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Phase 1 trial, pharmacokinetics, and pharmacodynamics of dasatinib combined with crizotinib in children with recurrent or progressive high-grade and diffuse intrinsic pontine glioma

Alberto Broniscer[1,2] | Sujuan Jia[3] | Belinda Mandrell[4] | Dima Hamideh[1] | Jie Huang[5] | Arzu Onar-Thomas[5] | Amar Gajjar[1,2] | Susana C. Raimondi[3] | Ruth G. Tatevossian[3] | Clinton F. Stewart[6]

[1]Department of Oncology, St. Jude Children's Research Hospital, Memphis, Tennessee

[2]Department of Pediatrics, University of Tennessee Health Science Center, Memphis, Tennesssee

[3]Department of Pathology, St. Jude Children's Research Hospital, Memphis, Tennessee

[4]Department of Pediatric Medicine, St. Jude Children's Research Hospital, Memphis, Tennessee

[5]Department of Biostatistics, St. Jude Children's Research Hospital, Memphis, Tennessee

[6]Department of Pharmaceutical Sciences, St. Jude Children's Research Hospital, Memphis, Tennessee

Correspondence
Alberto Broniscer, Department of Pediatrics, Children's Hospital of Pittsburgh, 4401 Penn Avenue, Pittsburgh, PA 15224.
Email: alberto.broniscer@chp.edu

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Abstract

Background: Progressive/recurrent high-grade and diffuse intrinsic pontine gliomas (DIPGs) are fatal. Treatments targeting molecular pathways critical for these cancers are needed.

Methods: We conducted a phase 1 study (rolling-six design) to establish the safety and maximum tolerated dose (MTD) of dasatinib, an oral platelet-derived growth factor receptor A (PDGFRA) inhibitor, and crizotinib, an oral c-Met inhibitor, in such patients. Pharmacokinetics of both agents were performed. Biomarkers of cellular pathway activation in peripheral-blood mononuclear cells (PBMC) were evaluated before and after administration of dasatinib. *PDGFRA* and *MET* amplification, and *PDGFRA* mutations were studied in tumor samples.

Results: Twenty-five patients were enrolled in this study (median age: 11.9 years). Eleven patients had DIPG. Glioblastoma accounted for 40% of cases. Dasatinib at 50 mg/m^2 and crizotinib at 130 mg/m^2 or 100 mg/m^2 were poorly tolerated when administered twice daily. Drug administration was then switched to once daily. Dasatinib administered at 50 mg/m^2 and crizotinib at 215 mg/m^2 once daily was the MTD. Dose-limiting toxicities consisted of diarrhea, fatigue, proteinuria, hyponatremia, rash, and grade 4 neutropenia. Only two patients received therapy for at least 6 months. No objective radiologic responses were observed. Pharmacokinetics of dasatinib and crizotinib were comparable to previous studies. A statistically significant decrease in the ratio of p-AKT/total AKT in PBMC occurred after dasatinib administration. *PDGFRA* and *MET* amplification were found in four and two cases, respectively. Only one of 10 tumors harbored a *PDGFRA* mutation.

Conclusions: This drug combination was poorly tolerated and its activity was minimal. We do not recommend further testing of this combination in children.

KEYWORDS
c-Met, children, crizotinib, dasatinib, diffuse intrinsic pontine glioma, high-grade glioma, PDGFRA

1 | INTRODUCTION

High-grade gliomas (HGGs), including diffuse intrinsic pontine gliomas (DIPGs), are among the most aggressive and lethal primary central nervous system (CNS) tumors in children.[1] The prognosis of affected patients remains dismal despite treatment with maximum safe surgical resection, radiation therapy (RT), and chemotherapy.[1] Children with recurrent or progressive HGGs and DIPGs are ideal candidates for investigational clinical trials since there are no standard chemotherapeutic regimens for these tumors.

