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Combination Testing of Cediranib (AZD2171) against Childhood Cancer Models by the Pediatric Preclinical Testing Program

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Abstract

Background—Cediranib (AZD2171) is a potent small molecule inhibitor of vascular endothelial growth factor VEGF receptors. Cediranib has demonstrated single agent activity in several adult cancers and is being studied in combination with standard cytotoxic agents in multiple disease settings.

Procedures—Cediranib was tested *in vivo* against six childhood tumor xenograft models (4 sarcomas, 1 glioblastoma, 1 neuroblastoma) alone or combined with cyclophosphamide (2 models), vincristine (3 models) or cisplatin (1 model), each administered at its maximum tolerated dose, or rapamycin (6 models).

Results—The combination of cediranib with standard cytotoxic agents was superior to the cytotoxic agent used alone for a single xenograft (one of three xenografts evaluated for the vincristine-cediranib combination). The cediranib-cyclophosphamide combination was inferior to single agent cyclophosphamide in time to event for both models studied and was significantly inferior for one of the models. Cediranib combined with rapamycin was superior to each of the agents used alone in 2 of 6 models and was determined to be additive or supra-additive with rapamycin in 4 models, although the effects were not large.

Conclusions—Cediranib combined with cytotoxic chemotherapy agents demonstrated little or no benefit (and in one case was significantly inferior) compared to chemotherapy alone for the six pediatric cancer xenografts studied. By contrast, the combination of cediranib with rapamycin was additive or supra-additive in 4 of 6 models in terms of prolongation of time to event, though tumor regressions were not observed for this combination.

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Keywords

Preclinical Testing; Developmental Therapeutics; Cediranib; AZD2171

INTRODUCTION

Cediranib (AZD2171) was developed as a specific inhibitor of the VEGF receptor (VEGFR) family of molecules. While cediranib abrogates VEGFR-1 (FLT1), VEGFR-2 (KDR) and VEGFR-3 (FLT4) kinase activity at low nanomolar concentrations, it also inhibits KIT at similar concentrations [1]. Cediranib has shown broad spectrum activity against a variety of adult human malignancies in preclinical xenograft models through an antiangiogenic mechanism [1].

Cediranib was the first antiangiogenic agent evaluated by the Pediatric Preclinical Testing program (PPTP). It was screened for antitumor activity against the PPTP's models to help establish priorities for additional preclinical testing and for clinical evaluations of cediranib against pediatric cancers [2]. As a single agent cediranib demonstrated largely cytostatic activity causing tumor growth to slow, induced infrequent regressions in solid tumors, and was without significant activity against leukemia models.

Antiangiogenic therapy may contribute to the treatment of pediatric malignancies not only through slowing the rate of tumor growth or through inhibiting metastases, but also through normalization of the tumor vasculature [3]. Such normalization may impact positively on distribution of standard chemotherapeutic agents into the tumor mass [4,5]. Indeed, combinations of angiogenesis inhibitors or vascular disrupting agents with cytotoxic agents have been reported to be synergistic in certain preclinical models [6,7].

Cediranib is currently being investigated in multiple tumor types, both as monotherapy and in combination with chemotherapy [8–17]. Phase 1 evaluations of cediranib in children have been initiated. Here we have evaluated the interaction between cediranib and cytotoxic agents used frequently in the treatment of childhood solid tumors.

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, osteosarcoma, rhabdomyosarcoma), neuroblastoma, and non-glioblastoma brain tumors, while BALB/c nu/nu mice were used for glioma models, as previously described [18–20]. 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 per group were used. Tumor volumes (cm³) were determined as previously described [21]. Responses were determined using three activity measures as previously described [21].

In Vivo Statistical Methods

The exact log-rank test, as implemented using Proc StatXact for SAS^R, was used to compare event-free survival distributions between treatment and control groups and between combinations and respective single-agent treatment groups. P-values were two-sided and considered significant if less than 0.01. Model-based analysis was performed using a linear regression model for time-to-event (T), with testing to determine the treatment interaction of the two-drug combination as previously described [22] (Supplemental Response Definitions). The regression model tests whether the interaction parameter is significantly

greater than or less than 0 ($p < 0.01$), indicative of supra-additivity or sub-additivity of the drug combination, respectively. Otherwise, the drug combination is considered additive. To allow comparison of the interaction parameters across xenografts and across agents, the interaction parameter values are normalized by using the ratio of the interaction parameter to the expected additive effect for the combination under the assumption of additivity. This “normalized interaction term” can be conceptualized as representing the percentage gain or loss of the expected treatment effect observed for the combination under additivity.

