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ORIGINAL REPORT

Phase I Pharmacokinetic and Pharmacodynamic Study of Pazopanib in Children With Soft Tissue Sarcoma and Other Refractory Solid Tumors: A Children's Oncology Group Phase I Consortium Report

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ABSTRACT

Purpose
Pazopanib, an oral multikinase angiogenesis inhibitor, prolongs progression-free survival in adults with soft tissue sarcoma (STS). A phase I pharmacokinetic and pharmacodynamic study of two formulations of pazopanib was performed in children with STS or other refractory solid tumors.

Patients and Methods
Pazopanib (tablet formulation) was administered once daily in 28-day cycles at four dose levels (275 to 600 mg/m^2) using the rolling-six design. Dose determination for a powder suspension was initiated at 50% of the maximum-tolerated dose (MTD) for the intact tablet. Ten patients with STS underwent dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) scanning at baseline and 15 ± 2 days after initiation of pazopanib at the tablet MTD.

Results
Fifty-three patients were enrolled; 51 were eligible (26 males; median age, 12.9 years; range, 3.8 to 23.9 years). Hematologic and nonhematologic toxicities were generally mild, with dose-limiting lipase, amylase, and ALT elevation, proteinuria, and hypertension. One patient with occult brain metastasis had grade 4 intracranial hemorrhage. The MTD was 450 mg/m^2 for tablet and 160 mg/m^2 for suspension. Steady-state trough concentrations were reached by day 15 and did not seem to be dose dependent. One patient each with hepatoblastoma or desmoplastic small round cell tumor achieved a partial response; eight patients had stable disease for ≥ six cycles, seven of whom had sarcoma. All patients with evaluable DCE-MRI (n = 8) experienced decreases in tumor blood volume and permeability ($P < .01$). Placental growth factor increased, whereas endoglin and soluble vascular endothelial growth factor receptor-2 decreased ($P < .01$; n = 41).

Conclusion
Pazopanib is well tolerated in children, with evidence of antiangiogenic effect and potential clinical benefit in pediatric sarcoma.

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INTRODUCTION

Pazopanib (Votrient; GlaxoSmithKline, London, United Kingdom) is a potent, selective, oral, ATP-competitive multitargeted receptor tyrosine kinase inhibitor of vascular endothelial growth factor receptors (VEGFR) -1, -2, and -3, c-kit, and platelet-derived growth factor receptors.[1] Pazopanib has dose-dependent inhibitory effects on cell proliferation, angiogenesis, and modest growth inhibition of xenografts derived from pediatric cancers.[1-3] Pazopanib is approved by the US Food and Drug Administration for the treatment of advanced renal cell carcinoma and soft tissue sarcoma (STS) in adults.[4-6] This multicenter clinical trial was performed to determine the maximum-tolerated dose (MTD) of pazopanib administered as a tablet (part 1) or suspension (part 2a) to children with cancer; to define and describe the toxicities, pharmacodynamics, and pharmacokinetics (PK) of the two formulations; and to explore pazopanib-induced changes in tumor blood volume and vascular permeability (K_i) using dynamic contrast-enhanced (DCE) magnetic resonance imaging (MRI) in a subset of patients with STS (part 2b).

PATIENTS AND METHODS

Study Participants

Patients ≥ 2 and less than 22 years of age with recurrent or refractory solid or primary CNS tumors were eligible. Patients with CNS involvement could not have new or ≥ three foci of punctate hemorrhage on baseline MRI. Patients in part 2b (DCE-MRI cohort) had a diagnosis of STS, did not require anesthesia for imaging, and were younger than age 25 years. Other eligibility criteria included the following: recovery from prior therapy; performance status ≥ 50; adequate baseline renal, hepatic, and hematologic function; normal blood pressure (BP) without antihypertensive agents; adequate cardiac function; QTc less than 450 milliseconds; and stable thyroxine supplementation if hypothyroid. Exclusion criteria included the following: active bleeding, intratumoral hemorrhage, or bleeding diathesis; history of thromboembolic events; treatment with antiplatelet or antithrombotic agents; recent or planned major surgery; impaired wound healing; abdominal fistula, perforation, or abscess; uncontrolled infection; pregnancy or lactation; treatment with strong CYP3A4 inhibitors or QTc-prolonging drugs; and concurrent use of other investigational or anticancer agents.

The trial was approved by the institutional review boards of participating sites. Written informed consent and assent were obtained in accordance with federal and institutional guidelines.

Drug Administration

Pazopanib was administered orally once daily without interruption on an empty stomach with clear liquids in 28-day cycles for a maximum of 24 cycles. Drug was supplied by GlaxoSmithKline and distributed by the Cancer Therapy Evaluation Program (National Cancer Institute, Bethesda, MD) as aqueous film-coated tablets (50, 200, or 400 mg) or in 5-g bottles. The starting tablet dose (part 1) was 275 mg/m^2, with planned escalations to 350, 450, and 600 mg/m^2. Prescribed doses were rounded to the nearest 50-mg tablet by body-surface area using a nomogram. Suspension (50 mg/mL; part 2a) was prepared by adding 90 mL of Ora-Sweet (Paddock Laboratories, Minneapolis, MN) to a 5-g bottle of pazopanib powder. Dose escalation was initiated at 50% of the tablet MTD, based on adult data for suspension showing increased bioavailability.[7] Patients in part 2b received whole tablets at the MTD (determined in part 1).

Toxicity Assessment and Disease Evaluations

Physical examinations, BP monitoring, and routine safety laboratory studies were performed weekly during cycle 1 and then biweekly. Periodic thyroid-stimulating hormone levels were also obtained. Cardiac toxicity was assessed at baseline, before cycles 2 and 5, and every sixth cycle thereafter by echocardiography and electrocardiogram. Radiographic studies of the knee for those with open growth plates (physes) at study entry were obtained at similar intervals. Adverse events were graded according to the National Cancer

Table 1. Demographics and Clinical Characteristics of Eligible Patients

Characteristic	No. of Patients			
	Dose Escalation, Part 1 (n = 25)	Suspension, Part 2a (n = 16)	Imaging, Part 2b (n = 10)	Total (N = 51)
Age, years				
Median	13.4	10.5	17.2	12.9
Range	5.0-21.7	3.8-19.2	8.3-23.9	3.8-23.9
Sex				
Male	13	8	5	26
Female	12	8	5	25
Prior chemotherapy regimens, No.				
Median	1	2	3	2
Range	0-7	1-15	1-6	0-15
Prior radiation therapy	18	14	7	39
Prior VEGF-blocking therapy	7	5	3	15
Diagnosis				
Sarcoma				28
Rhabdomyosarcoma		1	4	5
Osteosarcoma	1	3		4
Synovial sarcoma	3	1		4
Ewing sarcoma	2	1		3
Alveolar soft part sarcoma	2		1	3
Clear cell sarcoma			2	2
Desmoplastic small round cell		1	1	2
Other soft tissue sarcoma		3	2	5
Brain tumor				17
High-grade glioma	3	3		6
Ependymoma	3	1		4
Low-grade glioma	2			2
Germ cell	2			2
Medulloblastoma/PNET	2			2
Atypical teratoid/rhabdoid		1		1
Embryonal				3
Hepatoblastoma	1	1		2
Wilms tumor	1			1
Other				3
Melanoma	2			2
Renal cell	1			1

Abbreviations: PNET, primitive neuroectodermal tumor; VEGF, vascular endothelial growth factor.