Two major developments have stimulated the design of more rational therapies for children with HGGs and DIPGs. First, genome-wide studies have uncovered the molecular characteristics of these

Abbreviations: CNS, central nervous system; DIPG, diffuse intrinsic pontine glioma; DLT, dose-limiting toxicity; HGF, hepatocyte growth factor; HGG, high-grade glioma; HRQoL, health-related quality of life; MTD, maximum tolerated dose; PBMC, peripheral-blood mononuclear cells; PDGFR, platelet-derived growth factor receptor; RT, radiation therapy

cancers, which are mostly different from adult tumors.[2–6] Second, the establishment of preclinical models, including cell lines and human xenografts, has allowed for the high-throughput screening of new promising agents.[7] Therapies targeting abnormally activated cellular pathways have shown promising activity against preclinical models of pediatric HGGs and DIPGs including inhibitors of platelet-derived growth factor receptor (PDGFR) and c-Met.[7–9]

The PDGF pathway influences tumor formation by stimulating mitogenesis, dedifferentiation, and increased angiogenesis, and its modulation *in vivo* in combination with other oncogenic abnormalities led to the formation of HGGs and DIPGs in preclinical models.[10–13] While *PDGFRA* amplification can be found in one-third and 19% of DIPGs and pediatric nonbrainstem HGGs, respectively,[2,6] *PDGFRA* mutations occurred in approximately 5% and 14% of such tumors, respectively.[12]

Activation of the c-Met pathway has been recognized as a contributing mechanism for the formation of HGGs.[14] *MET* is the second most common amplified oncogene in DIPG.[2] *MET* fusion transcripts have recently been identified in approximately 10% of pediatric glioblastomas (WHO grade IV), particularly in those originating from the cerebral hemispheres.[9] *MET* fusion transcripts in combination with other oncogenic mechanisms also led to the formation of HGGs in an *in vivo* preclinical model.[9] Finally, cell lines and xenografts containing *MET* fusion transcripts were responsive to c-Met inhibition.[9]

Dasatinib (Sprycel, Bristol–Myers Squibb) is a potent oral inhibitor of PDGFRA and B, Src, and c-Kit, for which a phase 2 single-agent recommended dose has already been established in children.[15] Crizotinib (Xalkori, Pfizer) is a potent inhibitor of ALK and c-Met that has already undergone phase 1 testing in children.[16] Based on previously described data, we initiated a phase 1 clinical trial to test the tolerability and safety, and to establish the maximum tolerated dose (MTD) of the combination of dasatinib and crizotinib in pediatric patients with recurrent or progressive HGGs and DIPGs.

2 | PATIENTS AND METHODS

Patients between the ages of 2 and 21 years old with clinically and/or radiologically recurrent or progressive HGG or DIPG were eligible for this study. Other inclusion criteria consisted of the following: (1) performance score ≥ 50; (2) adequate hematologic (hemoglobin ≥ 8 g/dl [irrespective of previous transfusions], absolute neutrophil count ≥ 1,000/mm^3, and platelet count ≥ 100,000/mm^3 [transfusion independent]), renal (normal serum creatinine for age), and hepatic (transaminases and bilirubin <3× and <1.5× the institutional upper limit of normal, respectively, and albumin ≥ 2 g/dl) functions; (3) stable neurologic deficits on a fixed or decreasing dose of dexamethasone for ≥1 week; (4) no more than a grade 1 toxicity attributed to previous therapies; (5) interval from previous local, craniospinal, and palliative RT of ≥3 months, ≥6 months, and ≥2 weeks, respectively; (6) interval ≥ 4 weeks from previous chemotherapy (6 weeks if nitrosourea); (7) interval ≥ 1 week from previous small-molecule inhibitors with short half-lives; (8) interval ≥ 3 half-lives from previous monoclonal antibodies; (9) interval ≥ 3 months from previous high-dose chemotherapy with stem-cell rescue; (10) interval ≥ 1 week and ≥ 2 weeks from the use of filgrastim and pegfilgrastim, respectively; and (11) effective contraception for females of childbearing age and males of child fathering potential. Exclusion criteria consisted of the following: (1) use of other anticancer therapies; (2) use of enzyme-inducing anticonvulsants less than 10 days before start of protocol therapy; (3) previous treatment with a PDGFR or c-Met inhibitor; (4) presence of other medical conditions that could interfere with the study procedures or tolerance to therapy; and (5) pregnant or lactating patients.

