Published in final edited form as:

Pediatr Blood Cancer. 2014 November ; 61(11): 1972–1979. doi:10.1002/pbc.25175.

Initial Solid Tumor Testing (Stage 1) of AZD1480, an Inhibitor of Janus Kinases 1 and 2 by the Pediatric Preclinical Testing Program

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Abstract

Background—AZD1480 is an ATP competitive inhibitor of Janus kinases 1 and 2 (JAK1, 2) that has been shown to inhibit the growth of solid tumor models. This agent was selected for testing the putative role of JAK/STAT signaling in the standard PPTP solid tumor models.

Procedures—AZD1480 was tested against the PPTP *in vitro* cell line panel at concentrations from 1.0 nM to 10 M and against the PPTP *in vivo* solid tumor xenograft panels at (60 mg/kg once daily (SID) × 5) for 3 consecutive weeks. Additional studies evaluated 5 to 20 mg/kg BID × 5 with SID dosing at 7–30 mg/kg at weekends for 3 consecutive weeks.

Results—*In vitro* the median relative IC₅₀ (rIC₅₀) for the PPTP cell lines was 1.5 μM, with a range from 0.3 μM to 5.9 μM. The two cell lines with rIC₅₀ values of 0.3 μM both had ALK activating genomic alterations. AZD1480 demonstrated statistically significant differences (p<0.05) in EFS distribution compared to control in 89% of the solid tumor xenografts. AZD1480

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Conflict of interest statement: The authors consider that there are no actual or perceived conflicts of interest.

Supplemental Materials

Supplemental Table I. Efficacy of AZD1480 against PTP Solid Tumor Xenograft Models.

Supplemental Table II. Efficacy of AZD1480 (dose-response testing) in Wilms Tumor Xenograft Models.

Supplemental Response Definitions.

induced intermediate (EFS T/C > 2) or high-level growth inhibition in 15 of 30 (50%) solid tumor xenografts. Tumor regressions were observed in 3 of 6 Wilms tumor models at doses that induced inhibition of Stat3(Y705) phosphorylation.

Conclusions—AZD1480 demonstrated significant tumor growth inhibition against most PPTP solid tumor xenografts, similar to that observed for antiangiogenic agents tested by the PPTP. Tumor regressing activity was noted for Wilms tumor xenografts.

Keywords

Preclinical Testing; Developmental Therapeutics; JAK1/2 inhibitor

INTRODUCTION

The signal transducer and activator of transcription (Stat) proteins comprise a family of transcription factors that mediate cytokine and growth factor responses [1,2]. Compared with normal cells and tissues, constitutive Stat3 activation is found in a high frequency of human cancers including breast, prostate, head and neck as well as other solid tumors and hematologic malignancies. Further, forced activation of Stat3 is tumorigenic [3]. Stat3 is activated by Janus Kinases (Jaks), predominantly by Jak2. Stat3 activation is frequently found also in lymphomas, chronic lymphocytic leukemia, acute myelogenous leukemia and large granular lymphocytic leukemia [4]. The identification that about half of patients with myelofibrosis carry a gain-of-function mutation in the Janus kinase 2 gene (*JAK2* V617F) that contributes to the pathophysiology of the disease has led to the development of Jak2-selective small molecule inhibitors [5]. The FDA has recently granted marketing approval for ruxolitinib for the treatment of patients with intermediate or high-risk myelofibrosis (MF), including primary MF, post-polycythemia vera MF and post-essential thrombocythemia MF [6].

