

# 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 practice 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<sub>50</sub> for Kasumi-1 was 200 nM. Similar to IC<sub>50</sub> 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 16 cell lines had IC<sub>50</sub> 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 (2 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 N01CM42216)

## In Vitro Test Results for AZD6244

**Methods:** *In vitro* testing was performed using DMISCAN, a semi-automated fluorescence-based digital imaging microscopy system that quantifies viable (using fluorescent fluorescence) 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	IC50 (nM)	IC50 (μM)	IC50 (mM)
ALL-1	PD1	ALL	0.0001	0.0001	0.0001
ALL-2	PD1	ALL	0.0001	0.0001	0.0001
ALL-3	PD1	ALL	0.0001	0.0001	0.0001
ALL-4	PD1	ALL	0.0001	0.0001	0.0001
ALL-5	PD1	ALL	0.0001	0.0001	0.0001
ALL-6	PD1	ALL	0.0001	0.0001	0.0001
ALL-7	PD1	ALL	0.0001	0.0001	0.0001
ALL-8	PD1	ALL	0.0001	0.0001	0.0001
ALL-9	PD1	ALL	0.0001	0.0001	0.0001
ALL-10	PD1	ALL	0.0001	0.0001	0.0001
ALL-11	PD1	ALL	0.0001	0.0001	0.0001
ALL-12	PD1	ALL	0.0001	0.0001	0.0001
ALL-13	PD1	ALL	0.0001	0.0001	0.0001
ALL-14	PD1	ALL	0.0001	0.0001	0.0001
ALL-15	PD1	ALL	0.0001	0.0001	0.0001
ALL-16	PD1	ALL	0.0001	0.0001	0.0001
ALL-17	PD1	ALL	0.0001	0.0001	0.0001
ALL-18	PD1	ALL	0.0001	0.0001	0.0001
ALL-19	PD1	ALL	0.0001	0.0001	0.0001
ALL-20	PD1	ALL	0.0001	0.0001	0.0001
ALL-21	PD1	ALL	0.0001	0.0001	0.0001
ALL-22	PD1	ALL	0.0001	0.0001	0.0001
ALL-23	PD1	ALL	0.0001	0.0001	0.0001
ALL-24	PD1	ALL	0.0001	0.0001	0.0001
ALL-25	PD1	ALL	0.0001	0.0001	0.0001
ALL-26	PD1	ALL	0.0001	0.0001	0.0001
ALL-27	PD1	ALL	0.0001	0.0001	0.0001
ALL-28	PD1	ALL	0.0001	0.0001	0.0001
ALL-29	PD1	ALL	0.0001	0.0001	0.0001
ALL-30	PD1	ALL	0.0001	0.0001	0.0001
ALL-31	PD1	ALL	0.0001	0.0001	0.0001
ALL-32	PD1	ALL	0.0001	0.0001	0.0001
ALL-33	PD1	ALL	0.0001	0.0001	0.0001
ALL-34	PD1	ALL	0.0001	0.0001	0.0001
ALL-35	PD1	ALL	0.0001	0.0001	0.0001
ALL-36	PD1	ALL	0.0001	0.0001	0.0001
ALL-37	PD1	ALL	0.0001	0.0001	0.0001
ALL-38	PD1	ALL	0.0001	0.0001	0.0001
ALL-39	PD1	ALL	0.0001	0.0001	0.0001
ALL-40	PD1	ALL	0.0001	0.0001	0.0001
ALL-41	PD1	ALL	0.0001	0.0001	0.0001
ALL-42	PD1	ALL	0.0001	0.0001	0.0001
ALL-43	PD1	ALL	0.0001	0.0001	0.0001
ALL-44	PD1	ALL	0.0001	0.0001	0.0001
ALL-45	PD1	ALL	0.0001	0.0001	0.0001
ALL-46	PD1	ALL	0.0001	0.0001	0.0001
ALL-47	PD1	ALL	0.0001	0.0001	0.0001
ALL-48	PD1	ALL	0.0001	0.0001	0.0001
ALL-49	PD1	ALL	0.0001	0.0001	0.0001
ALL-50	PD1	ALL	0.0001	0.0001	0.0001
ALL-51	PD1	ALL	0.0001	0.0001	0.0001
ALL-52	PD1	ALL	0.0001	0.0001	0.0001
ALL-53	PD1	ALL	0.0001	0.0001	0.0001
ALL-54	PD1	ALL	0.0001	0.0001	0.0001
ALL-55	PD1	ALL	0.0001	0.0001	0.0001
ALL-56	PD1	ALL	0.0001	0.0001	0.0001
ALL-57	PD1	ALL	0.0001	0.0001	0.0001
ALL-58	PD1	ALL	0.0001	0.0001	0.0001
ALL-59	PD1	ALL	0.0001	0.0001	0.0001
ALL-60	PD1	ALL	0.0001	0.0001	0.0001
ALL-61	PD1	ALL	0.0001	0.0001	0.0001
ALL-62	PD1	ALL	0.0001	0.0001	0.0001
ALL-63	PD1	ALL	0.0001	0.0001	0.0001
ALL-64	PD1	ALL	0.0001	0.0001	0.0001
ALL-65	PD1	ALL	0.0001	0.0001	0.0001
ALL-66	PD1	ALL	0.0001	0.0001	0.0001
ALL-67	PD1	ALL	0.0001	0.0001	0.0001
ALL-68	PD1	ALL	0.0001	0.0001	0.0001
ALL-69	PD1	ALL	0.0001	0.0001	0.0001
ALL-70	PD1	ALL	0.0001	0.0001	0.0001
ALL-71	PD1	ALL	0.0001	0.0001	0.0001
ALL-72	PD1	ALL	0.0001	0.0001	0.0001
ALL-73	PD1	ALL	0.0001	0.0001	0.0001
ALL-74	PD1	ALL	0.0001	0.0001	0.0001
ALL-75	PD1	ALL	0.0001	0.0001	0.0001
ALL-76	PD1	ALL	0.0001	0.0001	0.0001
ALL-77	PD1	ALL	0.0001	0.0001	0.0001
ALL-78	PD1	ALL	0.0001	0.0001	0.0001
ALL-79	PD1	ALL	0.0001	0.0001	0.0001
ALL-80	PD1	ALL	0.0001	0.0001	0.0001
ALL-81	PD1	ALL	0.0001	0.0001	0.0001
ALL-82	PD1	ALL	0.0001	0.0001	0.0001
ALL-83	PD1	ALL	0.0001	0.0001	0.0001
ALL-84	PD1	ALL	0.0001	0.0001	0.0001
ALL-85	PD1	ALL	0.0001	0.0001	0.0001
ALL-86	PD1	ALL	0.0001	0.0001	0.0001
ALL-87	PD1	ALL	0.0001	0.0001	0.0001
ALL-88	PD1	ALL	0.0001	0.0001	0.0001
ALL-89	PD1	ALL	0.0001	0.0001	0.0001
ALL-90	PD1	ALL	0.0001	0.0001	0.0001
ALL-91	PD1	ALL	0.0001	0.0001	0.0001
ALL-92	PD1	ALL	0.0001	0.0001	0.0001
ALL-93	PD1	ALL	0.0001	0.0001	0.0001
ALL-94	PD1	ALL	0.0001	0.0001	0.0001
ALL-95	PD1	ALL	0.0001	0.0001	0.0001
ALL-96	PD1	ALL	0.0001	0.0001	0.0001
ALL-97	PD1	ALL	0.0001	0.0001	0.0001
ALL-98	PD1	ALL	0.0001	0.0001	0.0001
ALL-99	PD1	ALL	0.0001	0.0001	0.0001
ALL-100	PD1	ALL	0.0001	0.0001	0.0001

