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Pediatric Preclinical Testing Program (PPTP) evaluation of the MEK1/2 inhibitor AZD6244 (ARRY-142886)

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Abstract

Background: AZD6244 is a potent, selective, and uncompetitive inhibitor of MEK1/2 kinases that is currently in phase 2 clinical development. The activity of AZD6244 was evaluated against the PPTP's *in vitro* and *in vivo* panels.

Methods: The PPTP includes a molecularly characterized *in vitro* panel of cell lines (n=27) and *in vivo* panel of xenografts (n=61) representing most of the common types of childhood solid tumors and childhood acute lymphoblastic leukemia (ALL). AZD6244 was tested *in vitro* at concentrations from 1.0 nM to 10 µM and was tested against the PPTP *in vivo* panel using a BID schedule (excepting weekends for which a QD schedule was used), with oral administration for 6 weeks at a dose of 100 mg/kg. Three measures of antitumor activity were used: 1) an objective response measure modeled after the clinical setting; 2) a treated to control (T/C) tumor volume measure; and 3) a time to event (4-fold increase in tumor volume) measure based on the median event-free survival (EFS) of treated and control animals for each xenograft.

Results: AZD6244 demonstrated a clear cytotoxic effect against Kasumi-1, an AML cell line with an activating KIT mutation. The IC_{50} for Kasumi-1 was 200 nM, similar to IC_{50} values for AZD6244 in adult cancer cell lines with activating BRAF or RAS family mutations. Several other cell lines showed a limited response to AZD6244 that was consistent with a primarily cytostatic effect, while 18 cell lines had IC_{50} values > 10 µM. AZD6244 was well tolerated *in vivo* with toxicity in 2.6% of treated animals compared to 0% of control animals. AZD6244 significantly increased EFS in 10 of 37 (27%) evaluable solid tumor xenografts. Significant differences in EFS distribution occurred in the majority of xenografts in the glioblastoma panel (3 of 4) and in one-half of the xenografts from the osteosarcoma panel (3 of 6). None of the 6 evaluable ALL xenografts demonstrated significant increases in EFS. The EFS T/C values were below the criteria for intermediate activity for the time to event measure of activity (EFS T/C > 2) in all but three evaluable lines: the GBM xenograft BT-39 and two osteosarcoma xenografts (OS-1 and OS-33). The best objective response was PD2 (progressive disease with growth delay), with PD2 activity concentrated in the glioblastoma panel (2 of 4) and the osteosarcoma panel (3 of 6).

Conclusions: AZD6244 was highly active against a PPTP cell line with an activating KIT mutation, but was not active against the majority of the cell lines of the PPTP *in vitro* panel and did not significantly inhibit growth for most of the xenografts in the PPTP *in vivo* panel. These observations are consistent with the relative paucity of BRAF and RAS family mutations in the pediatric cancers included in this evaluation. Combinations of AZD6244 with agents targeting other signaling pathways involved in survival/proliferation are of interest for future PPTP evaluations of AZD6244. (Supported by NCI NO1CM42216)

In Vitro Test Results for AZD6244

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 1.0 nM to 10 µM with replicates of 6 per data point. Data were analyzed using Kaleidagraph (Synergy), fitting a non-linear regression model-sigmoidal dose-response model to the response-relative fluorescence values *vs.* the concentration.

Cell Line	Status	Histology	Minimum T/C	EC_{50} (nM)	IC_{50} (nM)
RD		Rhabdomyosarcoma	29.0	122	430
Rh41	Post-Therapy	Rhabdomyosarcoma	71.5	>10,000	>10,000
Rh18	Diagnosis	Rhabdomyosarcoma	27.2	1427	1433
Rh30	Diagnosis	Rhabdomyosarcoma	82.1	>10,000	>10,000
BT-12	Diagnosis	Rhabdoid	69.7	>10,000	>10,000
CHLA-266	Diagnosis	Rhabdoid	61.4	>10,000	>10,000
TC-71[1]	Post-Therapy	Ewing	81.8	>10,000	>10,000
CHLA-9	Diagnosis	Ewing	69.2	>10,000	>10,000
CHLA-10	Post-Therapy	Ewing	75.4	>10,000	>10,000
CHLA-258	Post-Bone Marrow Transplant	Ewing	75.1	>10,000	>10,000
SJ-GBM2	Post-Therapy	Glioblastoma	67.3	>10,000	>10,000
NB-1643	Diagnosis	Neuroblastoma	98.6	>10,000	>10,000
NB-EBc1	Post-Therapy	Neuroblastoma	39.9	36	356
CHLA-90	Post-Bone Marrow Transplant	Neuroblastoma	52.1	>10,000	>10,000
CHLA-136	Post-Bone Marrow Transplant	Neuroblastoma	81.1	>10,000	>10,000
COG-LL-317	Post-Therapy	ALL T-cell	68.2	>10,000	>10,000
NALM-6	Post-Therapy	ALL B-precursor	72.1	>10,000	>10,000
RS4;11[1]	Post-Therapy	ALL B-precursor	100.0	>10,000	>10,000
MOLT-4	Post-Therapy	ALL T-cell	41.5	149	3450
CCRF-CEM		ALL T-cell	93.6	>10,000	>10,000
Kasumi-1	Post-Bone Marrow Transplant	AML	2.4	199.91	200
Karpas-299	Post-Therapy	ALCL	52.6	>10,000	>10,000
Ramos-RA1		NHL	94.5	>10,000	>10,000
Median			29.0	>10,000	>10,000
Minimum			71.5	36	200
Maximum			27.2	>10,000	>10,000