Less is known regarding activation of Stats in pediatric cancers. Stat3 was observed to be activated (as determined by positive staining for Y705 phosphorylation) in 18 of 31 primary Ewing sarcoma family of tumors (ESFT) and in each of 3 ESFT cell lines examined [7]. Stat3 phosphorylation was found to be elevated in 19% (21/113) of osteosarcoma, 27% (17/64) of rhabdomyosarcoma, and 15% (22/151) of other soft-tissue sarcoma tissues [8]. Phospho-Stat3 was found to be induced in rhabdomyosarcoma cases with FGFR4 mutations [9]. Stat3 activation is consistently observed in ALK⁺ anaplastic large cell lymphomas, and its activation is required for NPM-ALK tumorigenesis [10–12]. Inhibition of Jak2 decreased cell viability in all cell lines derived from neuroblastoma, rhabdomyosarcoma and ESFT [5]. These authors concluded that inhibition of Jak/Stat3 signal transduction could be a promising therapeutic target for high-risk pediatric solid tumors [13].

AZD1480 is a potent pyrazolyl pyrimidine ATP-competitive Jak1–2 inhibitor, with IC₅₀ values for inhibition of Jak2-signaling in cellular assays in the 50 to 100 nM range and with higher concentrations required to block Jak3-mediated signaling [5]. In vivo, AZD1480 has demonstrated direct antitumor effects for Jak2-driven models, and it also shows anti-angiogenic activity related in part to inhibition of endothelial cell function [5,14–16].

Consistent with these findings for AZD1480, antiangiogenic activity has been reported for a Stat3 inhibitor in an osteosarcoma xenograft model [17].

Here we examined AZD1480 against the solid tumor panels in the PPTP. Although AZD1480 was withdrawn from clinical development after these studies were completed [18], the data set is valuable for assessing the role of Jak1–2 signaling in these preclinical models. Characterisation of Jak–mutated leukemia models and testing results for AZD1480 against an expanded panel of leukemias will be reported in a subsequent publication.

MATERIALS AND METHODS

In vitro testing

In vitro testing was performed using DIMSCAN [20], as previously described in a characterized panel of 23 cell lines [19]. Cells were incubated in the presence of AZD1480 for 96 hours at concentrations from 1.0 nM to 10 μ M and analyzed as previously described [20].

In vivo tumor growth inhibition studies

CB17SC *scid*^{−/−} female mice (Taconic Farms, Germantown NY), were used to propagate subcutaneously implanted kidney/rhabdoid tumors, sarcomas (Ewing, osteosarcoma, rhabdomyosarcoma), neuroblastoma, and non-glioblastoma brain tumors, while BALB/c nu/nu mice were used for glioma models, as previously described [21]. 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 in each control or treatment group. Tumor volumes (cm³) were determined and responses were determined using three activity measures as previously described [21]. An in-depth description of the analysis methods is included in the Supplemental Response Definitions section.

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.

Drugs and Formulation

AZD1480 was provided to the Pediatric Preclinical Testing Program by AstraZeneca, through the Cancer Therapy Evaluation Program (NCI). AZD1480 was formulated in 0.5% hydroxypropyl methyl cellulose/0.1% Tween 80, adjusted to pH 2.0 with 1N HCl, and stored for up to 7 days at 4C, protected from light. AZD1480 was administered oral gavage (PO) at 60 mg/kg daily for 5 days for 3 consecutive weeks. For secondary testing (Wilms tumors) the dose was adjusted to 20 mg/kg BID (M-F) and 30 mg/kg once daily (SID) at weekends (designated 20/30). Dose responses were evaluated using the 20/30 dose/schedule and at 10/15 and 5/7 (mg/kg) dose levels. AZD1480 was provided to each consortium investigator in coded vials for blinded testing.

RESULTS

In vitro testing

AZD1480 was tested against the PPTP *in vitro* cell line panel at concentrations ranging from 1.0 nM to 10.0 μ M using the PPTP's standard 96 hour exposure period. The median relative IC₅₀ (rIC₅₀) for the PPTP cell lines was 1.5 μ M, with a range from 0.3 μ M (Karpas-299 and NB-1643) to 5.9 μ M (Ramos-RA1). The median rIC₅₀ values for the neuroblastoma panel were lower than those of the remaining PPTP cell lines (0.9 versus 1.8 μ M, $p=0.02$), Figure 1.