Drugs and Formulation

AstraZeneca provided cediranib to the PPTP through the Cancer Therapy Evaluation Program (NCI). Cediranib was dissolved in 1% Polysorbate 80 in water and administered p.o. daily for 3 weeks at a dose of 3 mg/kg. Rapamycin was purchased from LC Laboratories (Woburn, MA). Rapamycin was dissolved in DMSO (5% final concentration) and diluted in 5% Tween 80 in water and administered IP daily $\times 5$ for 3 consecutive weeks at a dose of 5 mg/kg. Cyclophosphamide, vincristine and cisplatin were obtained from the NCI Drug Repository. Cyclophosphamide was administered weekly for 3 weeks by I.P. injection (150 mg/kg q 7d $\times 3$; MTD), as was vincristine (1 mg/kg every 7 days (q7d) $\times 3$; MTD). Cisplatin was administered at 7 mg/kg on day 1. Cyclophosphamide, vincristine and cisplatin were dissolved in physiological saline.

RESULTS

Previously cediranib was tested at a daily dose of 6 mg/kg, however in preliminary toxicity testing it was determined that in combination with cytotoxic agents at their respective MTDs, the daily dose of cediranib had to be reduced to 3 mg/kg. Rapamycin was administered IP 5-days per week for 3 weeks. The tumor models were selected based on their intermediate sensitivity to each individual agent [23–25]. Response data from the initial PPTP evaluations for each of the single agents for 6 weeks of therapy are given in Supplemental Table I. For the combination experiments, agents were administered for 3 weeks to allow detection of tumor progression and events during the period of observation (12 weeks). A complete summary of results is provided in Supplemental Table II, including total numbers of mice, number of mice that died (or were otherwise excluded), numbers of mice with events and median times to event, tumor growth delay, as well as objective responses and EFS T/C. Administered at 3 mg/kg/day as a single agent, cediranib demonstrated slightly reduced antitumor activity compared to 6 mg/kg/day against 3 tumor lines. For Rh30 and D645 models the response dropped from PD2 to PD1, and for OS-33 osteosarcoma xenograft from SD to PD2 (see Supplemental Response Definitions). Cediranib significantly retarded growth in four of six tumor models at the reduced dose used in these experiments.

The combination of cediranib with rapamycin was tested using all six tumor models, and results are summarized in Table I. The combination was significantly more active than cediranib in 3 of 6 tumor models, whereas it was significantly superior to rapamycin in 4 of 6 xenografts. Using the model-based interaction assessment analysis, the cediranib and rapamycin combination showed either additive or supra-additive effects for each of the 4 models for which the model fit was adequate, Table II. The supra-additive interaction for the combination for NB-EBc1 is shown in Figure 1.

The interaction of cediranib with standard cytotoxic agents, cyclophosphamide, vincristine and cisplatin, was determined in the appropriate models. The cytotoxic agent was more effective at prolonging time to event compared to cediranib in 5 of 6 models studied, and the primary comparison of interest was whether the addition of cediranib to the cytotoxic agent improved outcome compared to the cytotoxic agent used alone, Table I. The combination of

cediranib and cyclophosphamide was significantly inferior to single agent cyclophosphamide against the NB-EBc1 neuroblastoma xenograft ($P < 0.01$; Figure 2) and was nominally inferior to cyclophosphamide against the EW-5 xenograft ($P = 0.32$) (Table I). The latter xenograft was evaluable for the model-based interaction assessment and showed sub-additivity (Table II). Cediranib combined with vincristine was significantly superior to single agent vincristine for one of three xenografts (D645), and it was considered additive for D645 using the model interaction assessment. Cisplatin was tested against OS-33 osteosarcoma xenografts, with the combination showing no significant difference in EFS distribution compared to single agent cisplatin.

DISCUSSION

Previous experience has shown that the dose response relationship for most cytotoxic agents is very steep in the PPTP xenograft models. For this reason we maintained the dose intensity of each of the standard agents close to their MTD and reduced the dose of cediranib. This strategy is also consistent with the approach most commonly taken in the clinical setting for novel combinations in which the dose of standard agents is maintained and the dose of the experimental agent is used at a dose as close to its single agent MTD as can be tolerated. Clinical experience with cediranib supports the PPTP finding that the cediranib dose needs to be somewhat reduced from its single agent MTD when it is used in combination with cytotoxic agents [8]. As a single agent, cediranib has primarily been studied at doses of 30 mg or 45 mg administered daily, while in combination regimens with cytotoxic agents the tolerable daily cediranib dose has generally been 20 mg or 30 mg. The 30 mg dose has now been established as the single agent dose for further clinical investigations, and the 20 mg dose has been established for clinical use in combinations [13,26,27]. The reason for the required lower doses for combination regimens is not clear. While cediranib and other tyrosine kinase inhibitors have been shown to inhibit certain ABC drug transporters [28–30], in clinical studies there were no pharmacokinetic interactions reported when cediranib was combined with agents known to be substrates for these efflux pumps [31].

