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Initial Testing of the Multitargeted Kinase Inhibitor Pazopanib by the Pediatric Preclinical Testing Program

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Abstract

Pazopanib is an oral angiogenesis inhibitor targeting VEGFR-1, -2, and -3, PDGFR- α , PDGFR- β , and KIT that has demonstrated activity against a variety of adult cancer xenografts. Pazopanib was tested against a panel of pediatric rhabdomyosarcoma and Ewing sarcoma xenografts at a dose of 108mg/kg/day or 100 mg/kg twice daily, administered orally for 28 days. While no objective responses were observed, pazopanib induced statistically significant differences in event-free survival compared to controls in approximately one-half of the sarcoma xenograft models tested. Though well tolerated, pazopanib showed limited activity against the sarcoma models evaluated, with the best tumor responses being growth delay.

Keywords

Preclinical Testing; Developmental Therapeutics; Pazopanib

INTRODUCTION

Pazopanib is a small molecule tyrosine kinase inhibitor. Its primary targets include vascular growth factor receptors (VEGFR-1, -2, and -3), platelet derived growth factor receptors (PDGFR- α , PDGFR- β), and KIT. Cell signaling pathways involving these molecules are important to the development and growth of blood vessels known as angiogenesis. It is widely accepted that angiogenesis plays a crucial role in the growth and spread of many cancer types, as the overexpression of VEGF and PDGF has been linked to multiple cancers including cancers of the liver, lung, breast, kidney, bladder, ovaries, and colon [1]. Consequently, the blockage of VEGF, PDGFR, and KIT may prevent tumor growth and inhibit angiogenesis thereby slowing or stopping the growth and spread of malignancies [2].

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Previous *in vivo* studies in mice demonstrated that pazopanib inhibits VEGF-induced VEGFR2 phosphorylation, tumor angiogenesis, and the growth of human tumor xenografts [3,4]. Kumar et al. evaluated the antitumor activity of pazopanib against a panel of human tumor xenografts (colon, melanoma, prostate, renal, breast and lung). Their results showed a clear dose dependent response for tumor growth inhibition, with pazopanib demonstrating significant activity in all 6 xenograft models tested with the renal cell cancer (RCC) xenograft being the most sensitive to pazopanib [3].

In Phase II studies pazopanib demonstrated monotherapy activity with response rates in the 30% to 40% range in patients with RCC and patients with thyroid cancer [5,6]. Lower response rates (< 10%) have been noted in phase II trials for breast cancer [7], cervical cancer [8], glioblastoma [9], and soft tissue sarcoma [10]. In a phase III trial, improvement in progression free survival and tumor response compared to placebo in treatment-naïve and cytokine-pretreated patients with advanced and/or metastatic RCC was observed [11]. Based on these results, in 2009 pazopanib was approved by the FDA for the treatment of advanced renal cell carcinoma (RCC). Pazopanib has progressed to phase 3 evaluations of other cancers, including inflammatory breast cancer, soft tissue sarcoma, and ovarian cancer [12]. The phase 3 trial for adults with previously treated metastatic soft tissue sarcoma observed that pazopanib significantly prolonged progression-free survival compared with placebo (4.6 vs. 1.5 months, respectively; hazard ratio [HR]: 0.31; $p < 0.0001$), with an interim analysis of overall survival showing a statistically non-significant improvement for pazopanib versus placebo (median: 11.9 versus 10.4 months, respectively) [13].

MATERIALS AND METHODS

In vivo tumor growth inhibition studies

CB17SC *scid*^{-/-} female mice (Taconic Farms, Germantown NY) were used to propagate subcutaneously implanted sarcomas (Ewing, rhabdomyosarcoma). Female mice were used irrespective of the gender from which the tumor sample was obtained. All mice were maintained under barrier conditions and experiments were conducted using protocols and conditions approved by the institutional animal care and use committee of the appropriate consortium member. Ten mice were used per group. Tumor volumes (cm³) determined as previously described [14]. Responses were determined using three activity measures as previously described [14]. An in-depth description of the analysis methods is included in the Supplemental Response Definitions section.