In vivo testing

AZD1480 was initially tested against the PPTP solid tumor xenografts using a dose of 60 mg/kg dose administered by oral gavage daily $\times$ 5 for 3 weeks. The total planned treatment period was 3 weeks with an additional 3 weeks observation. Overall, AZD1480 induced a 6.6% toxicity rate (26/394) in the groups treated at 60 mg/kg. Control animals had a toxicity rate below 1%.

Thirty-six of 39 tested xenograft models were considered evaluable for efficacy. Lines inevaluable because of toxicity included two rhabdomyosarcoma xenografts (Rh18 and Rh30), and Ewing xenograft (TC71). 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.

AZD1480 induced statistically significant differences ($p<0.05$) in EFS distribution compared to control in 32 of 36 (89%) of the evaluable solid tumor xenografts treated (Table II). For those xenografts with a significant difference in EFS distribution between treated and control groups, the EFS T/C activity measure additionally requires an EFS T/C value of > 2.0 for intermediate activity and indicates a substantial agent effect in slowing tumor growth. High activity additionally requires a reduction in final tumor volume compared to the starting tumor volume. AZD1480 induced tumor growth inhibition meeting criteria for intermediate or high EFS T/C activity in 15 of 30 (50%) solid tumor xenografts evaluable for this measure at the 60 mg/kg dose. Intermediate or high activity for the EFS T/C metric was observed across all solid tumor panels except for the osteosarcoma panel (Table II).

The only solid tumor models with an objective response during the initial testing were two Wilms tumor xenografts (KT-10 and KT-11 with each achieving MCR) and the NB-EBc1 neuroblastoma xenograft (CR). The latter showed a heterogeneous pattern of growth among animals and overall the treatment effect was modest. The objective response results for both solid tumor and leukemia models are represented using Heatmap and COMPARE formats, based on the objective response scoring criteria centered around the midpoint score of 0 that represents stable disease Figure 2.

Because of the interesting, and unexpected, activity against the KT-10 and KT-11 Wilms tumor xenografts, testing against Wilms tumor models was expanded using the 20/30 dose/schedule [20 mg/kg BID (M-F) and 30 mg/kg SID at weekends]. The additional Wilms

tumor models studied were KT-5 (nuclear unrest), KT-6, and KT-18. AZD1480 induced PR in KT-18, inhibited growth of KT-5 during treatment (PD2), and induced stable disease in the slow-growing KT-6 xenograft. Responses of Wilms tumor xenografts to AZD1480 are shown in Figure 3. The dose-response relationship for AZD1480 was examined in three Wilms tumor xenograft models (KT-5, KT-10, and KT-18) using the same schedule at doses of 20/30, 10/15, and 5/7 (mg/kg). Responses were dose dependent, with the lowest dose inducing statistically significant growth delay in all models, however, tumor regression (noted for KT-10 and KT-18) was observed only at the highest dose level, Supplemental Table II.

Pharmacodynamic studies

One substrate for Jak2 is Stat3 where it phosphorylates tyrosine 705 (Y705) leading to homo-dimerization and translocation to the nucleus. We therefore examined Stat3 phosphorylation (Y705) as a marker of pathway activation in many of the solid tumor xenografts, **Figure 4A**. Phospho-Stat3(Y705) was relatively high in sarcomas (Ewing, rhabdomyosarcoma and osteosarcoma) as well as many of the brain tumors. It was low in the kidney tumor panel, and below detection in several neuroblastoma xenograft models. To examine the in vivo inhibition of Jak2 by AZD1480, mice bearing KT-10 or KT-13 Wilms tumors that were highly responsive to AZD1480 treatment, were studied. Mice were administered drug at 20 mg/kg or 10 mg/kg twice daily for 2 days. Tumors were harvested from untreated mice (control), 12 hr post dose 4 (designated 4 + 12 Hr), or 4, 8 and 24 Hr post dose 5 (single dose on day 3, designated 5 + 4 Hr etc.). As shown in Figure 4B, phospho-Stat3 was low in control KT-10 xenografts, consistent with the survey result (Figure 4A). Stat3 was relatively high, although 12 Hr post dose 4 (20 mg/kg; designated 4 + 12 Hr) only 1 of 3 tumors demonstrated detectable Stat3 signal. Subsequent to dose 5, Stat3 levels were undetectable at 4 and 8 Hr, but were detected at 24 Hr. Treatment on day 3 was associated with elevated cleaved caspase-3. Similar results were obtained with the 10 mg/kg dose, with a significant loss of Stat3 protein following dose 5, but less activation of caspase-3. Similar results were obtained with KT-13 xenografts, although in this tumor line inhibition of phospho-Stat3 following dose 5 was more readily detected, and associated with activation of caspase-3 at the 20 mg/kg dose level.