At the dose and schedule used for this report cediranib had a modest effect as a single agent, causing significant inhibition of tumor growth, although the best responses were PD2 (progressive disease 2) with no objective regressions. Responses to standard cytotoxic agents were similar to those reported previously, with the exception of the historical response of NB-EBc1 xenografts to cyclophosphamide (PD2 vs CR). The interaction between cediranib and each cytotoxic agent was primarily analyzed in terms of whether the addition of cediranib to the cytotoxic agent produced significantly better EFS compared to the cytotoxic agent used alone, as this mimics how pediatric clinical trials utilizing cediranib in combination with other agents would likely be designed and analyzed. The second analytic approach used a model based interaction assessment [22]. The combination of cediranib with cyclophosphamide in two models (NB-EBc1 and EW5) demonstrated some degree of sub-additivity. This was particularly striking for NB-EBc1 for which the median time to event was reduced from 55.8 days for cyclophosphamide alone to 32.3 days ($P < 0.01$). For EW5, the model based analysis method showed that the combination effect was sub-additive to the effect expected from the observed activity of each agent used alone. The mechanism for a negative interaction between cediranib and cyclophosphamide is not known.

For cediranib in combination with vincristine, a single xenograft (D645) among three evaluated demonstrated significantly better EFS for the combination compared to single agent vincristine. The combination effect for this xenograft was categorized as additive using the model-based interaction analysis. There was no potentiation of cisplatin antitumor activity in the one xenograft tested (OS-33).

While the antiangiogenic agent bevacizumab is approved for treatment of several adult carcinomas in conjunction with cytotoxic therapies, the observed lack of potentiation of the activity of cytotoxic agents by cediranib is consistent with recent clinical results for combinations of standard chemotherapy agents with either bevacizumab or with cediranib. A three-armed phase 3 trial in patients with ovarian cancer demonstrated that concurrent bevacizumab and chemotherapy was no more effective than chemotherapy alone, and that only if bevacizumab was continued as maintenance therapy was a significant effect on progression-free survival observed [32]. A phase 3 trial for patients with colorectal cancer showed that adjuvant chemotherapy plus bevacizumab caused only a transient improvement in disease-free survival compared to adjuvant chemotherapy alone, a result also consistent with a failure of bevacizumab to potentiate chemotherapy activity [33]. For cediranib, a phase 3 trial in patients with recurrent glioblastoma failed to demonstrate improved progression-free survival (PFS) for cediranib plus lomustine compared to lomustine alone [27]. A phase 3 trial evaluating the addition of cediranib to standard chemotherapy in the treatment of first-line metastatic colorectal cancer achieved a statistically significant, albeit small, improvement in PFS (8.6 versus 8.2 months), but there was no difference in overall survival [34].

When cediranib was combined with rapamycin there was additive or supra-additive activity for four models for which there was adequate data fit to the interaction model. The combination activity was significantly better than single agent rapamycin in four models and significantly better than both single agents in two models. However, it should be noted that the effects for the combination were not striking and that the best result was PD2. Rapamycin and related mTOR inhibitors have also been shown to have anti-angiogenic activity [35,36]. This activity may be through a direct effect on tumor cells (e.g., through regulating HIF1A protein expression) [37,38], or it may be through an effect on VEGF receptor signaling in endothelial cells [39,40]. The prolonged EFS in the absence of tumor regression for the rapamycin and cediranib combination is consistent with a more pronounced anti-angiogenic effect for the combination compared to the agents used alone. Of note, whereas cediranib had modest activity in enhancing vincristine, no effect on cisplatin, and antagonistic activity with cyclophosphamide, the combination of rapamycin with cyclophosphamide or vincristine was significantly more active than the cytotoxic agent alone for most evaluable models [22]. The combination of rapamycin with cisplatin at the cisplatin MTD produced excessive toxicity [22].

In summary, combination of cediranib with cytotoxic agents did not result in enhanced antitumor activity, and in one model was significantly inferior to cyclophosphamide as a single agent. Cediranib combined with rapamycin was additive or supra-additive in 4 of 6 models prolonging time to event, although the combination did not cause tumor regressions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

Responses of NB-EBc1 neuroblastoma xenografts to treatment with cediranib and rapamycin. A. Kaplan-Meier plots for event-free survival (EFS), plotted as probability for being event-free against time (days) after initiation of treatment. Control (black); cediranib (blue); rapamycin (red); cediranib + rapamycin (green). B. Relative tumor volume plots showing the median growth of tumors in each group (color code as for panel A). Individual tumor growth curves C: Control; D: Cediranib 3 mg/kg qd $\times$ 21; E: Rapamycin 5 mg/kg d $\times$ 5 $\times$ 3; F: Cediranib 3 mg/kg qd $\times$ 21 with rapamycin 5 mg/kg d $\times$ 5 $\times$ 3.