- AZD6244 *in vitro* activity was limited to a minority of the 23 cell lines tested.
- Kasumi-1 was the most responsive cell line and the only cell line with a clear cytotoxic response to AZD6244.
- Kasumi-1 has an activating KIT point mutation and is also sensitive to RTK small molecule inhibitors that block KIT activity.
- Other PPTP cell lines that had T/C values < 50% at the highest concentration tested included two rhabdomyosarcoma cell lines (RD and Rh18), a neuroblastoma cell line (NB-EBc1), and a T-cell ALL cell line (MOLT-4).
- Each of the cell lines had minimum T/C values > 25%, suggesting a growth inhibitory rather than a cytotoxic response to AZD6244.

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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.

➢Drug: AZD6244 was provided to the Pediatric Preclinical Testing Program by AstraZeneca through the Cancer Therapy Evaluation Program (NCI). AZD6244 was dissolved in a mixture of 0.5% hydroxypropyl methyl cellulose, 0.1% Polysorbate 80, and administered twice daily (except weekends, which were SID) by oral gavage for 42 days, at a dose of 100 mg/kg. AZD6244 was provided to each testing site in coded vials for blinded testing according the PPTP's standard operating procedures.

➢Solid Tumor Response Criteria:

Response		Definition	Score
PD1	Progressive Disease 1	>25% increase in tumor volume, TGD value of ≤1.5	0
PD2	Progressive Disease 2	>25% increase in tumor volume, TGD value of >1.5	2
SD	Stable Disease	≤25% increase, <50% regression	4
PR	Partial Response	≥50% regression	6
CR	Complete Response	<0.1 cm^3 tumor volume	8
MCR	Maintained CR	<0.1 cm^3 tumor volume at the end of study	10

➢Leukemia Response Criteria:

Response		Definition	Score
PD1	Progressive Disease 1	CD45% never drops below 1%, events before end of study, TGD value of ≤1.5	0
PD2	Progressive Disease 2	CD45% never drops below 1%, events 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% <1% for only 1 week	6
CR	Complete Response	CD45% <1% for 2 consecutive weeks	8
MCR	Maintained CR	CD45% <1% for 2 consecutive weeks and at end of study CD45% <1%	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.