Table 2. Toxicity Related to Pazopanib Therapy

Toxicity Type	No. of Patients							
	Maximum Grade Observed per Patient During Cycle 1				Maximum Grade Observed per Patient Across All Subsequent Cycles			
	Grade 1	Grade 2	Grade 3	Grade 4	Grade 1	Grade 2	Grade 3	Grade 4
Tablet formulation (parts 1 and 2b)*								
GI/metabolic†								
Diarrhea	12	4			7	2 (1‡)		
Nausea	9	2			6	1		
Vomiting	9	3			5	2		
Abdominal pain	2	2			1	2		
Anorexia	2	2			1		1‡	
ALT increased	8	1			4	1‡	1‡	
AST increased	8				7	1		
Serum amylase increased	3	1	1‡		1	3		
Lipase increased	1	2		1‡	1	1	1‡	
Hyperglycemia	4				7	1		
Hypophosphatemia	2	1	1		2	4		
Constitutional†								
Fatigue	9	4			3	2		
Headache	6	5			1	3		
Dizziness	6							
Back/tumor pain	2	1	1‡				2‡	
Rash maculopapular/hand-foot	1	3			1	1	1‡	
Hematologic§								
Thrombocytopenia	8	1			11			
Absolute lymphocyte count	5		1		5	7		
Absolute neutrophil count	3	5	1		3	6	4	1‡
Anemia	1	1			6	1	2 (1‡)	
MTKI class targeted†								
Proteinuria	5	2	1‡		8	1		
Hypertension	3	6	2‡		3	5		
Left ventricular systolic dysfunction	3	1			4	2		
Sinus bradycardia	4				3			
Hypothyroidism	4	1			5	3		
Suspension formulation (part 2a)‖								
GI/metabolic†								
Diarrhea	4				2			
Nausea	3	1			2			
Vomiting	3	1						
Abdominal pain	2							
ALT increased	3		2‡		2	1		
AST increased	2	1			2			
Constitutional†								
Fatigue	2	1			1			
Headache	2				1	1		
Hematologic§								
Thrombocytopenia	3							
Absolute lymphocyte count	2		2		1			
Absolute neutrophil count	2	1	1		1			
Anemia	1				1	1		
MTKI class targeted†								
Proteinuria	4				2			

Abbreviation: MTKI, multitargeted tyrosine kinase receptor inhibitor.
*Cycle 1, n = 33 cycles; cycles 2 to 23, n = 148 cycles.
†Nonhematologic toxicities related to protocol therapy that occurred in more than 10% of patients as determined in the first cycle of protocol therapy.
‡Dose-limiting toxicity.
§Hematologic toxicities independent of frequency and attribution.
‖Cycle 1, n = 15 cycles; cycles 2 to 8, n = 22 cycles.

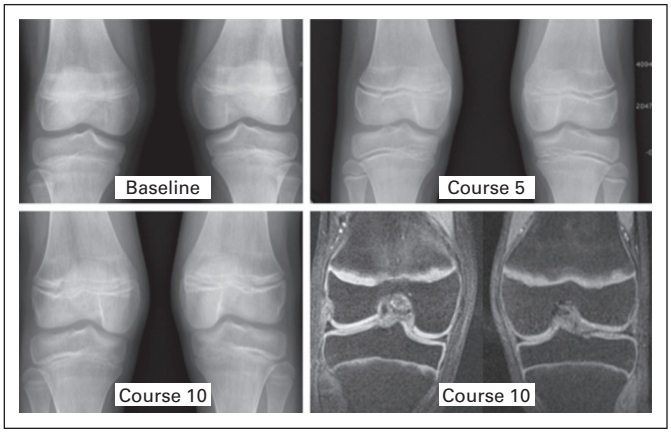

Fig 1. Growth plate expansion in an 11-year-old patient with alveolar parameningeal rhabdomyosarcoma treated with pazopanib for 10 cycles.

Institute Common Terminology Criteria for Adverse Events, version 4 (http://evs.nci.nih.gov/ftp1/CTCAE). Tumor response was evaluated using RECIST or two-dimensional measurement for CNS tumors. All patients with an objective response or stable disease for ≥ 6 months had central radiographic review.

Definitions of Dose-Limiting Toxicity and MTD

Hematologic dose-limiting toxicity (DLT) was defined as grade 4 neutropenia, grade 4 thrombocytopenia, or myelosuppression causing treatment delay of more than 14 days. Nonhematologic DLTs included any grade 4 toxicity; any grade 3 toxicity with exceptions of nausea and vomiting less than 3 days, fever or infection less than 5 days, and grade 3 electrolyte abnormalities responsive to oral supplementation; grade 2 arterial thrombosis; or grade 2 ALT in the presence of grade 2 hyperbilirubinemia (with > 35% direct). Dose-limiting hypertension was defined as grade 4 hypertension, confirmed systolic or diastolic BP more than 25 mmHg above the 95th percentile for age,[8] or an elevated BP not controlled by antihypertensive medication within 14 days, according to a previously described algorithm.[9] Cycle 1 toxicities were used for the purpose of dose escalations according to the rolling-six design.[10] Patients who developed a DLT or received at least 85% of the prescribed dose during cycle 1 were considered fully evaluable for toxicity. The MTD was defined as the highest dose level at which fewer than one third of patients experienced DLT.

PK

Blood for trough plasma concentrations was obtained before pazopanib administration on days 1, 15 (± 2), 22 (± 2), and 27 (± 1) of cycle 1, and before odd-numbered cycles. Extended PK sampling was optional in parts 1 and 2b but required in part 2a. For patients participating in full PK studies, plasma was obtained during cycle 1 before the pazopanib dose on days 1 and 15 or 22 (± 2) and at 30 minutes and 1, 2, 4, 6, 8, 10 to 12, and 24 (± 2) hours after

Table 3. Pazopanib Pharmacokinetic Parameters After Single and Repeated Daily Doses

Dose	No. of Patients*	T_{max} (hours)		C_{max} (μg/mL)		$AUC_{0\text{-}8\ h}$ (μg/mL · h)		$AUC_{0\text{-}24\ h}$ (μg/mL · h)		C_{ss}† (μg/mL)	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Tablet (part 1)											
275 mg/m^2											
Day 1	5	7.6	8.4	36.5	16.5	184	88	484	244	33.0	16.2
Day 15/22	5	2.2	1.1	48.9	13.2	309	58				
350 mg/m^2											
Day 1	2	3.5		34.7		199		511		30.8	19.2
Day 15/22	1	2.0		11.6		61.4					
450 mg/m^2											
Day 1	2	4.0		66.7		354		999		23.9	13.5
Day 15/22	2	3.5		59.8		394					
600 mg/m^2											
Day 1	3	6.1	2.0	23.0	8.4	314	253	832	496	42.8	18.8
Day 15/22	3	4.8	2.4	86.5	21.3	601	151				
Suspension (part 2a)											
160 mg/m^2											
Day 1	12	3.8	3.0	22.9	13.8	128	69	401	264	24.4	8.5
Day 15/22	10	3.4	2.6	40.0	13.5	262	94				
225 mg/m^2											
Day 1	4	3.0	2.0	30.8	14.1	181	80	419	200	28.4	11.5
Day 15/22	2	2.0		46.0		295					
Imaging (part 2b)											
450 mg/m^2											
Day 1	3	5.4	4.2	21.4	7.6	119	34	375	166	45.9	15.9
Day 15/22	3	2.0	1.7	45.9	20.0	331	144				

NOTE. Participation in extended pharmacokinetic studies was optional on parts 1 and 2b but required for part 2a. Participation in steady-state studies was mandatory for all patients.