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.

2.1 | Study design

This single-institution clinical trial followed the rolling-six design. The MTD was defined as the highest dosage at which no more than one of six evaluable patients experienced a dose-limiting toxicity (DLT). Two regimens of this combination were tested. Dasatinib and crizotinib were initially administered twice daily and the DLT evaluation period lasted for 6 weeks. Drug administration was switched to once daily and the DLT-evaluation period to 4 weeks as this initial regimen was not tolerable. Dose-limiting toxicities consisted of the following side effects attributable to study drugs: (1) grade 4 neutropenia; (2) grade 3 or 4 thrombocytopenia; (3) any grade 3 or 4 nonhematologic toxicity except for grade 3 weight change, grade 3 elevation in transaminases that returned to baseline or ≤grade 1 within 7 days of drug interruption, grade 3 or 4 electrolyte abnormalities that returned to ≤grade 2 within 7 days, and grade 3 fever or infection lasting < 5 days; and (4) any grade 2 nonhematologic toxicity lasting > 7 days and causing significant clinical repercussion. Toxicities were graded based on the National Cancer Institute Common Toxicity Criteria for Adverse Events (version 4.0).

For the twice-daily regimen, the starting doses of dasatinib and crizotinib were 50 mg/m^2 and 130 mg/m^2, respectively. For the once-daily regimen, the starting doses of dasatinib and crizotinib were 50 mg/m^2 and 165 mg/m^2, respectively (Table 1). In both regimens, patients received the first dose of crizotinib on day 1 and then treatment was held for 48 hr to allow for completion of pharmacokinetic studies. The administration of dasatinib was delayed for 48 hr as well. Treatment with both drugs continued uninterrupted on day 3 of therapy except for the omission of the evening dose of crizotinib on day 14 in the twice-daily regimen so that extended pharmacokinetic studies could be completed.

Treatment was divided into 28-day cycles except for the first two cycles on the twice-daily regimen that comprised 6 weeks. The maximum planned treatment duration was 2 years.

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. Crizotinib was administered with or without food as 200 mg and 250 mg capsules or as a solution (10 mg/ml) depending

TABLE 1 Proposed dose escalation schema

	Dosage level	Dasatinib (mg/m^2 per dose)	Crizotinib (mg/m^2 per dose)
Twice-daily regimen	0	50	100
	1[a]	50	130
	2	65	130
	3a	65	165
	3b	85	130
	4	85	165
	5	85	215
Once-daily regimen	0	50	130
	1[a]	50	165
	2a	50	215
	2b	65	165
	3	65	215
	4a	65	280
	4b	85	280

[a]Starting dosage level.

on the planned dose and the ability to swallow capsules. The dose of both medications was rounded to the nearest 10 mg. The actual dose of both medications was calculated based on the body surface area (BSA) at the start of therapy. Changes in dose based on variations of BSA were allowed after 6 months of therapy. When applicable, an interval of at least 4 hr was recommended between the administration of dasatinib and histamine receptor 2 antagonists. Use of septra was flexible, depending on the physician's preferences and patients' needs. Loperamide was strongly recommended if patients developed diarrhea.

A clinical assessment, complete blood counts with differential, chemistry panel, urinalysis, and electrocardiogram were obtained before the start of therapy and weekly during cycle 1, every 2 weeks during cycle 2, and every 4 weeks thereafter. Magnetic resonance imaging (MRI) scans for tumor evaluation were obtained before the start of therapy, before cycle 2, and after every other cycle thereafter. An echocardiogram was obtained before the start of therapy, at the completion of cycle 1, and every 6 months thereafter during treatment. Chest X-ray was obtained before the start of therapy and every 6 months thereafter.

2.2 | Pharmacokinetic studies

For the twice-daily regimen, mandatory pharmacokinetic studies of crizotinib were scheduled on days 1 and 14, and studies of crizotinib and dasatinib were to occur approximately at the end of the DLT-evaluation period (day 42 ± 3 days). In the once-daily regimen, the pharmacokinetic studies of crizotinib were scheduled on days 1 and 14, and the pharmacokinetic studies of dasatinib occurred on day 14.