DISCUSSION

The Jak1–2 inhibitor ruxolitinib has been approved for treatment of myelofibrosis, and other Jak inhibitors are in development. Although AZD1480 has recently been withdrawn from development, it serves as a valuable probe compound to evaluate the role of Jak/Stat signaling in pediatric tumor models. AZD1480 was withdrawn from development because of neuropsychiatric dose-limiting toxicities (dizziness, ataxia, hallucinations, and anxiety) observed during phase 1 testing [18]. It is unclear whether these toxicities were related to inhibition of Jak signaling in the brain or to an effect of AZD1480 on other kinases that it potently inhibits (e.g., TRK family kinases) [18]. Two other Jak2 inhibitors, XL-019 and SAR302503, have been dropped from clinical development because of central neurotoxicity [22,23].

The median rIC_{50} value for the PPTP cell lines was 1.5 μM . To put the PPTP rIC_{50} values in context, the IC_{50} for a cell line driven by JAK2 (Ba/F3 TEL-JAK2) was approximately 0.06 μM , compared to 2.1 μM for a JAK3 driven cell line and 0.7 μM for a JAK1 driven cell line (Ba/F3 TEL-JAK3 and Ba/F3 TEL-JAK1), respectively [5]. The SET-2 cell line (a human megakaryoblastic cell line derived from an essential thrombocytopenia (ET) patient and heterozygous for JAK2 V617F) showed 50% inhibition of STAT5 and STAT3 at ~ 0.025 μM AZD1480, which correlated with its GI_{50} concentration of 0.016 μM [24]. By contrast, prior reports have described IC_{50} values for JAK wildtype cell lines in viability/proliferation assays generally between 1 μM and 10 μM despite near complete inhibition of STAT signaling at 0.5 μM concentrations [5,13,14,25,26]. The body of previous work suggests specific inhibitory effects for AZD1480 related to Jak/Stat signaling at submicromolar concentrations, with nonspecific effects observed at higher concentrations. Only two PPTP cell lines showed rIC_{50} values < 0.5 μM : NB-1643 and Karpas-299, with the former having an activating ALK mutation (R1275Q) and the latter having the NPM-ALK translocation. The relative *in vitro* sensitivity of the NPM-ALK expressing Karpas-299 cell line is consistent with prior reports describing a role for this fusion oncogene in activating the Jak-Stat pathway through Jak2 and Jak3 [27–31]. ALK-activating mutations in neuroblastoma cells have also been shown to signal through Stat3 [32]. The potential clinical relevance of these *in vitro* observations is unclear, given the lack of tumor-regressing *in vivo* activity for AZD1480 against Karpas-299 and NB-1643 grown as a xenograft.