Abbreviations: $AUC_{0\text{-}8\ h}$, area under the curve from 0 to 8 hours; $AUC_{0\text{-}24\ h}$, area under the curve from 0 to 24 hours; C_{max}, maximum serum concentration; C_{ss}, steady-state plasma trough concentration; SD, standard deviation; T_{max}, time to maximum serum concentration.

*Number of patients who participated in extended pharmacokinetic sampling.

†The C_{ss} column shows the mean and SD values of the trough concentrations across days 15 to 27 at each dose level. At least one trough value on day 15, 22, and/or 27 was available for 43 patients (31 patients had all three values). Specifically for C_{ss}, the numbers of patients were as follows: tablet 275 mg/m^2 (n = 6), 350 mg/m^2 (n = 6), 450 mg/m^2 (n = 5), and 600 mg/m^2 (n = 5); suspension 160 mg/m^2 (n = 11) and 225 mg/m^2 (n = 3); and imaging 450 mg/m^2 (n = 7).

dosing. Blood was collected into EDTA tubes and centrifuged at 2,500 × *g* at 5°C for 10 minutes; plasma was stored at −20°C. Pazopanib concentrations were measured using a validated liquid chromatography/tandem mass spectrometry method[11] (Covance Central Laboratory Services, Tarrytown, NY), and concentration-time data were analyzed via standard noncompartmental analysis methods using WINNonlin (Pharsight, Cary, NC). Half-life values were predicted by rearrangement of the equation: $R = \frac{1}{(1-e^{-(0.693\times\tau/t_{1/2})})}$, where R is the accumulation based on the area under the curve (AUC) from 0 to 8 hours ($AUC_{0\text{-}8\ hours}$) on day 15 or 22 versus day 1, $t_{1/2}$ is the elimination half-life, and τ is the dosing interval (24 hours). Associations between dose, PK variables, and toxicity were established using Pearson correlation coefficients and compared using the two-tailed *t* test.

Pharmacodynamic Studies

Angiogenic cytokines VEGF, soluble VEGFR-1, soluble VEGFR-2, endoglin (ENG), and placental growth factor (PlGF) were measured in plasma using commercially available enzyme-linked immunosorbent assay kits (R&D Systems, Minneapolis, MN) before dose on days 1 and 15 ± 2, coincident with PK trough sampling in consenting patients. Gene expression study samples were collected in PAXgene tubes (PreAnalytiX; Qiagen/BD, Hilden, Germany), gently inverted, and shipped on ice within 24 hours of collection together with whole blood (EDTA) for analysis of total, progenitor, and apoptotic circulating endothelial cells. Cells were immediately analyzed using previously described four-color flow cytometry methods.[12,13] cDNA preparation was carried out using First Strand cDNA Synthesis Kits for RT-PCR (Roche Diagnostics, Indianapolis, IN) and Superscript II (Invitrogen, Carlsbad, CA). Gene expression of *ENG*, *PlGF*, and reference gene B-actin (*ACTB*) was measured using specific primers and TaqMan probes. Changes in angiogenesis biomarkers were assessed using the paired *t* test for normally distributed data and the Wilcoxon signed rank test for non-normal data.

DCE-MRI

DCE-MRI scans were performed on non-necrotic target lesions (> 2 cm and not sensitive to motion artifact) at baseline and day 15 ± 2, coincident with PK trough sampling, using previously published methods.[14] DCE-MRI data sets were centrally reviewed and analyzed using a two-compartment kinetic model yielding estimates of fractional blood volume and the permeability transfer constant (K_i). A scaled K_i was used ($K_i \div$ voxel volume × 6,000) to correct for the different voxel sizes across patients and anatomic location of the target lesion.

RESULTS

Patient Characteristics

From July 2009 to May 2011, 53 patients were enrolled; two were ineligible as a result of inadequate documentation of baseline BP. Characteristics of the remaining 51 patients are listed in Table 1. Three patients were not evaluable for toxicity (early disease progression,