Day 1 serial blood samples (3 mL) for crizotinib were collected before and at 1, 2, 4, 8, 24, and 48 hr after the administration of crizotinib. On day 14, serial samples of crizotinib were obtained before and at 1, 2, 4, 8, and 24 hr after the morning dose of crizotinib. For pharmacokinetic studies of dasatinib, serial samples were obtained before and at 1, 2, 4, 8, and 24 hr after their morning dose. Other details are provided in Supplementary Appendix S1.

2.3 | Pharmacodynamic studies

Optional studies consisted of serial evaluation of the plasma concentration of the hepatocyte growth factor (HGF) obtained concurrently with brain MRIs before the start and during therapy, and analysis of total and phosphorylated AKT and ERK in peripheral-blood mononuclear cells (PBMCs) obtained before and 3 hr after the administration of dasatinib on day 14 of therapy and at the end of the DLT-evaluation period. Details about sample processing, collection, and analysis are provided in Supplementary Appendix S1.

2.4 | Molecular studies

Analysis of amplification of *PDGFRA* and *MET* by FISH was performed as previously described.[17] The methods used for *PDGFRA* sequencing are provided in Supplementary Appendix S1.

2.5 | Assessment of quality of life

Health-related quality of life (HRQoL) was assessed through patient and parent-proxy report using the Pediatric Quality of Life Inventory (PedsQL 4.0) and the Pediatric Quality of Life Brain Tumor Module (PedsQL BT module).[18,19] Patients and parent-proxy reported on HRQoL and symptoms over the previous 7 days. The HRQoL assessments were obtained at the initiation of therapy, at weeks 2, 4, and 6 of therapy, and before every other cycle of therapy beginning at cycle 3.

2.6 | Statistical analysis

Descriptive statistics were provided. Random coefficient model was used to test whether HGF concentration changed with time. The Spearman correlation was used to estimate the association between HGF plasma concentrations and length of therapy. Sign test, a nonparametric analog of the *t*-test for paired samples, was used to compare p-ERK/total ERK (or p-AKT/total AKT) before and after administration of dasatinib, and to compare data obtained after administration of dasatinib on different days. A significance threshold of 0.05 was used without adjusting for multiplicity.

The Wilcoxon signed-rank test was used to compare the change in HRQoL from baseline to week 2, week 2 to week 4, and week 4 to week 6 of therapy for patients and parents. Patient (≥5 years of age) self-reported and parent-proxy reported HRQoL domain scores were compared at each therapy time point using the Wilcoxon signed-rank test, with data only reported for patients and parents with comparative data. The false discovery rate adjustment was used to account for multiple testing when appropriate. A two-sided significance level of $P < 0.05$ was used for all statistical tests. Statistical analyses were conducted using SAS Version 9.4 (SAS Institute, Cary, NC).

TABLE 2 Characteristics of 25 patients enrolled in this study

Characteristics	Number of patients (%)
Median age (in years) at study enrollment	11.9 (range: 2.9–21.3)
Gender	
Male	10 (40)
Female	15 (60)
Race/ethnicity	
Caucasian	17 (68)
African-American	3 (12)
Hispanic	5 (20)
Histologic diagnoses	
Anaplastic astrocytoma	3 (12)
Glioblastoma	10 (40)
High-grade glioma	4 (16)
No histologic confirmation	8 (32)
Previous RT	25 (100)
Previous regimens of chemotherapy	
0	2 (8)
1	16 (64)
2	5 (20)
≥3	2 (8)

Of note, 11 patients had a radiologic diagnosis of diffuse intrinsic pontine glioma.
Three patients with diffuse intrinsic pontine glioma had histologic confirmation at biopsy (n = 2) or at autopsy (n = 1).

3 | RESULTS

Twenty-five patients were enrolled in this study from November 2012 until September 2016. Tables 2 and 3 list their characteristics at study enrollment. All patients except one had measurable radiographic disease at the start of this therapy. Three (12%) of 25 patients had leptomeningeal metastases at the start of therapy although complete metastatic work-up was not mandatory.