In vivo, AZD1480 was well tolerated, and induced statistically significant growth delay in 32 of 36 solid tumor models, at the 60 mg/kg daily $\times$ 5 schedule, and 4 of 5 models at the 20/30 dose/schedule. This pattern of widespread tumor growth inhibition with low rates of tumor regression is similar to that observed by the PPTP to antiangiogenic agents targeting VEGF signaling (e.g., cediranib, sunitinib, and sorafenib) [33–35]. This observation is consonant with previous reports describing a prominent *in vivo* anti-angiogenic effect of AZD1480 with treatment (50 mg/kg) resulting in rapid reductions in VEGF and MMP9 expression in tumor cells [14]. Supporting an effect on angiogenesis, Xin, et al., demonstrated that *in vitro* treatment of human umbilical vein endothelial cells (HUVEC) with AZD1480 inhibited endothelial cell tube formation at submicromolar concentrations [14]. Thus, the tumor growth inhibitory activity that we and others [13] have observed for AZD1480 against pediatric solid tumor xenografts is consistent with the previously described prominent antiangiogenic activity of AZD1480.

Of note, in the original screen there was intermediate or high activity in two Wilms tumor models. Because of this signal, a larger panel of Wilms tumors was tested to include addition models representing Favorable Histology (FH) including a model having ‘nuclear unrest’ [36] as well as diffuse anaplastic histology. Overall, AZD1480 treatment caused 2 MCR (KT-10, KT-13 both FH), PR (KT-18; diffuse anaplastic), SD (KT-5, FH with nuclear unrest) and PD2 (KT-13, diffuse anaplastic). Tumor responses were dose proportionate using the 20/30 schedule, with objective regressions observed only at the highest dose level (Supplemental Table II).

In a survey of solid tumor models in the PPTP primary panels, Stat3 and phospho-Stat3 (Y705) were high in the sarcoma models (rhabdomyosarcoma, Ewing sarcoma and

osteosarcoma), intermediate in non-GBM brain tumors and low in neuroblastoma and kidney tumor models. The level of phospho-Stat3 in KT-10 tumors was low, hence it was difficult to demonstrate inhibition by AZD1480. However, at both 20 and 10 mg/kg the levels of Stat3 markedly decreased at 4 and 8 hours after dosing, with some recovery by 24 hours. Phospho-Stat3 was more readily detected in KT-13 xenografts and was inhibited by AZD1480 treatment. As with KT-10 tumors, total Stat3 also decreased post-dosing. The reason for loss of Stat3 is unclear, although inhibition of Stat3 dimerization by an oxazole-based small-molecule Stat3 inhibitor modulates Stat3 stability and processing [37]. Inhibition of JAK2 would lead to hypophosphorylation of Stat3 (Y705) required for dimerization. Schaefer et al have proposed destabilization of STAT3 as a mechanism for regulating STAT3 isoforms [38], and Stat3 stability is sensitive to osmolarity changes [39]. For both KT-10 and KT-13 Wilms tumor models, there was an increase in cleaved caspase 3 at the higher dose level, consistent with the observed tumor responses. Although the KT-13 results demonstrate target inhibition, further studies are required to determine whether responses in these tumors are a consequence of Jak2/Stat3 inhibition, or whether other kinases inhibited by AZD1480 may be important in mediating the antitumor activity of this agent.

In summary, AZD1480 had modest single agent activity against pediatric solid tumor models, retarding tumor growth in a similar manner as antiangiogenic agents tested by the PPTP, but inducing few objective regressions. Interesting tumor-regressing activity was observed against an expanded panel of Wilms tumor xenografts, and further evaluations to understand the basis of this activity are warranted.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by NO1-CM-42216, CA21765, and CA108786 from the National Cancer Institute and used AZD1480 supplied the AstraZeneca. In addition to the authors this paper represents work contributed by the following: Sherry Ansher, Joshua Courtright, Kathryn Evans, Edward Favours, Henry S. Friedman, Danuta Gasinski, Doris A. Phelps, Melissa Sammons, Joe Zeidner, Jianrong Wu, Ellen Zhang, and Jian Zhang.

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Figure 1. AZD1480 *in vitro* activity

The median rIC₅₀ ratio graph shows the relative rIC₅₀ values for the cell lines of the PPTP panel. Each bar represents the ratio of the panel rIC₅₀ to the rIC₅₀ value of the indicated cell line. Bars to the right represent cell lines with higher sensitivity, while bars to the left indicate cell lines with lesser sensitivity.