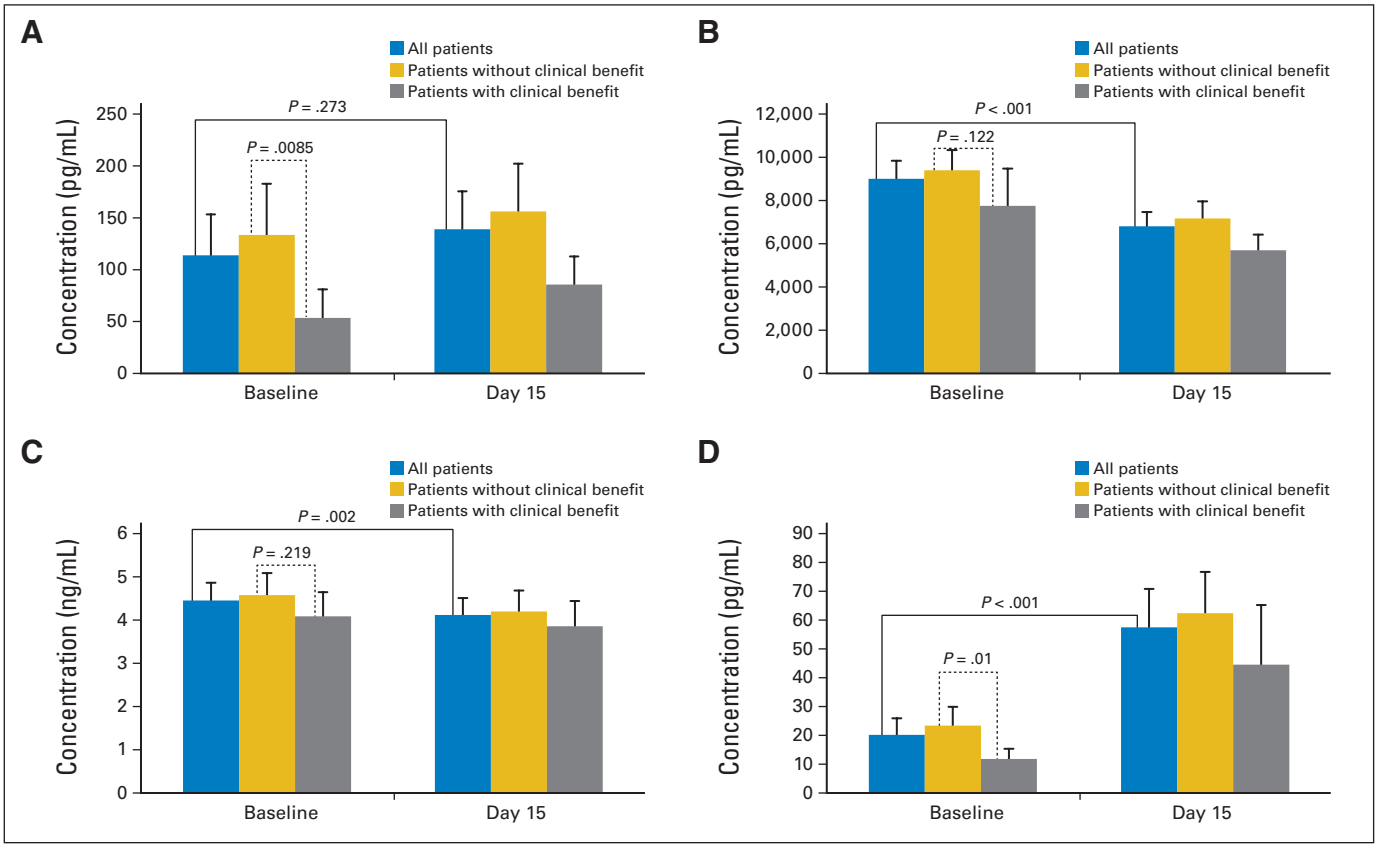

Fig 2. Plasma angiogenesis biomarkers and gene expression. Mean plasma levels (with SE) for (A) vascular endothelial growth factor (VEGF), (B) soluble VEGF receptor-2 (sVEGFR-2), (C) endoglin (ENG), and (D) placental growth factor (PlGF) on days 1 and 15 are displayed for all patients (blue) and those without (yellow) and with (gray) clinical benefit (partial response or stable disease > 6 months). Paired analysis of individual change from baseline to day 15 after pazopanib therapy demonstrated significant decreases in sVEGFR-2 and ENG (n = 41; $P < .001$ and $P = .002$, respectively) and significant increases in plasma PlGF (n = 35; $P < .001$) by two-sided *t* test. Patients deriving clinical benefit from pazopanib had lower baseline mean VEGF and PlGF ($P = .0085$ and $P = .01$, respectively). Statistically significant (E) decreases in relative *ENG* (β-actin; n = 35; $P < .001$) and (F) increases in relative *PlGF* (n = 35; $P = .021$) gene expression in peripheral-blood mononuclear cells were demonstrated from baseline to day 15.

n = 2; noncompliance with supportive care recommendations, n = 1).

Dose Escalation and Toxicity

The escalation schema, toxicity by dose level, and DLTs are listed in Appendix Tables A1 to A3 (online only). Toxicities attributable to pazopanib during all cycles are listed in Table 2. Cycle 1 DLTs included grade 3 or 4 lipase, grade 3 amylase and ALT elevations, grade 3 proteinuria, and hypertension. One patient with occult brain metastasis had grade 4 intracranial hemorrhage. Because two of five patients enrolled at 600 mg/m^2 experienced DLTs and only one of five patients at the prior dose level experienced DLT, the MTD for pazopanib tablets was determined to be 450 mg/m^2. Dose escalation for suspension was initiated at 225 mg/m^2. Two of four patients receiving suspension experienced grade 3 ALT. Interim PK data did not suggest marked differences, but the dose was de-escalated according to study design. There were no DLTs among the first six patients entered at 160 mg/m^2, which was defined as the suspension MTD.

Twenty patients required dose modification (10 patients during cycle 1, five during cycles 2 and 3, and five during cycles 7 to 17). Twelve patients were removed from protocol therapy for toxicity (Appendix Tables A2 and A3). Toxicities requiring dose modification and/or discontinuation in later cycles included tumor pain (n = 2), ALT elevation (n = 2), diarrhea, myalgia associated with microangiopathic hemolytic anemia, hand-foot syndrome, weight loss, fistula formation at a previously irradiated site, growth plate abnormality, and recurrent neutropenia (each n = 1). Three of 27 patients treated at the MTD had asymptomatic, reversible decreases in left ventricular systolic function, and pazopanib was successfully reintroduced in two patients. Asymptomatic sinus bradycardia occurred in four patients, all ≥ 12 years old. No QTc prolongation was observed.

Serial knee radiographs were obtained and centrally reviewed in 25 patients with open physes (median age, 11 years; range, 4 to 17 years). One 11-year-old, Tanner II patient had progressive physeal widening, with a three-fold expansion by cycle 10, confirmed by MRI (Fig 1), and a decrease in height velocity. Pazopanib was discontinued but resumed compassionately by the treating physician, as a result of rapid tumor regrowth. In addition, three patients ages 11, 8, and 4 years each had growth plate widening radiographically after four cycles but were removed as a result of progressive disease. The remaining 21 patients had normal radiographs, including three patients receiving 8, 12, and 13 cycles.

PK

Pazopanib PKs were characterized on day 1 and at steady-state (day 15 or 22) in 31 patients (Table 3; Appendix Table A4, online

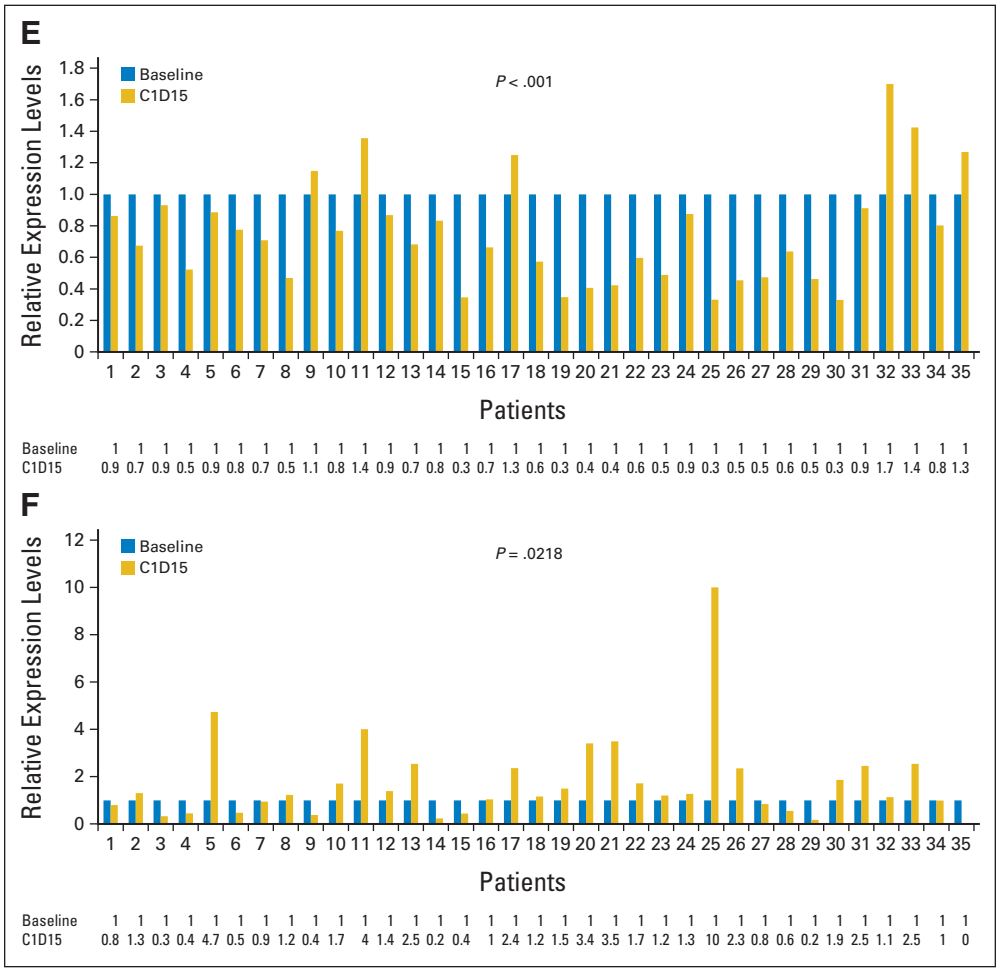

Fig 2. Continued.