Of five patients enrolled on dosage level 1 of the twice-daily regimen, three of four evaluable patients experienced DLTs consisting of grade 3 diarrhea (n = 1) and fatigue (n = 2). Therefore, three patients started treatment on dosage level 0, two of whom developed DLTs. One patient experienced grade 3 hyponatremia concurrently with disease progression; further evaluation showed that hyponatremia was at least partially related to inappropriate secretion of antidiuretic hormone. Another patient with normal urinalysis at the start of therapy developed grade 3 proteinuria within the nephrotic range which improved with discontinuation of treatment.

Since the twice-daily regimen was poorly tolerated even at the lowest planned dosage level, the clinical trial was amended to test doses of the study drugs closer to their single-agent MTD administered once daily. Six patients were enrolled on dosage level 1 of the once-daily regimen, three of whom were unevaluable due to early disease progression. None of the three evaluable patients experienced significant toxicities. Therefore, eight patients started treatment on dosage level 2a. Only one of six evaluable patients at this dosage level had a DLT consisting of grade 3 diarrhea and rash. Hence, three patients were treated at dosage level 2b, two of whom experienced DLTs consisting of grade 3 rash (n = 1) and grade 4 neutropenia (n = 1). Therefore, the MTD was established as dasatinib 50 mg/m^2 and crizotinib 215 mg/m^2 administered once daily. Table 4 provides a summary of the most significant toxicities attributed to the study agents. Diarrhea was common during and after the completion of the DLT-evaluation period despite use of loperamide. Fatigue related to study agents was often difficult to distinguish from clinical progression and was suspected based on rapid onset without other signs of clinical deterioration and improvement by holding the dose of dasatinib, the medication with the shortest half-life. Only one patient experienced QT_c prolongation grade 1 at dosage level 2a. No pleural effusions related to dasatinib were observed.

3.1 | Pharmacokinetic studies

Pharmacokinetic studies of crizotinib were collected from 25 patients on day 1 of therapy. The median (range) T_{max}, apparent oral clearance, and elimination half-life of crizotinib were 4.1 hr (1.0–23.9), 56.5 l/hr/m^2 (17.7–113.2), and 13.1 hr (9.8–34.7), respectively. Nineteen patients had pharmacokinetic studies of crizotinib performed on day 14 of therapy. The median (range) apparent oral clearance and elimination half-life were 50.3 l/hr/m^2 (12.0–126.9) and 12.8 hr (8.0–36.5), respectively.

Pharmacokinetic studies of dasatinib were performed in 15 patients. However, only those studies obtained for 12 patients on day 14 of therapy were deemed appropriate for analysis. The median (range) T_{max}, apparent oral clearance, and elimination half-life of dasatinib were 2.1 hr (1.1–4.0), 1,328 mL/min/m^2 (532–2,125), and 4.4 hr (2.9–7.9), respectively. The results of the C_{max} and $AUC_{0\rightarrow t}$ of crizotinib and dasatinib are summarized in Table 5.

3.2 | Pharmacodynamic studies

Twenty-one patients had 46 serial plasma samples analyzed for the concentration of HGF (median = 198.3 pg/ml; range: 88.1–3,925.8). There was no change in the log-transformed concentration of HGF in plasma over time for patients with serially collected samples ($P = 0.087$). We found a negative correlation between HGF plasma concentration at baseline and the duration of therapy (Spearman correlation = -0.59; $P = 0.0048$)

Twenty paired samples in 14 patients were available for analysis of the changes produced by dasatinib in markers of cellular pathway activation in PBMC. When all paired samples and only those collected on day 14 of therapy (n = 13) were analyzed, there was a statistically significant decrease in the ratio of p-AKT/total AKT ($P = 0.041$ and 0.003, respectively) 3 hr after the administration of dasatinib. No statistically significant changes in the ratio of p-ERK/total ERK were found after dosing.

