2 intolerable diarrhea.

decreased absorption of dasatinib rather than increased elimination accounted for its lower exposure on day 42.

Serial plasma and CSF samples were obtained concurrently in 2 patients treated at dosage level 1. Five and 6 consecutive collections were obtained within 24 hours in each patient. The CSF-to-plasma ratios of AUC_{0-24h} for

dasatinib were 0.028 and 0.016. The respective ratios for vandetanib were 0.012 and 0.024.

Pharmacodynamic studies

There was a statistically significant increase in the plasma levels of SDF1 α at the end of radiotherapy ($P < 0.0001$).

Table 4. Pharmacokinetic parameters of vandetanib and dasatinib

Drug	Dose (mg/m ²)	Day	No. of patients	C _{max} (ng/mL)	AUC _{0-9 h} (ng/h/mL)	AUC _{0-24 h} (ng/h/mL)	C _{ss} trough (ng/mL)	t _{1/2} (hours)	CL/F (mL/min/m ²)
Dasatinib	65	8	19	168 ± 89	545 ± 219	677 ± 278	NA	5.2 ± 1.3	2,243 ± 871
	65	42 (±3)	18	106 ± 83	352 ± 186	456 ± 217	NA	5.7 ± 2.1	4,021 ± 2,983
	85	8	6	200 ± 68	697 ± 290	843 ± 402	NA	4.6 ± 0.8	2,248 ± 1,114
	85	42 (±3) ^a	4	124 ± 67	529 ± 116	688 ± 143	NA	5.0 ± 0.8	2,505 ± 605
	50	† ^b	2	104; 106	320; 453	396; 549	NA	4.3; 5.5	1,520; 2,607
Vandetanib	65	42 (±3)	21	868 ± 431	ND	18,919 ± 9,101	684 ± 315	ND	67 ± 26
	85	42 (±3)	3	716 ± 271	ND	15,535 ± 5,430	558 ± 175	ND	93 ± 36

NOTE: Data were provided as mean values ± SD.

Abbreviations: AUC, area under concentration-time curve; t_{1/2}, half life, CL/F, apparent oral clearance; NA, not applicable; ND, not done.

^aOne patient had repeat pharmacokinetic studies done on day 28 of therapy.

^bDose was reduced because of DLTs. Repeat pharmacokinetic studies were obtained on days 97 and 153 of therapy.

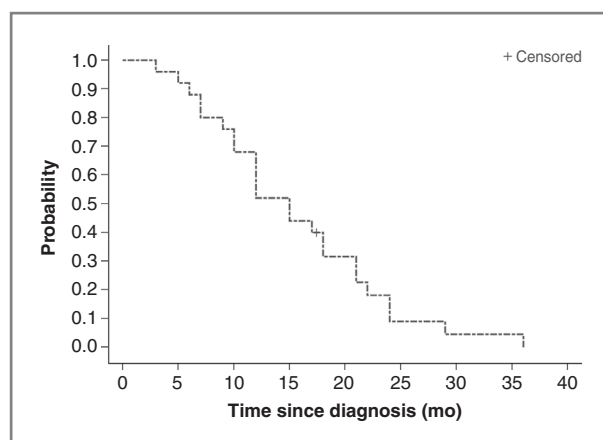


Figure 2. Overall survival for all patients.

We also observed a trend for increased plasma levels of VEGF ($P = 0.08$) and decreased plasma levels of PDGF-BB ($P = 0.06$) at the end of radiotherapy. There was a significant increase of log-transformed plasma levels of VEGF ($P = 0.0006$) and SDF1 α ($P = 0.0031$) during therapy. Patients whose plasma levels of SDF1 α increased during therapy had longer PFS ($P = .046$). Likewise, increases in SDF1 α ($P = 0.02$) and VEGF ($P = 0.03$) during therapy were associated with longer OS.

There was a statistically significant decrease in phosphorylated (p) 70S6K ($P = 0.0018$) and a trend for lower pAkt ($P = 0.07$) on day 28 compared with day 8 for patients with higher exposure to dasatinib on day 42. We did not observe any other associations between the pharmacokinetic and pharmacodynamic parameters analyzed. Although 7 of 17 patients had a decrease in pSrc during therapy, these findings were not statistically significant.

FISH analysis

Tissue obtained at autopsy was available for analysis in 12 patients. All attempts for hybridization failed in the samples from 2 patients. Only 1 of 10 tumors analyzed displayed *PDGFRA* amplification. No *MET* amplifications were observed.

Outcome

Treatment with dexamethasone was discontinued or its dose significantly reduced at the end of radiotherapy in all patients except one. All patients experienced disease progression and only one remains alive with progressive disease. The 1- and 2-year OS for all patients were $52\% \pm 10\%$ and $9\% \pm 6\%$, respectively (Figure 2). Five patients completed more than 1 year of therapy; 2 of them were on treatment for more than 2 years. All 5 patients had the typical clinical and radiologic characteristics of DIPG at diagnosis. The patient whose tumor harbored *PDGFRA* amplification survived for only 7 months.