Figure 2.

Responses of NB-EBc1 neuroblastoma xenografts to treatment with cediranib and cyclophosphamide. A. Kaplan-Meier plots for event-free survival (EFS), plotted as probability for being event-free against time (days) after initiation of treatment. Control (black); cediranib (blue); cyclophosphamide (red); cediranib + cyclophosphamide (green). B. Relative tumor volume plots showing the median growth of tumors in each group (color code as for panel A). Individual tumor growth curves C: Control; D: Cediranib 3 mg/kg qd $\times$ 21; E: Cyclophosphamide 150 mg/kg q7d $\times$ 3; F: Cediranib 3 mg/kg qd $\times$ 21 with cyclophosphamide 150 mg/kg q7d $\times$ 3.

Table 1

Event-Free Survival (EFS) for Single Agents and Combinations with Cediranib

EFS for treatment with Cediranib, Rapamycin and the Combination						
Xenograft	CED T-C	RAP T-C	CED+RAP T-C	P-value [combination vs. CED]	P-value [combination vs. RAP]	
EW5	10.5	6.3	23.9	0.04	<0.01	
Rh30	3.8	8.1	19.2	0.04	<0.01	
Rh41	8.2	10.2	16.7	0.21	0.30	
D645	0.7	4.5	17.3	<0.01	<0.01	
NB-EBc1	3.3	4.9	19.8	<0.01	<0.01	
OS-33	8.7	38.5	37.5	<0.01	0.93	

EFS for Treatment with Cediranib, Cyclophosphamide, Vincristine, Cisplatin and in Combination						
Agent	CED T-C	Agent T-C	CED+Agent T-C	P-value [combination vs. Agent]	P-value [combination vs. CED]	
EW5	cyclophosphamide	10.5	30.7	26	0.32	<0.01
Rh30	vincristine	3.79	37.1	41.7	0.07	<0.01
Rh41	vincristine	8.24	5.3	15.5	0.11	0.15
D645	vincristine	0.73	18.9	26.9	<0.01	<0.01
NB-EBc1	cyclophosphamide	3.25	51.1	27.6	<0.01	<0.01
OS-33	cisplatin	8.74	38	37.6	0.33	<0.01

CED: Cediranib; RAP: Rapamycin

Table II

Model based assessment of combination therapy with cediranib.

Model-based interaction assessment of combination therapy of cediranib with rapamycin in all xenograft models								
Xenograft	cediranib	Normalized Interaction Term	Total Effect Expected	Interaction Parameter Estimate	P	R ²	Adequate model fit (based on R ²)?	Interpretation
EW5	rapamycin	0.303	18.2	5.524	0.1086	0.75	Y	Additive
Rh30	rapamycin	0.463	11.1	5.156	0.2423	0.45	N	NE (poor model fit)
Rh41	rapamycin	-0.301	22.2	-6.691	0.2235	0.34	N	NE (poor model fit)
D645	rapamycin	1.418	6.0	8.522	0.0115	0.60	Y	Additive
NB-EBc1	rapamycin	0.884	9.9	8.775	0.0037	0.74	Y	Supra-additive
OS-33	rapamycin	-0.201	45.7	-9.176	0.0159	0.90	Y	Additive
Model-based interaction assessment of combination therapy of cediranib with cyclophosphamide, vincristine or cisplatin in all xenograft models								
Xenograft	cediranib +	Normalized Interaction Term	Total Effect Expected	Interaction Parameter Estimate	P	R ²	Adequate model fit (based on R ²)?	Interpretation
EW5	cyclophosphamide	-0.392	43.4	-17.039	0.0002	0.79	Y	Sub-Additive
Rh30	vincristine	0.167	37.4	6.258	0.3825	0.75	Y	NE (censoring)
Rh41	vincristine	-0.200	15.7	-3.139	0.4892	0.34	N	NE (censoring)
D645	vincristine	0.642	16.2	10.426	0.0169	0.77	Y	Additive
NB-EBc1	cyclophosphamide	-0.526	58.3	-30.666	<.0001	0.96	Y	NE (censoring)
OS-33	cisplatin	-0.163	43.6	-7.112	0.1565	0.82	Y	Additive