only). There was marked interpatient variability in maximum serum concentration (C_{max}) and $AUC_{0\text{-}24\ hours}$ (Appendix Figures A1A and A1B, respectively, online only), resulting in substantial overlap between dose levels. $AUC_{0\text{-}8\ hours}$ at steady-state compared with that following the day 1 dose revealed that pazopanib accumulated over time. A mean accumulation factor of 2.3 was calculated, consistent with an estimated half-life of 29.6 hours. Comparison of mean $AUC_{0\text{-}24\ hours}$ normalized to a dose of 275 mg/m^2 between patients who received suspension (639 $\pm$ 403 μg/mL $\cdot$ h; n = 14) versus tablet (418 $\pm$ 297 μg/mL$\cdot$h; n = 15) did not demonstrate statistically significant greater bioavailability for suspension (P = .1). The median body-surface area–adjusted apparent oral clearance (0.31 L/h/m^2) was identical for children $\leq$ 12 years old (0.03 to 5.07 L/h/m^2) compared with children more than 12 years old (0.17 to 0.65 L/h/m^2), although the variability was greater in younger patients (standard deviation, 1.20 *v* 0.15 L/h/m^2, respectively). There was a strong association between cycle 1 DLT and $AUC_{0\text{-}24\ hours}$ (n = 29; r = 0.595; P = .001); mean pazopanib $AUC_{0\text{-}24\ hours}$ was significantly higher for patients with DLT (n = 7) compared with those without DLT (896 *v* 367 μg/mL $\cdot$ h, respectively; P = .039).

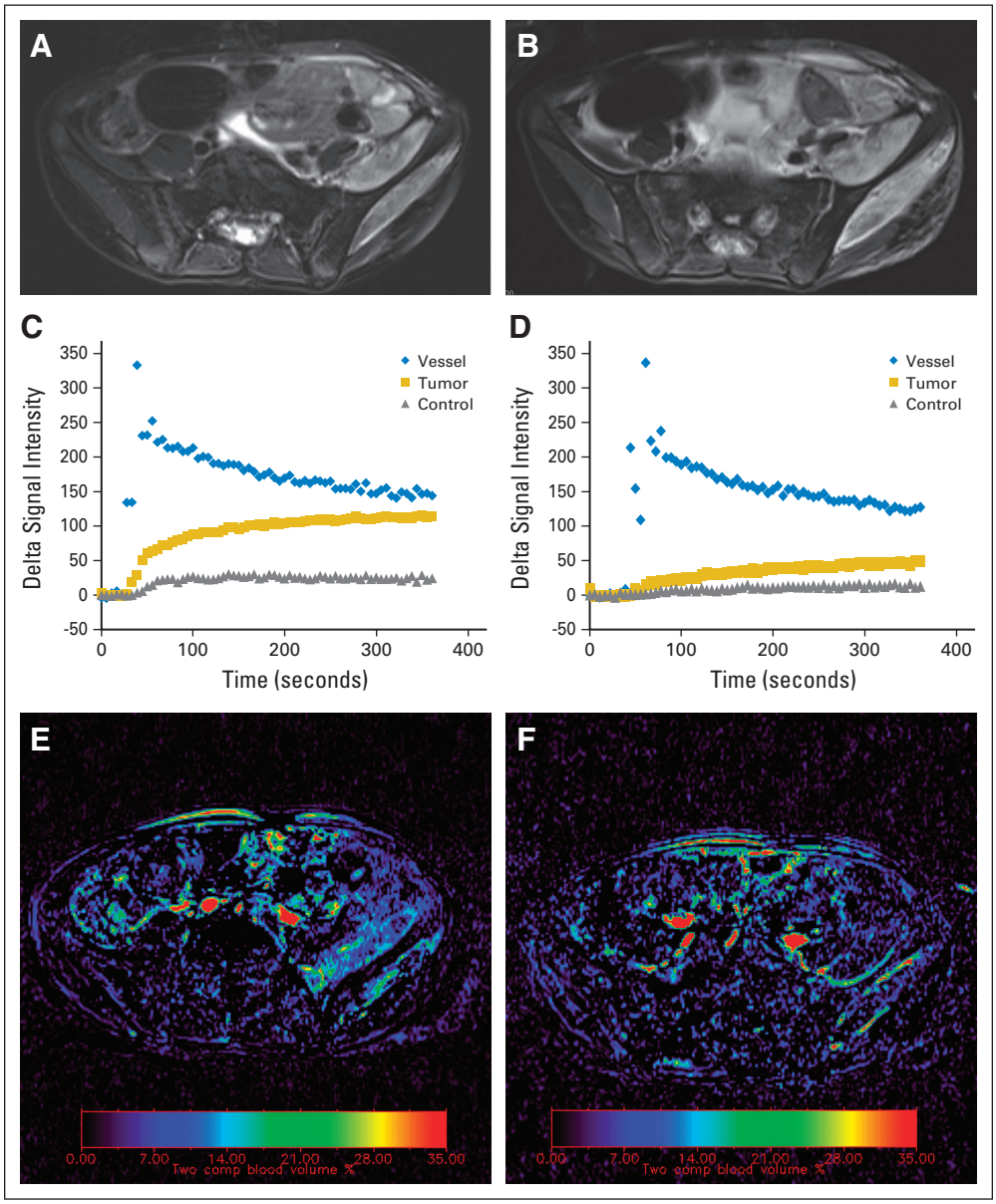

Fig 3. Dynamic contrast-enhanced T1-weighted acquisition was performed during quiet breathing over 5 to 6 minutes, with repeated imaging before, during, and after bolus administration of a single dose of gadopentetic acid (0.1 mmol/kg), at a rate of 1 cm^3/sec, followed by a 20-mL saline flush. Sequence parameters included dynamic, three-dimensional, T1-weighted gradient echo (FLASH, SPGR, VIBE, LAVA, or FFE) with minimum repetition time, minimum echo time, flip angle of 30 degrees, and slice thickness of 4 to 8 mm depending on anatomy. Representative imaging findings at (A, C, E) baseline and (B, D, G) day 15 in a patient with clear cell sarcoma and metastasis surrounding the left iliac bone and involving the left ilacus muscle anteriorly and the gluteus medius muscle posteriorly demonstrate no appreciable change on a standard fat-saturated T2 sequence. (C and D) The same patient shows significant decrease in enhancement on the delta signal intensity curve (graphs represent region of interest time curves for vessel, tumor, and adjacent normal tissue) and (E and F) the blood volume maps from the area of the lesions. All patients experienced a decrease in (G) fractional tumor blood volume (P = .004), (H) vascular permeability (K_i; P = .021), and (I) scaled K_i (P = .006).