Plasma sample analysis for pazopanib following a dose of 100 mg/kg was performed using triplicate samples as previously described [3].

Statistical Methods

The exact log-rank test, as implemented using Proc StatXact for SAS®, was used to compare event-free survival distributions between treatment and control groups. P-values were two-sided and were not adjusted for multiple comparisons given the exploratory nature of the studies.

Drug Information and Formulation

GlaxoSmithKline, through the Cancer Therapy Evaluation Program (NCI), provided pazopanib to the Pediatric Preclinical Testing Program. Pazopanib was tested using a daily $\times$ 28 schedule with a planned two week observation period. The agent was administered by oral gavage at a dose of 108 mg/kg using a vehicle of 0.5% hydroxypropyl methyl cellulose plus 0.1% Polysorbate 80 (Tween 80). Subsequently, pazopanib was tested using a daily $\times$ 28 schedule at 100 mg/kg BID. Pazopanib was provided to each consortium investigator in coded vials for blinded testing.

RESULTS AND DISCUSSION

Pazopanib was tested against a subset of sarcoma models, with most previously showing tumor growth delay of two-fold or greater compared to control for cediranib (AZD2171) and sunitinib (Rh28 was the exception in showing EFS T/C < 2 for cediranib and sunitinib) [15,16]. Initial testing of pazopanib was done using a daily $\times$ 28 schedule with a planned two week observation period. Pazopanib was well tolerated at the dose and schedule evaluated, with no toxicity observed in either treated or control groups. All 7 xenograft models tested were considered evaluable. Complete details of testing are provided in Supplemental Table I, including total numbers of mice, number of mice that died (or were otherwise excluded), numbers of mice with events and average times to event, tumor growth delay, as well as numbers of responses and T/C values.

Pazopanib at the 108 mg/kg daily dosing schedule induced significant differences in EFS distribution compared to controls in 4 of 7 evaluable solid tumor xenografts tested as shown in Table I. However, none of the 7 tested xenografts had EFS T/C values exceeding 2, the minimum value required for “intermediate activity” for this efficacy measure. Objective responses were not observed for any of the sarcoma xenografts studied.

Because of the lack of toxicity observed at the dose and schedule initially studied, pazopanib was further evaluated using the same 28-day duration of treatment but at approximately twice the daily dose (100 mg/kg BID). Complete details of testing are provided in Supplemental Table II. Greater toxicity was observed using the twice-daily schedule, with a toxic mortality rate in the treated group of 4.5% versus 0% for the vehicle-treated control group. Pazopanib significantly increased event-free survival in only two sarcoma models in the initial daily testing (SK-NEP-1, Rh41), Table I. Testing pazopanib at 100 mg/kg BID initially showed significant growth delay for Rh18 tumors only. The SK-NEP-1 and Rh30 studies were repeated at 100 mg/kg BID, and in these experiments pazopanib induced significant growth delay with EFS T/C values of 2.2 and 2.4, respectively.

The activity observed for pazopanib by the PPTP is less than that described previously by Kumar et al against a variety of adult cancer xenografts using a dose of 100 mg/kg administered either daily or twice-daily [3]. Other multi-targeted kinase inhibitors with similar spectrum of inhibition to pazopanib [cediranib (VEGFR1-3, KIT), sunitinib (VEGFR1-3, PDGFR, FLT3, KIT) and sorafenib (VEGFR1-3, PDGFR, FLT3, RET, BRAF, KIT)] were tested against the same pediatric sarcoma models used to evaluate pazopanib [15–17]. Cediranib and sunitinib, in these experiments, each produced a somewhat greater

delay in time to event compared to pazopanib. The level of tumor growth inhibition for pazopanib was similar to that previously reported for sorafenib for these same models [17].