Discussion

Unlike other clinical trials which had previously combined local radiotherapy with conventional chemother-

apeutic agents or single-agent small-molecule inhibitors (1), this was the first clinical trial to use a rational combination of small-molecule inhibitors in children with newly diagnosed DIPG. We chose the study drugs based on their potential to target biologic characteristics of glioblastoma and relevant molecular abnormalities recently unveiled in DIPG, which are distinct from those found in adult and nonbrainstem pediatric high-grade gliomas (7–11). The selection of vandetanib and dasatinib followed multiple other criteria, including the knowledge of the pediatric phase II recommended dose for each agent, the good tolerance of both medications by children at comparable or higher doses than in adults, the presence of few overlapping toxicities, the opportunity of oral dosing, and the different mechanisms of action and possibility of targeting cancer as well as stromal cells (4, 22).

We conducted detailed plasma pharmacokinetic studies to evaluate potential interactions between vandetanib and dasatinib. The trough steady-state concentrations of vandetanib at 65 mg/m^2 were similar to those seen in our previous experience with it as a single agent (4). We could not compare the other steady-state parameters of vandetanib in the current study because these data were not available in children. Moreover, the steady-state exposure and C_{max} of vandetanib in our patients were slightly higher than those in adults who received an equivalent dose of the medication (31). There was a significant decrease in the exposure of dasatinib between days 8 and 42 of therapy, which could be due to interaction with vandetanib. The efflux transporter ABCC4 in the stomach facilitates the absorption of dasatinib (32). As vandetanib is an inhibitor of ABCB1 and ABCG2 (33), it is plausible that it could affect the absorption of dasatinib by cross-inhibiting ABCC4. Alternatively, a time-dependent decrease in the exposure of imatinib was seen after chronic administration (34). Although a similar phenomenon could account for the decrease in the exposure of dasatinib, this is unlikely because such a decrease was not observed after chronic exposure to dasatinib in adults (23). The steady-state exposure of dasatinib in our patients remained similar to that seen in adults who received equivalent doses (23). Despite the decrease in steady-state exposure of dasatinib, the C_{max} and AUC of dasatinib in our study also remained quite similar to that observed after a single dose of dasatinib only in another pediatric study (22).

We showed for the first time that the CSF exposure of vandetanib and dasatinib in humans is modest. Our findings were similar to the CSF exposure of most other tyrosine kinase inhibitors (35, 36). Although drug levels in the CSF are commonly used as surrogates of brain parenchyma penetration, this assumption is not completely valid particularly for brain tumors with a disrupted blood-brain barrier. Nevertheless, our findings are important not only for patients with CNS tumors but also for those with other cancers (e.g., leukemia). Although the C_{max} of dasatinib in the CSF was low, it reached sustained levels similar to those

causing significant inhibition in other drug targets (e.g., Bcr-abl) *in vitro* (17).

We observed multiple longitudinal changes in plasma angiogenic factors, some of which (e.g., an increase in SDF1 α) had already been observed in our previous study (4). The association between longitudinal changes in VEGF and SDF1 α and outcome were intriguing. Unlike our previous study (4), increases in VEGF during therapy were associated with a longer (and not shorter) OS in the current study. We speculate that this association of increases in VEGF with better OS may reflect an improved inhibition of alternative VEGF-mediated angiogenic pathways by combining dasatinib with vandetanib compared with vandetanib only (i.e., an improved anti-angiogenic effect may lead to better survival). The inhibition of pAkt and p70S6K in PBMCs during therapy was interesting and may reflect an indirect effect of the inhibition of RTKs. Unfortunately, the evaluation of PDGFR phosphorylation in PBMCs was logistically impossible. Of note, we commonly observed a decrease in skin pigmentation and hair discoloration among our patients, a previously described effect of dasatinib on c-Kit (37).

One of the main strengths of this study was our ability to evaluate short- and long-term toxicities of this drug combination since half of our patients received therapy for more than 6 months. In fact, some of the most concerning side effects (e.g., diarrhea, gastrointestinal bleeding, and hypoalbuminemia) were seen after the completion of the DLT evaluation period. Diarrhea was one of the few overlapping toxicities between vandetanib and dasatinib. We considered gastrointestinal hemorrhage and hypoalbuminemia to be more likely secondary to dasatinib since these side effects had been previously associated with this medication (38) and were reported in the phase I pediatric study (22). Myelosuppression was a common toxicity of dasatinib (38). Interestingly, we did not observe some toxicities of dasatinib commonly described in patients with hematologic malignancies (e.g., pleural effusions, edema, and skin rash; ref. 38). Similar to what was said in a recent publication (39), we emphasized the importance of adequate evaluation of long-term toxicities of new targeted agents.

We believe that the larger radiotherapy margins used in the current study led to the occurrence of symptomatic radiation necrosis in uninvolved areas of the brain in 3 young patients. To the best of our knowledge, no other patients with DIPG treated at our institution with more standard radiotherapy fields had ever developed symptomatic radiation necrosis in uninvolved areas of the brain.

Therefore, we do not recommend the use of enlarged radiotherapy fields as used in the current study.

Although the combination of radiotherapy, vandetanib, and dasatinib did not change the poor prognosis for children with DIPG, the 1-year OS of our patients compared favorably with those seen in other studies (1). New preclinical models of DIPG were recently generated (40). We hope that drug testing in preclinical models that closely mimic DIPG, the discovery of new promising molecular tumor targets, and the ability to select the most appropriate therapies for specific molecular subgroups of this heterogeneous cancer may lead to improvements in the treatment of children with DIPG.

Disclosure of Potential Conflicts of Interest

A. Broniscer has a commercial research grant from AstraZeneca. No potential conflicts of interest were disclosed by the other authors.

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