Xenograft Line	Histology	Log-rank p-value	Median Score	Midpoint Difference	Overall Group Response	Heat Map
BT-29	Rhabdoid	<0.001	2	-3	PD2	
KT-16	Rhabdoid	<0.001	2	-3	PD2	
KT-14	Rhabdoid	<0.001	2	-3	PD2	
KT-12	Rhabdoid	<0.001	2	-3	PD2	
KT-10	Wilms	<0.001	10	5	MCR	
KT-11	Wilms	<0.001	10	5	MCR	
KT-13	Wilms	<0.001	2	-3	PD2	
SK-NEP-1	Ewing	<0.001	2	-3	PD2	
EW5	Ewing	0.004	2	-3	PD2	
EW8	Ewing	0.033	1	-4	PD1	
CHLA258	Ewing	<0.001	2	-3	PD2	
Rh10	ALV Rhabdomyosarcoma	<0.001	2	-3	PD2	
Rh28	ALV Rhabdomyosarcoma	<0.001	2	-3	PD2	
Rh30R	ALV Rhabdomyosarcoma	<0.001	1	-4	PD1	
Rh41	ALV Rhabdomyosarcoma	<0.001	2	-3	PD2	
BT-28	Medulloblastoma	<0.001	2	-3	PD2	
BT-45	Medulloblastoma	<0.001	2	-3	PD2	
BT-50	Medulloblastoma	0.474	2	-3	PD2	
BT-50	Medulloblastoma	<0.001	2	-3	PD2	
BT-36	Ependymoma	1.000	4	-1	SD	
BT-41	Ependymoma	1.000	2	-3	PD2	
GBM2	Glioblastoma	0.003	2	-3	PD2	
BT-39	Glioblastoma	<0.001	2	-3	PD2	
D645	Glioblastoma	0.063	0	-5	PD1	
D456	Glioblastoma	0.003	2	-3	PD2	
NB-SD	Neuroblastoma	0.010	2	-3	PD2	
NB-1771	Neuroblastoma	<0.001	2	-3	PD2	
NB1691	Neuroblastoma	0.036	2	-3	PD2	
NB-EBc1	Neuroblastoma	0.017	8	3	CR	
NB-1643	Neuroblastoma	<0.001	2	-3	PD2	
OS-1	Osteosarcoma	<0.001	0	-5	PD1	
OS-2	Osteosarcoma	<0.001	1	-4	PD1	
OS-17	Osteosarcoma	<0.001	2	-3	PD2	
OS-9	Osteosarcoma	0.007	0	-5	PD1	
OS-33	Osteosarcoma	<0.001	0	-5	PD1	
OS-31	Osteosarcoma	<0.001	0	-5	PD1	
Karpas-299	Anaplastic Large Cell Lymphoma	0.164	0	-5	PD1	
Median			2.0

Figure 2. Summary of antitumor activity for AZD1480 administered at 60 mg/kg daily $\times$ 5 for 3 consecutive weeks

Left: The colored heat map depicts group response scores. A high level of activity is indicated by a score of 6 or more, intermediate activity by a score of 2 but <6 , and low activity by a score of <2 . Right: representation of tumor sensitivity based on the difference of individual tumor lines from the midpoint response (stable disease). Bars to the right of the median represent lines that are more sensitive, and to the left are tumor models that are less sensitive. Red bars indicate lines with a significant difference in EFS distribution between treatment and control groups, while blue bars indicate lines for which the EFS distributions were not significantly different.

Figure 3. AZD1480 *in vivo* objective response activity for Wilms tumor models

Wilms tumors KT-6, and KT-18 were treated using the 20/30 schedule whereas KT-10 and KT-11 were treated using the 60 mg/kg SID $\times$ 5 schedule: Kaplan-Meier curves for EFS (left), median relative tumor volume graphs (center), and individual tumor volume graphs (right) are shown for selected lines. Controls (gray lines); Treated (black lines), statistical significance (p values) of the difference between treated and control groups are included.