TABLE 3 Clinical and molecular data for all 25 patients

	Gender/race or ethnicity	Age at study enrollment (years)	Histologic diagnosis	Molecular abnormalities	Duration of therapy (weeks)
1	F/Caucasian	11.9	Anaplastic astrocytoma	No PDGFRA or MET ampl	6
2	F/African-American	15.5	Not available	Not available	11
3	F/Caucasian	8	Glioblastoma	No PDGFRA or MET ampl	12
4	M/Caucasian	14.9	Not available	Not available	6
5	F/Caucasian	16.8	Glioblastoma	No PDGFRA or MET ampl	<1
6	F/Caucasian	5	Not available	Not available	6
7	F/African-American	7.7	Anaplastic astrocytoma	No PDGFRA or MET ampl	5
8	F/Hispanic	5.9	Not available	Not available	2
9	M/Caucasian	21.3	Glioblastoma	MET ampl; no PDGFRA ampl	3
10	M/Caucasian	5.3	Glioblastoma	No PDGFRA or MET ampl	<1
11	M/Caucasian	6.5	Not available	Not available	4
12	F/Hispanic	10.1	High-grade glioma	No PDGFRA or MET ampl	16
13	M/Caucasian	7.4	Not available	Not available	2
14	M/Caucasian	16.1	Glioblastoma	No PDGFRA or MET ampl	48
15	F/Hispanic	16.9	High-grade glioma	Not available	1
16	F/Caucasian	5.7	Not available	Not available	4
17	M/Caucasian	13.1	Glioblastoma	PDGFRA ampl; no MET ampl	8
18	F/Caucasian	7.1	Not available	Not available	4
19	M/African-American	19.1	High-grade glioma	No PDGFRA or MET ampl	4.5
20	F/Hispanic	13.1	Glioblastoma	No PDGFRA or MET ampl[a]	3
21	F/Caucasian	13.8	Anaplastic astrocytoma	No PDGFRA or MET ampl	16
22	F/Caucasian	13	Glioblastoma	PDGFRA and MET ampl	9
23	M/Hispanic	14.9	High-grade glioma	Focal PDGFRA ampl; no MET ampl	25
24	M/Caucasian	2.9	Glioblastoma	PDGFRA ampl; no MET ampl	8
25	F/Caucasian	9.3	Glioblastoma	No PDGFRA or MET ampl	4

[a]A PDGFRA nonsynonymous mutation was only identified in one of 10 tumors tested (patient 20).
Eleven patients (patients 2–6, 8, 10, 11, 13, 16, and 18) had a radiological diagnosis of diffuse intrinsic pontine glioma.
F, female; M, male; ampl, amplification.

3.3 | Molecular studies

Seventeen patients underwent histologic confirmation of their tumors. Of 10 (59%) tumors that could undergo sequencing, only one harbored a nonsynonymous *PDGFRA* mutation (*c.1432T > C* p.S478P).

FISH analysis of *PDGFRA* and *MET* amplifications were available in 16 (94%) cases. While four tumors harbored *PDGFRA* amplification, one of them focal, *MET* amplification occurred in two cases. Only one tumor harbored co-amplification of both oncogenes (Table 3).

3.4 | Outcome

The median number of cycles of therapy received was one (range: 0–12). Seven patients experienced clinical progression before completion of cycle 1 of therapy. Only two patients received at least six cycles of therapy (Table 3). No objective radiologic responses were observed with this therapy. Only three patients remain alive.

3.5 | Quality of life

HRQoL measures were collected from 23 patients. Only median total scores were reported due to the small sample size. We included evaluations up to week 6 of therapy because by that time point only nine participants remained in the study. Only the physical, emotional, and social domains were reported for the PedsQL 4.0, as these patients were not attending school.

Patients reported physical function decline starting at week 6 of treatment. Patients did not report a significant increase in brain tumor-specific symptom scores through the first 6 weeks of therapy. Parent-proxy reports were then compared to those obtained from patients. Parent-proxy reported their child as having significantly lower physical function at week 6 and significantly lower emotional and social function at baseline and at weeks 2 and 4 compared to patients' report. Parent-proxy reported their child as experiencing poorer cognitive function at weeks 2 and 4, more pain and hurt at week 2, poorer movement and balance at baseline and at weeks 2 and 4, more nausea at week 4, more worry at baseline and at week 2, and overall lower

