102 Pediatric Preclinical Testing Program (PPTP) Evaluation of the VEGFR-2 Inhibitor AZD2171

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Abstract

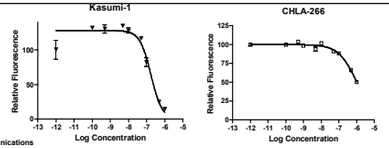
Background: AZD2171 is an oral, highly potent and selective VEGF signaling inhibitor of all VEGFR tyrosine kinases (VEGFR-1,-2 and 3) and effectively blocks VEGF-induced angiogenesis and neovascular survival. AZD2171 inhibits the growth of a wide range of established adult tumor xenografts in a dose-dependent manner and is in clinical evaluation for adults with cancer.
Methods: The PPTP includes an *in vitro* panel (23 lines) as well as panels of xenografts (n=61) representing most of the common types of childhood solid tumors and childhood ALL. AZD2171 was tested against the PPTP *in vivo* tumor panels at a dose of 6 mg/kg p.o. daily for 6 weeks. Three measures of antitumor activity were used: 1) response criteria modeled after the clinical setting [e.g., partial response (PR), complete response (CR), etc.]; 2) treated to control (T/C) tumor volume at day 21; and 3) a time to event measure based on the median EFS of treated and control lines (intermediate activity required EFS T/C > 2, and high activity additionally required a net reduction in median tumor volume at the end of the experiment).
Results: AZD2171 induced significant tumor growth delay in 84% of the solid tumor xenografts tested, with growth delay observed in each of the solid tumor panels. Using a time to event measure of efficacy, AZD2171 had intermediate and high levels of activity against 10 and 1 of 26 solid tumor xenografts evaluable for this measure, respectively. Intermediate activity was observed in 4 of 5 rhabdomyosarcoma, 3 of 3 Ewing sarcoma, and 2 of 3 Wilms tumor (WT) xenografts, with high level activity observed in 1 of 2 evaluable rhabdoid tumor (RT) xenografts. AZD2171 induced CR against 1 of 3 osteosarcoma (OS), 1 of 3 RT, and 1 of 3 WT xenografts, but had no effect on *in vivo* growth of any ALL xenografts. Kasumi-1, the only PPTP *in vitro* panel cell line with an EC50 <1 μM (EC50 0.175 μM) is known to have a gain-of-function KITAsn822Lys mutation.
Conclusions: AZD2171 demonstrated broad activity against the PPTP's solid tumor panel. Antitumor activity was manifested primarily as tumor growth delay, although tumor regressions were observed in the OS, RT, and WT panels. Further preclinical evaluation of AZD2171 is warranted and will include studies of AZD2171 in combination with clinically relevant agents for selected xenografts in which activity was observed. The Pediatric Brain Tumor Consortium is planning clinical evaluations of AZD2171 in children. (Supported by NCI NO1CM42216)

In Vitro Test Results for AZD2171

Methods: *In vitro* testing was performed using DIMSCAN, a semiautomatic fluorescence-based digital image microscopy system that quantifies viable (using fluorescein diacetate [FDA]) cell numbers in tissue culture multiwell plates (Keshelava, et al. Methods Mol.Med., *110*: 139-153, 2005). Testing was for 96 hours at concentrations from 0.1 nM to 1.0 μM with replicates of 6 per data point. Data were analyzed using GraphPad Prism, fitting a non-linear regression model-sigmoidal dose-response model to the response-relative fluorescence values *vs.* the concentration.

Results: AZD2171 showed little or no evidence for drug effect against 20/22 cell lines of the PPTP *in vitro* panel. The exceptions were Kasumi-1 (AML), which is known to have a gain-of-function c-Kit mutation (Asn822Lys), with an EC50 of 0.175 μM and CHLA-266 (rhabdoid tumor) with an estimated EC50 of approximately 1 μM. All other lines had EC50 values substantially above the highest concentration of AZD2171 tested (1 μM).

Examples of Dose Response Curves



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Methods for PPTP *In Vivo* Testing

Stage 1 testing involves testing an agent across the entire PPTP panel of childhood cancer xenograft lines at its MTD (or at a dose selected based on PK/PD studies using adult preclinical models).

- **Solid tumor testing**: For each xenograft line, 10 mice bearing SC tumors initiated treatment when the tumors were between 0.2–0.5 cm^3. Two perpendicular tumor diameters were measured at either once or twice weekly intervals with digital vernier calipers. Assuming tumors to be spherical, volumes were calculated from the formula (π/6)×d3, where d represents the mean diameter.
- **Acute lymphoblastic leukemia testing**: For each xenograft line, 8 mice were inoculated with 3-5 x 10^6 mononuclear cells purified from the spleens of secondary recipient mice. Engraftment was monitored weekly by flow cytometry, and treatment was initiated when the proportion of human CD45+ cells in the peripheral blood reached 1%. The proportion of human CD45+ cells in the peripheral blood was monitored weekly throughout the course of treatment.
- **AZD2171** was provided by AstraZeneca through the Cancer Therapy Evaluation Program (NCI). AZD2171 was dissolved in 1% Polysorbate 80 in water and administered p.o. daily x 6 weeks at a dose of 6 mg/kg in the solid tumor models and 3 mg/kg in the ALL models. AZD2171 was provided to each consortium investigator in coded vials for blinded testing according the PPTP program standard operating procedures.
- **Solid Tumor Response Criteria:**