Figure 4. Pharmacodynamics study

A. Expression of STAT3 and phospho-STAT3(Y705) in solid tumor xenografts in the PPTP.

B. Dynamic changes in pSTAT3(Y705), STAT3 and cleaved caspase 3 in KT-10 (upper) and KT-13 (lower) Wilms tumor xenografts after administration of AZD1480 at 20 mg/kg (left panels) or 10 mg/kg (right panels) BID. Tumors were harvested from untreated mice (control), 12 hr post dose 4 (designated 4 doses + 12 Hr), or 4, 8 and 24 Hr post dose 5 (single dose on day 3, designated 5 doses + 4 Hr etc.). Three tumors per time point were used. GAPDH is used as a loading control. AM380C1 cells were either not stimulated, or stimulated with IL-6 in the absence or presence of AZD1480 (10 M) as a control for phospho-STAT3(Y705).

Table I

In vitro activity of AZD1480 against PPTP cell lines

Cell Line	Histotype	rIC ₅₀ (μ M)	Panel rIC ₅₀ / Line rIC ₅₀	Y _{min} (%) (Observed)
RD	Rhabdomyosarcoma	4.6	0.3	26.5
Rh41	Rhabdomyosarcoma	0.7	2.1	0.2
Rh18	Rhabdomyosarcoma	2.1	0.7	7.4
Rh30	Rhabdomyosarcoma	1.9	0.7	5.2
BT-12	Rhabdoid	1.0	1.5	25.0
CHLA-266	Rhabdoid	1.1	1.3	19.0
TC-71	Ewing sarcoma	3.5	0.4	2.0
CHLA-9	Ewing sarcoma	1.2	1.2	2.8
CHLA-10	Ewing sarcoma	1.4	1.0	10.6
CHLA-258	Ewing sarcoma	1.4	1.0	1.8
SJ-GBM2	Glioblastoma	1.6	0.9	9.8
NB-1643	Neuroblastoma	0.3	4.6	3.8
NB-EBc1	Neuroblastoma	1.1	1.3	6.0
CHLA-90	Neuroblastoma	1.0	1.4	0.7
CHLA-136	Neuroblastoma	0.8	1.9	11.4
NALM-6	ALL	1.9	0.8	4.0
COG-LL-317	ALL	2.6	0.5	0.1
RS4;11	ALL	2.4	0.6	2.5
MOLT-4	ALL	1.2	1.2	0.2
CCRF-CEM (1)	ALL	2.3	0.6	5.1
CCRF-CEM (2)	ALL	1.5	0.9	3.0
Kasumi-1	AML	1.6	0.9	3.4
Karpas-299	ALCL	0.3	5.1	1.3
Ramos-RA1	NHL	5.9	0.2	4.3
Median		1.5	1.0	3.9
Minimum		0.3	0.2	0.1
Maximum		5.9	5.1	26.5