A dose-dependent increase in pazopanib steady-state plasma trough concentration (C_{ss}) was not observed over the dose ranges administered (Table 3). Steady-state concentrations were reached by day 15 and exceeded 20 μg/mL at all dose levels (Appendix Fig A1C). The mean C_{ss} in patients with drug-related grade 2 or 3 hypertension after a median of two cycles (range, one to five cycles) was 43.7 ± 13.3 μg/mL (n = 11) versus 29.4 ± 13.0 μg/mL in normotensive patients (n = 27; $P = .004$), suggesting a significant relationship between BP and C_{ss}. Mean C_{ss} was also higher in patients who experienced any DLT across all cycles versus those who did not (38.8 ± 11.1 *v* 29.6 ± 13.6 μg/mL, respectively; $P = .04$).

Response Evaluation

One patient with desmoplastic small round cell tumor achieved a sustained partial response (PR) after 14 cycles and completed 24 cycles of protocol therapy. Another patient with hepatoblastoma had a PR by cycle 4, maintained for 12 cycles, but was removed from study for recurrent neutropenia. Eight patients had stable disease for ≥ 6 months (alveolar soft part sarcoma, n = 2; synovial sarcoma, osteosarcoma, alveolar rhabdomyosarcoma, mesenchymal chondrosarcoma, gastrointestinal stromal sarcoma, and myxopapillary ependymoma, n = 1 each). All but one patient with clinical benefit had C_{ss} more than 20 μg/mL, and all five patients who received therapy for a year or more had $C_{ss} \geq 30$ μg/mL.

Blood Biomarkers

Paired samples for plasma biomarker analysis were available for 41 patients (Figs 2A to 2D). Plasma soluble VEGFR-2 (sVEGFR-2) and ENG decreased significantly in patients receiving pazopanib ($P < .001$ and $P = .002$, respectively). Change from baseline to day 15 ± 2 in sVEGFR-2, coincident with PK trough sampling, was strongly correlated with the day 15 trough concentration ($r = 0.515$, $P = .002$). PlGF increased significantly over the course of the first cycle of therapy (n = 35; $P < .001$). VEGF and sVEGFR1 did not significantly change with pazopanib therapy. Gene expression analysis of peripheral-blood mononuclear cells (n = 35) confirmed the enzyme-linked immunosorbent assay findings, demonstrating a 0.75-fold decrease from baseline in the mean relative expression level of *ENG* ($P = .001$) and a 3.12-fold increase in the mean relative expression level of *PlGF* ($P = .02$; Figs 2E and 2F). Patients with clinical benefit from pazopanib had lower baseline plasma VEGF and PlGF ($P = .0085$ and $P = .01$, respectively). Paired samples for total and subsets of circulating endothelial cells (n = 33) did not reliably change with therapy or correlate with clinical benefit (data not shown).

Functional Imaging

Eight of 10 patients on the imaging strata had interpretable DCE-MRI (one baseline dynamic scan not performed; one technical failure). The mean C_{ss} for these patients was 45.9 μg/mL (range, 29.7 to 66.7 μg/mL). Representative data from a patient with clear cell sarcoma and metastasis infiltrating the iliacus and gluteus medius muscles are presented in Figures 3A to 3F. All patients with evaluable DCE-MRI experienced a decrease in fractional tumor blood volume after initiation of pazopanib, with a mean pretreatment level of 16% (range, 1% to 29%) versus 7% (range, 0% to 15%) after treatment ($P = .004$). All patients also had a decrease in scaled K_i, with mean pretreatment values of 7.75 mL/100 g/min (range, 2.32 to 20.39 mL/100 g/min) decreasing after treatment to 4.28 mL/100 g/min (range, 0.19 to 12.60 mL/100 g/min; $P = .006$; Figs 3G to 3I).

DISCUSSION

Pazopanib administered daily to children and adolescents with STS and other refractory solid tumors was generally well tolerated with common adverse effects including diarrhea, nausea, vomiting, fatigue, proteinuria, and hypertension. An MTD of 450 mg/m^2 was determined for the tablet formulation, comparable to the adult recommended flat dose of 800 mg. At this dose, the median day 1 C_{max} and $AUC_{0\text{-}24\ hours}$ values for children were 21.5 μg/mL and 377.6 μg/mL · h, respectively, similar to the mean day 1 C_{max} and $AUC_{0\text{-}24\ hours}$ values of 19.4 μg/mL and 275.1 μg/mL · hr, respectively, for adults treated at the equivalent dose.[15] Dose-proportional increases in AUC and C_{ss} were not demonstrated, which may be a result of the wide age range of children enrolled onto this study, the large variability in oral clearance for children ≤ 12 years old, the effect of food on absorption,[16] or saturable absorption. Overall, toxicity seemed to correlate with exposure rather than dose. Adults receiving suspension demonstrated exposures of approximately 133% that of tablet.[7] Similarly, pazopanib bioavailability in children seemed to be higher with the suspension, but the difference was not statistically significant, most likely because of the limited data and broad range in AUC values among patients.

Preclinical models suggest that optimal in vivo antitumor and antiangiogenic activity occurs when total pazopanib C_{ss} exceeds 17.5

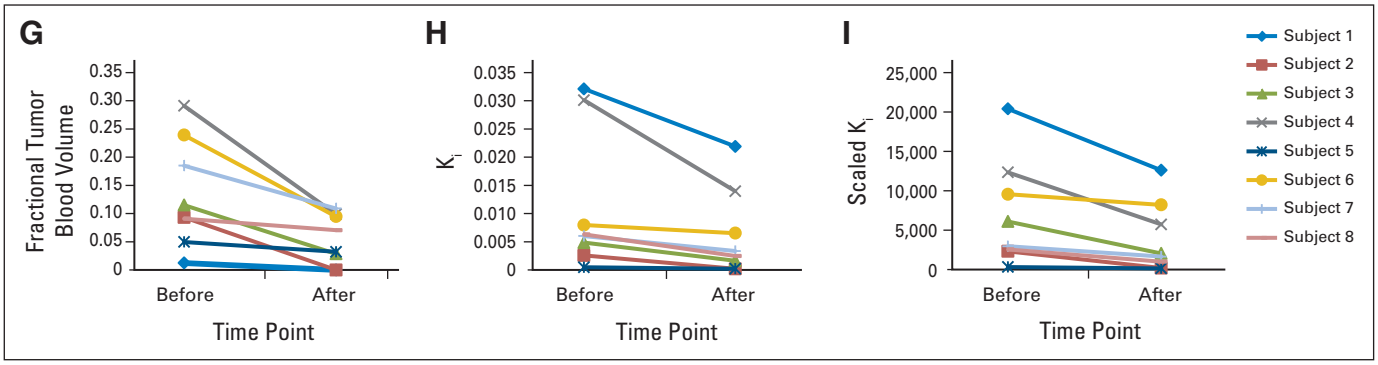

Fig 3. Continued.

μg/mL (40 μmol/L).[1] On the basis of its protein binding of 99.9%, this level should yield free pazopanib concentrations that exceed the half maximal inhibitory concentration of 0.02 μmol/L for VEGFR kinase–induced cell proliferation in vitro.[17] In our study, this C_{ss} was achieved in the majority of patients at all dose levels. In a phase II study, the threshold C_{ss} demonstrating efficacy in adult patients with renal cell carcinoma was $\geq$ 20 μg/mL.[15,18] With one exception, the threshold for clinical benefit in our pediatric study was also 20 μg/mL, and 30 μg/mL for those with disease stabilization over a year. Given interpatient PK variability, future studies might consider individually dosing pazopanib to achieve target C_{ss}. The incidence of hypertension, a mechanism-based toxicity and pharmacodynamic marker of effective VEGF inhibition, could also be correlated with higher mean C_{ss}. In adults, elevated BP has been suggested as a correlative marker for improved antitumor efficacy of VEGF pathway inhibitors.[19-21] Additional studies are warranted to determine whether hypertension can be used to optimize dose or predict clinical benefit in children.