	Response	Definition	Score
PD1	Progressive Disease 1	>50% regression at all measurements and >25% increase in tumor volume at the end of the study period, TGD value of ≤1.5	0
PD2	Progressive Disease 2	>50% regression at all measurements and >25% increase in tumor volume at the end of the study period, TGD value of >1.5	2
SD	Stable Disease	>50% regression at all measurements and ≤25% increase at the end of the study	4
PR	Partial Response	≥50% regression but with tumor volume ≥0.1 cm^3 during study	6
CR	Complete Response	Tumor volume <0.1 cm^3 for at least one study measurement	8
MCR	Maintained Complete Response	Tumor volume <0.1 cm^3 at the end of study	10

- **Leukemia Response Criteria:**

	Response	Definition	Score
PD1	Progressive Disease 1	CD45% never dr ops below 1%, event s before end of study, TGD value of ≤1.5	0
PD2	Progressive Disease 2	CD45% never dr ops below 1%, eve nts before end of study, TGD value of >1.5	2
SD	Stable Disease	CD45% never drops below 1%, no events before end of study	4
PR	Partial Response	CD45% drops below 1% for only 1 week	6
CR	Complete Response	CD45% drops be low 1% for 2 consecut ive weeks	8
MCR	Maintained Complete Response	CD45% drops be low 1% for last 3 consecutive measurements of the study	10

- **Median Group Response:** Each individual mouse in the treatment group was assigned a response score (see Tables above) and an median score for the treatment group was calculated and then each treatment group was assigned an overall response according to the table below.

If Average Score (AS) from (1):	Overall Group Response
0 ≤ AS ≤1	PD1
1 < AS ≤3	PD2
3 < AS ≤5	SD
5 < AS ≤7	PR
7 < AS ≤9	CR
9 < AS	MCR

- **Statistical Methods:** Event-free survival (EFS) distributions of each treatment group were compared to the EFS distribution of the respective control group using the exact log rank test. P-values were 2-sided & were not adjusted for multiple comparisons given the exploratory nature of this study. P-values < 0.05 were considered to be significant. Relative tumor volumes (RTV) for control (C) and treatment (T) mice were calculated at day 21 or when all mice in the control and treated groups still had measurable tumor volumes (if less than 21 days). The mean relative tumor volumes for control and treatment mice for each study were then calculated and the T/C value was the mean RTV for the treatment group divided by the mean RTV for the control group.

AZD2171 Activity: PPTP *In Vivo* Lines

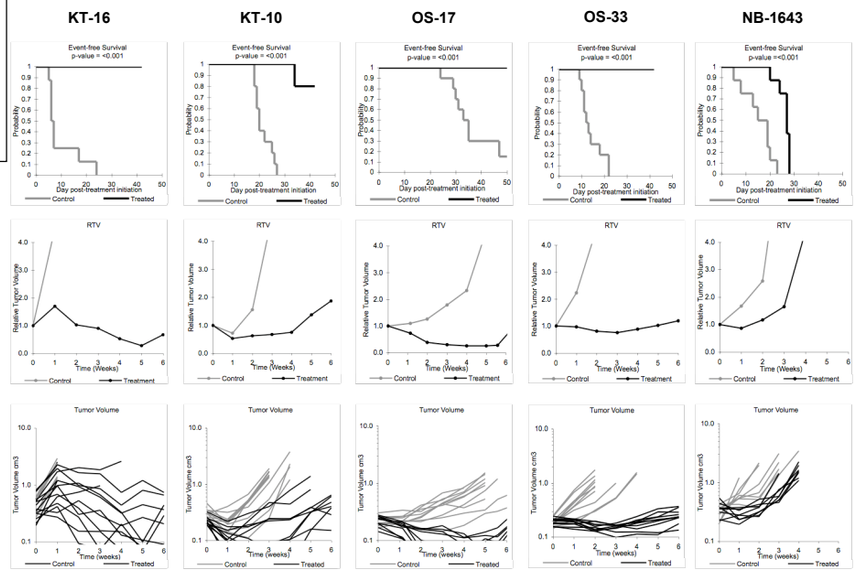
- **AZD2171 induces significant prolongation of time to event in 27 of 32 solid tumor xenografts tested (84%).**
- **AZD2171 primarily induced decreased tumor growth rates without tumor regression.**
- **KT-16 (Rhabdoid) and OS-17 (Osteosarcoma) achieved complete responses, and two additional xenografts demonstrated stable disease: KT-10 (Wilms) and OS-33 (Osteosarcoma).**
- **11 of 27 evaluable xenografts met criteria for intermediate activity for the EFS T/C measure of activity, with KT-16 of the Rhabdoid Panel meeting criteria for high activity.**
- **AZD2171 had no significant effect on growth for any of the ALL xenografts.**