Table II

In vivo activity of AZD1480 against PPTP xenograft models

Line Description	Tumor Type	Median Time to Event	P-value	EFS T/C	Median RTV/CD4 5 at End of Study	Tumor Volume T/C	Median Group Response	EFS Activity
60 mg/kg SID × 21								
BT-29	Rhabdoid	> EP	<0.001	> 1.8	2.9	0.5	PD2	NE
KT-16	Rhabdoid	23.6	<0.001	2.7	>4	0.4	PD2	Int
KT-14	Rhabdoid	> EP	<0.001	> 2.1	2.7	0.4	PD2	Int
KT-12	Rhabdoid	> EP	<0.001	> 2.3	1.5	0.3	PD2	Int
KT-10	Wilms	> EP	<0.001	> 4.2	0.0	0.0	MCR	High
KT-11	Wilms	> EP	<0.001	> 2.3	0.0	0.0	MCR	High
KT-13	Wilms	32.9	<0.001	3.0	>4	0.2	PD2	Int
SK-NEP-1	Ewing	27.5	<0.001	2.6	>4	0.3	PD2	Int
EW5	Ewing	11.1	0.004	1.6	>4	0.6	PD2	Low
EW8	Ewing	15.2	0.033	1.6	>4	0.5	PD1	Low
CHLA258	Ewing	27.6	<0.001	2.0	>4	0.5	PD2	Int
Rh10	Alveolar RMS	29.3	<0.001	2.6	>4	0.5	PD2	Int
Rh28	Alveolar RMS	> EP	<0.001	> 1.9	3.6	0.3	PD2	NE
Rh30R	Alveolar RMS	29.3	<0.001	1.5	>4	0.4	PD1	Low
Rh41	Alveolar RMS	21.0	<0.001	1.8	>4	0.5	PD2	Low
BT-28	Medulloblastoma	33.5	<0.001	2.1	>4	0.3	PD2	Int
BT-45	Medulloblastoma	> EP	<0.001	> 1.3	1.5	0.3	PD2	NE
BT-50	Medulloblastoma	> EP	<0.001	> 1.2	2.7	0.4	PD2	NE
BT-36	Ependymoma	> EP	1.000	.	1.1	0.57	SD	NE
BT-41	Ependymoma	> EP	1.000	.	1.9	0.7	PD2	NE
GBM2	Glioblastoma	17.0	0.003	1.8	>4	0.5	PD2	Low
BT-39	Glioblastoma	17.3	<0.001	2.2	>4	0.4	PD2	Int
D645	Glioblastoma	8.7	0.063	1.5	>4	0.6	PD1	Low
D456	Glioblastoma	13.2	0.003	2.1	>4	0.5	PD2	Int

Line Description	Tumor Type	Median Time to Event	P-value	EFS T/C	Median RTV/CD4 S at End of Study	Tumor Volume T/C	Median Group Response	EFS Activity
NB-SD	Neuroblastoma	23.8	0.010	1.9	>4	0.9	PD2	Low
NB-1771	Neuroblastoma	18.6	<0.001	2.1	>4	0.4	PD2	Int
NB-1691	Neuroblastoma	10.3	0.036	1.8	>4	0.6	PD2	Low
NB-EBc1	Neuroblastoma	13.7	0.017	2.7	>4	0.3	CR	Int
NB-1643	Neuroblastoma	25.9	<0.001	2.1	>4	0.3	PD2	Int
OS-1	Osteosarcoma	40.8	<0.001	1.4	>4	0.6	PD1	Low
OS-2	Osteosarcoma	26.6	<0.001	1.5	>4	0.6	PD1	Low
OS-17	Osteosarcoma	30.0	<0.001	1.5	>4	0.6	PD2	Low
OS-9	Osteosarcoma	24.2	0.007	1.3	>4	0.8	PD1	Low
OS-33	Osteosarcoma	20.4	<0.001	1.3	>4	0.8	PD1	Low
OS-31	Osteosarcoma	28.8	<0.001	1.4	>4	0.7	PD1	Low
Karpas-299	ALCL	10.0	0.164	1.1	>4	0.9	PD1	Low
20/30 × 3 weeks								
KT-5	Wilms	42.5	<0.001	1.8	>4	0.37	PD2	Low
KT-6	Wilms tumor	.	1.000	NE	0.9	0.63	SD	NE
KT-10	Wilms	38.6	<0.001	3.5	>4	0.03	CR	Int
KT-13	Wilms tumor	23.9	<0.001	1.8	>4	0.33	PD2	Low
KT-18	Wilms	> EP	<0.001	> 1.8	1.8	0.17	PR	NE

Solid tumor xenografts were treated at 60 mg/kg daily for 5 days for 3 consecutive weeks. Xenografts designated 20/30 were treated with AZD1480 at 20 mg/kg BID (M-F) and 30 mg/kg SID at weekends for 3 consecutive weeks. NE, Not evaluable.