One potential explanation for the relatively modest level of tumor growth inhibition observed for pazopanib is the failure to achieve expected systemic exposures in the SCID mice utilized for testing. To rule out the possibility that pazopanib drug levels were inappropriately low, pharmacokinetic characterization of pazopanib in non-tumored SCID mice was performed. Drug levels in excess of 100 μ M were maintained for up to 8 hours after dosing, before dropping to <10 μ M at 24 hours (Table II). A plasma concentration of 40 μ M has been associated with effective VEGF receptor inhibition in mouse models [3], and 800 mg daily or 300 mg BID dosing in patients achieved trough levels 40 μ M [3]. Thus, while daily administration of pazopanib at 100 mg/kg would not produce continuous VEGF receptor inhibition, twice-daily administration should provide near continuous VEGF receptor inhibition.

Pazopanib has entered clinical evaluation in children with cancer, with a pediatric phase 1 trial showing that children tolerate doses (adjusted for body surface area) similar to those tolerated in adults and that pazopanib has a similar toxicity profile in children as in adults [18]. A partial response was noted in a patient with hepatoblastoma, and several patients with various types of sarcomas showed stable disease persisting for 6 months or more. A phase 2 study of pazopanib that is in development will allow the single agent activity of pazopanib to be determined for multiple types of pediatric solid tumors. Further pediatric development of pazopanib will depend in part on the results from the phase 2 study.

In conclusion, pazopanib induced modest tumor growth inhibition for a series of sarcoma xenografts. The level of tumor growth inhibition was no better than those achieved for other VEGFR2 targeted agents studied by the PPTP and in some cases was nominally less for pazopanib [15–17]. For the pediatric preclinical sarcoma models that the PPTP has evaluated against small molecule VEGFR2 inhibitors, tumor regression is uncommon and the best response is tumor growth delay.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Antitumor activity of pazopanib administered at 108 mg/kg daily (no shading) or 100 mg/kg twice daily (gray shading) against *in vivo* models of solid tumors

Table 1

Xenograft Line	Histology	EFS T/C	P-value	Median Final RTV	T/C	P-value	T/C Activity	EFS Activity	EFS Activity	Response
SK-NEP-1	Ewing	1.8	<0.001	>4	0.45	0.005	Int	Low	Low	PD2
EW8	Ewing	1.2	0.003	>4	0.63	0.005	Low	Low	Low	PDI
Rh28	ALV RMS	0.8	0.943	>4	1.10	0.393	Low	Low	Low	PDI
Rh30	ALV RMS	1.9	0.188	>4	0.79	0.684	Low	Low	Low	PD2
Rh30R	ALV RMS	1.6	<0.001	>4	0.48	<0.001	Low	Low	Low	PD2
Rh41	ALV RMS	1.1	0.374	>4	0.93	0.912	Low	Low	Low	PDI
Rh18	EMB RMS	1.4	0.041	>4	0.84	0.034	Low	Low	Low	PDI
SK-NEP-1	Ewing	1.4	0.012	>4	0.56	0.004	Low	Low	Low	PDI
SK-NEP-1 *	Ewing	2.2	<0.001	>4	0.55	0.023	Low	Int	Int	PD2
EW8	Ewing	1.3	0.084	>4	0.54	0.029	Low	Low	Low	PDI
Rh28	ALV RMS	1.3	0.167	>4	0.84	0.481	Low	Low	Low	PDI
Rh30	ALV RMS	1.0	0.485	>4	0.97	0.829	Low	Low	Low	PDI
Rh30*	ALV RMS	2.4	<0.001	>4	0.54	0.015	Int	Int	Int	PD2
Rh30R	ALV RMS	1.1	0.338	>4	0.77	1.00	Low	Low	Low	PDI
Rh41	ALV RMS	1.5	<0.001	>4	0.51	<0.001	Low	Low	Low	PDI
Rh18	EMB RMS	1.3	0.051	>4	0.63	0.022	Low	Low	Low	PDI

* Repeat experiment

Table II

Pazopanib drug levels following a 100 mg dose of pazopanib.

Hour	Pazopanib (μ M)	Standard Deviation (μ M)
1	111	34.3
2	139	16.0
4	112	8.0
8	112	20.5
24	16	1.3