Xenograft	Histology	P-value	EFS T/C	Median Final RTV	Tumor Volume T/C	Overall Group Response
BT-29	Rhabdoid	<0.001	> 1.8	1.7	0.66	PD2
KT-16	Rhabdoid	<0.001	> 6.5	0.7	0.43	CR
KT-14	Rhabdoid	0.140	1.8	>4	0.78	PD2
KT-10	Wilms	<0.001	> 2.1	1.9	0.21	SD
KT-11	Wilms	0.224	1.1	>4	0.73	PD1
KT-13	Wilms	0.001	2.8	>4	0.61	PD2
SKNEP	Ewings/Kidney	<0.001	3.0	>4	0.22	PD2
EW5	Ewings	<0.001	2.3	>4	0.43	PD2
EW8	Ewings	<0.001	3.6	>4	0.32	PD2
Rh28	ALV RMS	<0.001	1.6	>4	0.58	PD2
Rh30	ALV RMS	<0.001	> 3.3	3.8	0.25	PD2
Rh30R	ALV RMS	<0.001	2.9	>4	0.53	PD2
Rh41	ALV RMS	<0.001	3.3	>4	0.49	PD2
Rh18	EMB RMS	0.001	2.7	>4	0.38	PD2
BT-28	Medulloblastoma	0.008	1.2	>4	0.61	PD1
BT-45	Medulloblastoma	0.005	> 1.5	1.7	0.68	PD2
BT-46	Medulloblastoma	0.093	1.2	>4	0.78	PD1
GBM2	Glioblastoma	0.006	1.6	>4	0.70	PD1
BT-39	Glioblastoma	0.872	1.1	>4	0.95	PD1
D645	Glioblastoma	<0.001	2.6	>4	0.43	PD2
D456	Glioblastoma	<0.001	1.6	>4	0.62	PD2
NB-SD	Neuroblastoma	<0.001	1.6	>4	0.28	PD2
NB-1771	Neuroblastoma	<0.001	2.0	>4	0.49	PD2
NB-1691	Neuroblastoma	0.076	1.1	>4	0.91	PD1
NB-EBc1	Neuroblastoma	<0.001	1.7	>4	0.43	PD2
CHLA-79	Neuroblastoma	<0.001	1.8	>4	0.68	PD2
NB-1643	Neuroblastoma	<0.001	1.6	>4	0.36	PD2
OS-1	Osteosarcoma	<0.001	> 1.4	2.0	0.40	PD2
OS-2	Osteosarcoma	0.003	1.3	>4	0.55	PD1
OS-17	Osteosarcoma	<0.001	> 1.5	1.6	0.21	CR
OS-33	Osteosarcoma	<0.001	> 3.3	1.2	0.18	SD
OS-31	Osteosarcoma	<0.001	> 1.7	3.8	0.66	PD2
ALL-2	ALL B-precursor	0.841	1.0	>25	.	PD1
ALL-3	ALL B-precursor	0.754	1.5	>25	.	PD1
ALL-4	ALL B-precursor	0.224	1.5	>25	.	PD1
ALL-7	ALL B-precursor	0.831	0.9	>25	.	PD1
ALL-8	ALL T-cell	0.161	1.6	>25	.	PD1
ALL-17	ALL B-precursor	0.405	0.7	>25	.	PD1
ALL-19	ALL B-precursor	0.867	1.0	>25	.	PD1

Red shading in the p-value column indicates a significant difference in EFS distribution between treated and control groups.
Shading in the EFS columns indicates xenografts that have either high (dark blue), intermediate (light blue), or indeterminate (gray) activity.

Examples of AZD2171 *In Vivo* Activity

KM curves for EFS, median relative tumor volume, and individual tumor volumes for KT-16, KT-10, OS-17, OS-33, and NB-1643. Controls (gray lines); Treated (black lines)



DISCUSSION & CONCLUSIONS

- **The broad-spectrum activity for AZD2171 against the PPTP's solid tumor xenografts is similar to the effect seen in adult epithelial xenografts.**
- **The absence of an in vitro effect of AZD2171 on most pediatric cell lines supports an anti-angiogenic rather than a direct tumor effect as an explanation for the in vivo activity of AZD2171.**
- **Tumor regression was rare, with the majority of lines showing some degree of decreased growth rate.**
- **Two xenografts achieved complete responses (osteosarcoma & rhabdoid tumor). Neither of these histologies is known to have activating mutations in the kinases that AZD2171 inhibits at low nanomolar concentrations.**
- **AZD2171 demonstrated little or no activity against the PPTP's ALL xenografts. Although previous reports suggested a possible role for angiogenesis in general, and VEGF specifically, in the growth and progression of pediatric ALL, our results do not support a therapeutically relevant effect for VEGF signaling inhibition in the pediatric ALL setting.**
- **The PPTP is actively pursuing studies combining AZD2171 with conventional chemotherapy agents, as this appears to be the leading approach for seeking clinical benefit from anti-angiogenic agents in the pediatric setting.**

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