- 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 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 these cell lines had minimum T/C values > 25%, suggesting a growth inhibitory rather than a cytotoxic response to AZD6244.

## 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 model).

> **Solid tumor testing:** For each xenograft line, 10 mice bearing SC tumors initiated treatment when the tumors were between 0.2-0.5 cm<sup>3</sup>. 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)×d<sup>3</sup>, where d represents the mean diameter.

> **Acute lymphoblastic leukemia testing:** For each xenograft line, 5 mice were inoculated with 3-5 × 10<sup>6</sup> 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 BID) 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 to 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, >25% regression	4
PR	Partial Response	≥35% regression	6
CR	Complete Response	<0.1 cm <sup>3</sup> tumor volume	8
MCR	Maintained CR	<0.1 cm <sup>3</sup> tumor volume at 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 3 consecutive weeks	8
MCR	Maintained CR	CD45+ <1% for consecutive weeks and 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
1 + AS 01	PD1
1 + AS 02	PD2
3 + AS 03	SD
1 + AS 07	PR
3 + AS 08	CR
1 + AS 09	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
EWS	Ewing	0.317	0.9	>4	1.19	0.247	PD1
EWS	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	0.833	0.6	>4	1.19	0.353	PD1
Rh20	ALV RMS	0.299	1.9	>4	0.7	0.315	PD1
Rh30	ALV RMS	0.002	1.4	>4	0.65	0.007	PD1
Rh39R	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	PD2
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			