The best biomarker of antiangiogenic effect remains unknown. We demonstrate that pazopanib therapy is associated with significant changes in sVEGFR-2, ENG, and PlGF, and for the latter two, this is accompanied by changes in host angiogenic cytokine expression. Similarly, pazopanib-treated adults with non–small-cell lung cancer showed decreases in sVEGFR-2 and increases in PlGF, and it was suggested that post-treatment changes in plasma sVEGFR-2 could be significantly correlated with tumor shrinkage.[22] In our study, degree of change in sVEGFR-2 was associated with C_{ss} but was not predictive of efficacy. Lower baseline levels of VEGF and PlGF did correlate with prolonged stable disease.

Vascular and permeability changes on DCE-MRI have gained acceptance as pharmacodynamic markers, but their value as predictors of clinical outcome have not been established.[23,24] To our knowledge, this is the first pediatric, multicenter trial to systematically evaluate DCE-MRI in STS, demonstrating the feasibility of performing such studies in a clinical trial network. Within the imaging stratum, all patients with interpretable studies had a decrease in tumor blood volume and permeability consistent with the antiangiogenic mechanism of pazopanib. Because of the small cohort, no correlation with benefit could be made.

Our study is also the first, to our knowledge, to document physeal expansion in young patients with prolonged exposure to VEGF-inhibiting therapy. As a result of preclinical findings that VEGF blockade diminishes capillary invasion into the hypertrophic chondrocyte zone, impairs chondrocyte apoptosis, and reduces long bone growth velocity,[25] pediatric trials of angiogenic inhibitors have incorporated growth plate monitoring for physeal expansion.[9,13,26-29] Although the majority of these studies have shown no change, they have been limited by the short duration of therapy typically administered on phase I trials.[28] This finding highlights that ongoing growth plate monitoring remains warranted in patients who have not attained adult height.

The clinical activity of pazopanib, which included PRs in refractory desmoplastic small round cell tumor and hepatoblastoma and stable disease exceeding 6 months in another 16% of patients, is encouraging in this heavily pretreated pediatric population. A phase II trial to further investigate PK and pharmacodynamic relationships and define the spectrum of antitumor activity among pediatric tumors of mesenchymal origin, including STS, osteosarcoma, Ewing sarcoma, hepatoblastoma, and neuroblastoma, is in development.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Although all authors completed the disclosure declaration, the following author(s) and/or an author's immediate family member(s) indicated a financial or other interest that is relevant to the subject matter under consideration in this article. Certain relationships marked with a "U" are those for which no compensation was received; those relationships marked with a "C" were compensated. For a detailed description of the disclosure categories, or for more information about ASCO's conflict of interest policy, please refer to the Author Disclosure Declaration and the Disclosures of Potential Conflicts of Interest section in Information for Contributors.
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Final approval of manuscript: All authors

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Appendix

Table A1. Dose Escalation and DLT Summary

Formulation	Dose Level (mg/m^2)	No. of Patients Entered	No. of Patients Evaluable	No. of Patients With DLT	Type of DLT
Tablet					
Part 1	275	7	6	1	Grade 3/4 lipase (n = 1)
Part 1	350	6	6	0	
Part 1	450	7	6	1	Grade 3 hypertension (n = 1), grade 3 proteinuria (n = 1)
Part 2b	450	10	10	1	Grade 3 sensory neuropathy (n = 1), grade 3 back pain (n = 1), grade 3 extremity pain (n = 1)
Part 1	600	5	5	2	Grade 3 hypertension (n = 1), grade 3 amylase (n = 1)
Suspension					
Part 2a	160	12	11	1	Grade 4 CNS hemorrhage (n = 1)
Part 2a	225	4	4	2	Grade 3 ALT increase (n = 2)

Abbreviation: DLT, dose-limiting toxicity.

Table A2. Cycle 1 Toxicity by Dose Level

	Maximum Grade Observed per Patient by Dose Level During Course 1 (No. of patients)																							
	Tablet																Suspension							
	275 mg/m^2 (n = 6)				350 mg/m^2 (n = 6)				450 mg/m^2 (n = 16)				600 mg/m^2 (n = 5)				160 mg/m^2 (n = 11)				225 mg/m^2 (n = 4)			
Toxicity Type	Grade 1	Grade 2	Grade 3	Grade 4	Grade 1	Grade 2	Grade 3	Grade 4	Grade 1	Grade 2	Grade 3	Grade 4	Grade 1	Grade 2	Grade 3	Grade 4	Grade 1	Grade 2	Grade 3	Grade 4	Grade 1	Grade 2	Grade 3	Grade 4
GI/metabolic*																								
Diarrhea	4	2			2				6	1				1			3				1			
Nausea	2	1			1				6	1							3	1						
Vomiting	3	1			1				4	2			1				2	1			1			
Abdominal pain						1			2					1							2			
Anorexia									2	2														
ALT increased	1								5	1			2				2				1		2†‡	
AST increased	1				1				5				1				1				1	1		
Amylase increased	1				2									1	1†									
Lipase increased		1		1†‡	1					1														
Hyperglycemia									3				1											
Hypophosphatemia	1				1					1					1									
Constitutional*																								
Fatigue	4				1				4	2				2			2	1						
Headache	1	1			1	2			4	2							1				1			
Dizziness					3				3															
Back/tumor pain	1				1					1	1†													
Rash maculopapular	1	1								2														
Hematologic§																								
Thrombocytopenia	2				1				4	1			1				2				1			
ALC	1				2				2		1						2		2					
ANC	1	1			1				1	4	1						2	1					1	
Anemia									1	1							1							
MTKI class targeted*																								
Proteinuria	2								2	2	1†‡		1				3				1			
Hypertension		2			2	2			1	1	1†‡			1	1†									
LVSD	1				1				1	1														
Sinus bradycardia									4															
Hypothyroidism					1	1			2				1											
CNS bleed																				1†‡				

Abbreviations: ALC, absolute lymphocyte count; ANC, absolute neutrophil count; LVSD, left ventricular systolic dysfunction; MTKI, multitargeted tyrosine kinase receptor inhibitor.
*Nonhematologic toxicities related to protocol therapy that occurred in more than 10% of patients as determined in the first cycle of protocol therapy.
†Dose-limiting toxicity.
‡Required removal from protocol therapy.
§Hematologic toxicities independent of frequency and attribution.