TABLE 4 Most significant toxicities attributed to study agents during and after DLT-evaluation period

	Twice-daily regimen				Once-daily regimen					
	0		1		1		2a		2b	
Dosage level	n = 3		n = 5		n = 6		n = 8		n = 3	
	Grade 1/2	Grade 3/4	Grade 1/2	Grade 3/4	Grade 1/2	Grade 3/4	Grade 1/2	Grade 3/4	Grade 1/2	Grade 3/4
Anemia	2 (67%)	0	3 (60%)	0	3 (50%)	0	5 (62%)	0	3	0
Neutropenia	0	0	0	0	1 (16%)	0	1 (12%)	0	1 (33%)	1 (33%)[a]
Thrombocytopenia	0	0	0	0	1 (16%)	0	1 (12%)	0	0	0
Diarrhea	2 (67%)	0	3 (60%)	1 (20%)[a]	4 (67%)	0	7 (87%)	1 (12%)[a]	3 (100%)	0
Nausea/vomiting	1 (33%)	0	2 (40%)	0	5 (83%)	0	6 (75%)	0	3 (100%)	0
Increase in transaminases	2 (67%)	0	3 (60%)	0	1	0	1 (12%)	0	2 (67%)	0
Hypoalbuminemia	3	0	4 (80%)	0	1 (16%)	0	6 (75%)	0	3 (100%)	0
Hyponatremia	2 (67%)	1 (33%)[a]	2 (40%)	1 (20%)	1 (16%)	0	0	1 (12%)	0	0
Hypokalemia	1 (33%)	0	2 (40%)	2 (40%)	2 (33%)	0	4 (50%)	0	0	0
Hypophosphatemia	2 (66%)	1 (33%)	3 (60%)	1 (20%)	0	1 (16%)	5 (62%)	1 (12%)	2 (67%)	0
Proteinuria	2 (66%)	1 (33%)[a]	3 (60%)	0	0	0	3 (37%)	0	3 (100%)	0
Rash	0	0	0	0	1 (16%)	0	6 (75%)	1 (12%)[a]	1	1 (33%)[a]
Fatigue	3	0	0	2 (40%)[a]	4	0	2 (25%)	0	1 (33%)	0

[a]Dose-limiting toxicity.

TABLE 5 Summary of dasatinib and crizotinib pharmacokinetic parameters

Drug	Dose (mg/m^2)	Day of therapy	Number of patients	C_{max} (ng/ml)	$AUC_{0\rightarrow t}$ (ng/ml/hr)
Dasatinib	50	14	10	119.8 (54.1–385.8)	593.8 (388.1–1,521.1)
	65	14	2	209.5 (95.7–323.2)	863.9 (721.9–1,006)
Crizotinib	100	1	3	49.1 (42–97.8)	1,288.9
	100	14	3	392.6 (383–487.4)	5,706 (4,740.9–6,671.9)
	130	1	5	95.7 (57.5–155.5)	2,189.4 (1,261.5–2,668.5)
	130	14	5	443.9 (254.4–793.7)	5,486.1 (4,286.6–10,869)
	165	1	9	155.5 (55.8–324.4)	3,360.1 (1,457.5–4,939.1)
	165	14	7	155.9 (95.9–343.1)	1,933.8 (1,678.1–6,210.4)
	215	1	8	278.8 (123.1–690.2)	5,326.3 (2,068.3–12,182.9)
	215	14	4	261.9 (93.8–356.2)	3,118.9 (1,693.8–4,278.5)

Of note, results are shown as median (ranges in parentheses).
$AUC_{0\rightarrow t}$ of crizotinib were t = ∞ for day 1 and t = 24 hr for day 14.

HRQoL from baseline until week 4 when compared to the patients' reports.

4 | DISCUSSION

The rational design of innovative clinical trials using small-molecule inhibitors for the treatment of children with HGGs and DIPGs should be ideally based on multiple premises including selection of agents that inhibit a biologically relevant target, use of medications with sufficient CNS penetration, and if possible, proven activity against relevant preclinical models. Since HGGs and DIPGs are highly refractory to therapy, there is great enthusiasm for the use of promising treatment combinations.