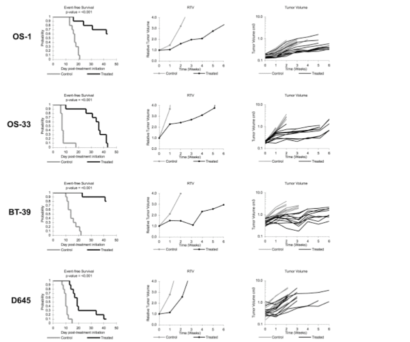
AZD6244 in Vivo Activity

Xenograft Line	Histology	P-value	EFS T/C	Median Final RTV	Tumor Volume T/C	P-Value	Heat Map
BT-29	Rhabdoid	<0.001	1.8	>4	0.43	0.005	PD2
KT-14	Rhabdoid	0.668	1.1	>4	0.95	0.739	PD1
KT-12	Rhabdoid	0.104	1.4	>4	0.86	0.447	PD1
KT-10	Wilms	0.759	1.2	>4	0.96	0.971	PD1
KT-11	Wilms	0.229	1.2	>4	0.73	0.258	PD1
KT-13	Wilms	0.582	1	>4	0.93	0.965	PD1
SK-NEP-1	Ewing	0.368	1.3	>4	0.75	0.218	PD1
EW5	Ewing	0.317	0.9	>4	1.19	0.247	PD1
EW8	Ewing	0.246	1.1	>4	0.95	0.684	PD1
TC-71	Ewing	0.166	1.2	>4	0.83	0.353	PD1
CHLA258	Ewing	0.883	0.8	>4	1.19	0.353	PD1
Rh28	ALV RMS	0.299	1.9	>4	0.7	0.315	PD2
Rh30	ALV RMS	0.002	1.4	>4	0.65	0.007	PD1
Rh30R	ALV RMS	0.747	1	>4	0.88	0.912	PD1
Rh65	ALV RMS	0.482	1	>4	0.97	0.853	PD1
Rh18	EMB RMS	0.06	1.1	>4	0.94	0.360	PD1
Rh36	EMB RMS	0.486	0.8	>4	1.13	0.393	PD1
BT-28	Medulloblastoma	0.35	1	>4	0.87	0.315	PD1
BT-45	Medulloblastoma	0.112	0.8	>4	1.1	0.436	PD1
BT-46	Medulloblastoma	0.582	0.9	>4	1.05	0.853	PD1
BT-44	Ependymoma	0.237	1	>4	1.02	0.280	PD1
GBM2	Glioblastoma	0.139	1.2	>4	0.87	0.353	PD1
BT-39	Glioblastoma	<0.001	> 3.0	2.9	0.4	<0.001	PD2
D645	Glioblastoma	<0.001	1.9	>4	0.38	<0.001	PD2
D456	Glioblastoma	0.011	1.3	>4	0.77	0.015	PD1
NB-SD	Neuroblastoma	0.003	1.3	>4	0.55	0.113	PD1
NB-1771	Neuroblastoma	0.996	1	>4	0.85	0.182	PD1
NB-1691	Neuroblastoma	0.09	1.2	>4	0.68	0.280	PD1
NB-EBc1	Neuroblastoma	0.916	1	>4	0.86	0.863	PD1
CHLA-79	Neuroblastoma	0.302	1.2	>4	0.61	0.165	PD1
NB-1643	Neuroblastoma	0.004	1.4	>4	0.37	0.007	PD1
OS-1	Osteosarcoma	<0.001	> 2.5	3.4	0.55	<0.001	PD2
OS-2	Osteosarcoma	0.252	1.2	>4	0.77	0.007	PD1
OS-17	Osteosarcoma	0.025	1.5	>4	0.74	0.113	PD2
OS-9	Osteosarcoma	0.091	1.1	>4	0.87	0.105	PD1
OS-33	Osteosarcoma	<0.001	4.9	>4	0.56	<0.001	PD2
OS-31	Osteosarcoma	0.287	1.2	>4	0.76	0.182	PD1
ALL-2	ALL B-precursor	0.01	0.8	>25	.	.	PD1
ALL-3	ALL B-precursor	0.096	5.1	>25	.	.	PD2
ALL-4	ALL B-precursor	0.225	0.9	>25	.	.	PD1
ALL-7	ALL B-precursor	0.149	1.4	>25	.	.	PD1
ALL-16	ALL T-cell	0.13	1.6	>25	.	.	PD1
ALL-19	ALL B-precursor	0.208	4.2	>25	.	.	PD2

Red shading in the p-value columns indicates a significant difference in EFS distribution or Tumor Volume T/C 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.

AZD6244 was provided to the PPTP by AstraZeneca. Testing was supported by NCI NO1CM42216

AZD6244 *In Vivo* Activity



CONCLUSIONS

- AZD6244 *in vitro* activity was most pronounced for Kasumi-1, an AML cell line with an activating KIT mutation. The response of Kasumi-1 to AZD6244 is similar to that previously described for AZD6244 against selected B-Raf and Ras mutant cell lines. Other PPTP cell lines showed only limited response to AZD6244.
- AZD6244 was well tolerated at the dose and schedule used for *in vivo* testing.
- Significant differences in EFS distribution occurred in the majority of xenografts in the glioblastoma panel (3 of 4) and in one-half of the xenografts from the osteosarcoma panel (3 of 6), but in none of the evaluable xenografts in the Ewing, Wilms, neuroblastoma, and ALL panels.
- AZD6244 did not induce objective responses in any of the solid tumor panels or in the ALL panel. The best response to AZD6244 was PD2 (progressive disease with growth delay), with PD2 activity concentrated in the glioblastoma panel (2 of 4) and the osteosarcoma panel (3 of 6).
- Constitutive phosphorylation of ERK was documented in the PPTP osteosarcoma xenografts (data not shown), indicating baseline MEK activation for the xenografts in this panel.
- Potential areas of future focus in PPTP evaluations of AZD6244 include:
  - Documenting the extent and duration of MEK inhibition at the dose/schedule evaluated for efficacy testing, and
  - Evaluating selected combinations of AZD6244 with other signal transduction inhibitors (e.g., rapamycin).