Table A3. Later Toxicity by Dose Level

	Maximum Grade Observed per Patient by Dose Level During Cycles 2-23 (148 cycles; No. of patients)																							
	Tablet																Suspension							
	275 mg/m^2 (n = 3)				350 mg/m^2 (n = 5)				450 mg/m^2 (n = 13)				600 mg/m^2 (n = 3)				160 mg/m^2 (n = 7)				225 mg/m^2 (n = 1)			
Toxicity Type	Grade 1	Grade 2	Grade 3	Grade 4	Grade 1	Grade 2	Grade 3	Grade 4	Grade 1	Grade 2	Grade 3	Grade 4	Grade 1	Grade 2	Grade 3	Grade 4	Grade 1	Grade 2	Grade 3	Grade 4	Grade 1	Grade 2	Grade 3	Grade 4
GI/metabolic*																								
Diarrhea	1	1†‡							5	1			1				1				1			
Nausea	1								4	1			1				1				1			
Vomiting	1								2	2			2											
Abdominal pain	1					1								1										
Anorexia									1		1†													
ALT increased	1								2	1†	1†‡		1				2	1						
AST increased	1				1				3	1			2				1				1			
Amylase increased		1			1					1				1						1†				
Lipase increased									1	1	1†													
Hyperglycemia	1	1			2				4															
Hypophosphatemia	1					1				2			1	1										
Constitutional*																								
Fatigue					1				2	2											1			
Headache									1	2				1			1					1		
Dizziness																								
Back/tumor pain											1†													
Rash/hand-foot syndrome							1†‡		1	1														
Hematologic§																								
Thrombocytopenia	1				3				5				2											
ALC	1				1				3	5				2			1							
ANC				1†‡		1	1		2	4	3		1	1			1				1			
Anemia		1							5		1		1		1†‡		1	1				1		
MTKI class targeted*																								
Proteinuria	2				2				2	1			2				1				1			
Hypertension		1			1	3			1	1			1											
LVSD	1	1							1	1			2											
Sinus bradycardia									2				1											
Hypothyroidism						2			3				2	1										
Tissue necrosis																							1†‡	
Growth plate											1†‡													

Abbreviations: ALC, absolute lymphocyte count; ANC, absolute neutrophil count: LVSD, left ventricular systolic dysfunction; MTKI, multitargeted tyrosine kinase receptor inhibitor.
*Nonhematologic toxicities related to protocol therapy that occurred in more than 10% of patients as determined in the first cycle of protocol therapy.
†Dose-limiting toxicity.
‡Required removal from protocol therapy.
§Hematologic toxicities independent of frequency and attribution.

Table A4. Pazopanib Trough Plasma Concentrations After Single and Repeated Daily Doses

Formulation and Dose (mg/m^2)	Trough Plasma Concentration (μg/mL)							
	Day 2		Day 15		Day 22		Day 27	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Tablet (part 1)								
275	14.7	9.1	32.7	12.6	34.5	21.2	31.4	16.8
350	11.1	14.7	32.4	20.1	28.8	18.4	31.4	24.3
450	28.1	30.1	25.7	15.5	19.1	12.6	26.0	14.1
600	13.0	7.6	41.5	13.0	46.4	16.8	49.8	18.7
Suspension (part 2a)								
160	13.2	10.7	23.0	9.3	25.2	7.9	25.2	8.7
225	9.7	5.0	27.3	11.4	23.9		40.7	
Imaging (part 2b)								
450	13.0	7.6	41.5	13.0	46.4	16.8	49.8	18.7

NOTE. The numbers of patients with measurements were as follows: tablet 275 mg/m^2 level, day 2 (n = 4), day 15 (n = 6), day 22 (n = 6), and day 27 (n = 5); tablet 350 mg/m^2 level, day 2 (n = 2), day 15 (n = 6), day 22 (n = 6), and day 27 (n = 4); tablet 450 mg/m^2 level, day2 (n = 2), day 15 (n = 5), day 22 (n = 4), and day 27 (n = 5); tablet 600 mg/m^2 level, day 2 (n = 2), day 15 (n = 3), day 22 (n = 3), and day 27 (n = 3); suspension 160 mg/m^2 level, day 2 (n = 11), day 15 (n = 10), day 22 (n = 8), and day 27 (n = 8); suspension 225 mg/m^2 level, day 2 (n = 4), day 15 (n = 3), day 22 (n = 2), and day 27 (n = 1); imaging 450 mg/m^2 level, day 2 (n = 3), day 15 (n = 7), day 22 (n = 7), and day 27 (n = 7).

Abbreviation: SD, standard deviation.

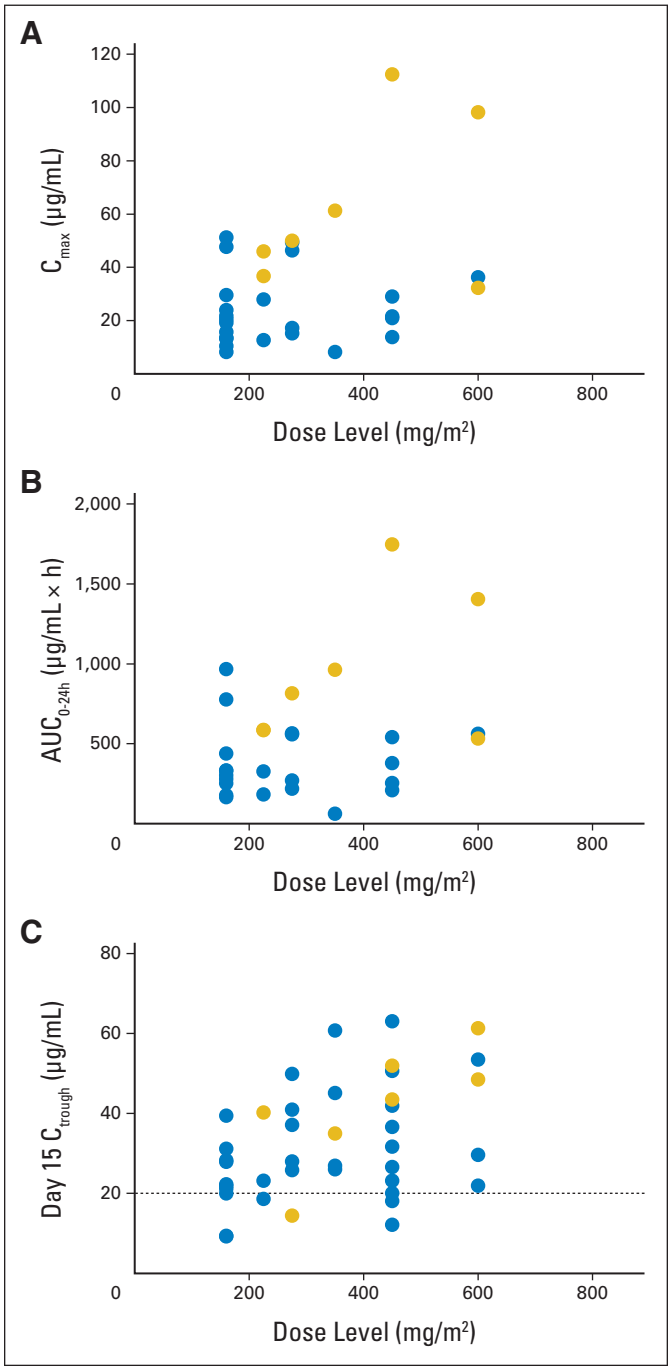

Fig A1. Pazopanib pharmacokinetics. (A) Maximum serum concentration (C_{max}). (B) Area under the curve from 0 to 24 hours (AUC_{0-24h}). (C) Day 15 trough concentration (C_{trough}). Gold circles represent patients who experienced dose-limiting toxicities, and blue circles represent patients who did not.