We report detailed clinical, pharmacokinetic, and pharmacodynamic data about the combination of dasatinib and crizotinib. Unfortunately, this combination was poorly tolerated and the recommended daily phase 2 dose represented 29% and 38% of the MTD of dasatinib and crizotinib, respectively, when used as single agents in children with solid tumors.[15,16] Most of the toxicities observed, including diarrhea, nausea and vomiting, electrolyte abnormalities, rash, and hypoalbuminemia were predictable based on the adverse events previously reported with these medications.[15,16] However, the observation of severe proteinuria in the nephrotic range and hyponatremia was unexpected. No objective radiologic responses were observed.

Although we initially designed our pharmacokinetic studies to evaluate potential interactions between dasatinib and crizotinib, the limited number of patients evaluated prevented us from drawing firm conclusions. The C_{max} and AUC of dasatinib in our patients showed similar results to those previously reported in children with DIPG.[20] Likewise, several pharmacokinetic parameters of crizotinib in our patients (for example, C_{max}, T_{max}, $AUC_{0\rightarrow t}$, and apparent oral clearance)

were comparable to those reported recently in children with solid tumors despite significant methodologic differences between both studies (for example, drug formulation and pharmacokinetic sampling strategy).[21]

Our results demonstrating a decrease in p-AKT in PBMC after administration of dasatinib are interesting, although significant concern remains that dasatinib does not reach clinically effective concentrations in the CNS.[20,22] The longitudinal analysis of plasma HGF was performed based on the report that its expression was predictive of response to c-Met inhibition in HGG preclinical models.[23] Future studies using c-Met inhibitors in the treatment of patients with CNS cancers should further explore the association between concentrations of HGF in plasma and cerebrospinal fluid and treatment response.

The results of this clinical trial need to be interpreted in the context of data available at its design. Dasatinib is one of the most potent commercially available PDGFRA inhibitors. Multiple *in vitro* studies in HGG or DIPG-derived cell lines with or without *PDGFRA* abnormalities have shown activity of this agent, which seems to be mostly cytostatic.[8,12,24] However, the promiscuity of dasatinib in inhibiting multiple targets probably accounts for a large share of its toxicities. Furthermore, as a substrate of p-glycoprotein and BCRP,[22] it is now recognized that its CNS penetration is more limited than initially recognized.[20] Finally, the recommended phase 2 dose reached in this clinical trial provided short exposure to this agent considering that its $t_{1/2}$ is quite short.[20]

Likewise, crizotinib was the only c-Met inhibitor commercially available at the start of this study. Although there was anecdotal evidence of its activity against *MET*-amplified glioblastoma,[25] it is now clear that the CNS penetration of crizotinib is minimal and probably inadequate for the treatment of primary CNS cancers.[25,26] One study recently reported promising activity of the combination of dasatinib and cabozantinib, another c-Met inhibitor, against DIPG-derived cell lines.[8]

We believe that specific and better CNS-penetrant PDGFRA and c-Met inhibitors warrant further testing in children with CNS cancers. It would also be beneficial if preclinical studies could determine the subgroup of patients whose tumors are more likely to respond to PDGFRA and/or c-Met inhibition (for example, those harboring *PDGFRA* and/or *MET* amplification). Although we performed detailed molecular analyses of available tumors in the context of a phase 1 study, we did not select patients based on the presence of specific genetic abnormalities within their tumors. Of note, a focal amplification of *PDGFRA* was found in one of the patients who received this therapy for 6 months. Although no *PDGFRA* or *MET* amplifications were found in the tumor of the patient who received therapy for 12 cycles, we were unable to sequence *PDGFRA* in his tumor. We were unable to evaluate tumors for the recently described *MET* fusions either.[9]

Based on the data provided, particularly the significant toxicity and low recommended phase 2 dose, we do not feel that further testing of the combination of dasatinib and crizotinib in children with CNS cancers is warranted.

CONFLICT OF INTEREST

The authors have no conflicts of interest to disclose in association with the work presented in this manuscript.

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ORCID

Alberto Broniscer http://orcid.org/0000-0001-9646-0463

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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