Abstract: LB-318

Pediatric Preclinical Testing Program (PPTP) Evaluation of the JAK Inhibitor AZD1480

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AZD1480

- ❑AZD1480 is a potent, competitive small molecule inhibitor of JAK1/2 kinase that has entered clinical evaluation.
- ❑The JAK/STAT pathway is active in a number of childhood cancers. JAK inhibition is of particular interest given the activating JAK1/2 mutations observed in a subset of pediatric acute lymphoblastic leukemia (ALL) cases.
- ❑AZD1480 was evaluated against the PPTP's *in vitro* and *in vivo* panels.

AZD1480 *IN VITRO* ACTIVITY

- ❑The median relative IC_{50} (rIC_{50}) for AZD1480 against the PPTP cell lines was 1.5 μM, with a range from 0.3 μM to 5.9 μM.
- ❑Neuroblastoma cell lines were relatively sensitive to AZD1480 (median rIC_{50} = 0.9 μM) with each cell line having a rIC_{50} value lower than the median for the entire panel.

Cell Line	Histotype	rIC_{50} (μM)	Panel rIC_{50} /Line rIC_{50}	Ymin (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**

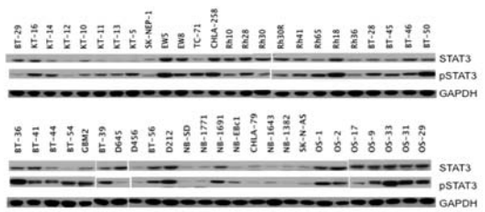
AZD1480 was provided for testing by AstraZeneca, and JAK-mutated ALL xenografts were developed in collaboration with the Children's Oncology Group. Testing was supported by NCI NO1CM42216.

AZD1480 *IN VIVO* ACTIVITY

Line	Tumor Type	Median Time to Event	P-value	EFS T/C	EFS Activity	Response
BT-29	Rhabdoid	> EP	<0.001	> 1.8	NE	PD2
KT-14	Rhabdoid	> EP	<0.001	> 2.1	Int	PD2
KT-10	Wilms	> EP	<0.001	> 4.2	High	MCR
KT-13	Wilms	32.9	<0.001	3.0	Int	PD2
SK-NEP-1	Ewing	27.5	<0.001	2.6	Int	PD2
EW5	Ewing	11.1	0.004	1.6	Low	PD2
EW8	Ewing	15.2	0.033	1.6	Low	PD1
CHLA258	Ewing	27.6	<0.001	2.0	Int	PD2
Rh10	ALV RMS	29.3	<0.001	2.6	Int	PD2
Rh30R	ALV RMS	29.3	<0.001	1.5	Low	PD1
Rh41	ALV RMS	21.0	<0.001	1.8	Low	PD2
BT-28	Medulloblastoma	33.5	<0.001	2.1	Int	PD2
GBM2	Glioblastoma	17.0	0.003	1.8	Low	PD2
BT-39	Glioblastoma	17.3	<0.001	2.2	Int	PD2
D645	Glioblastoma	8.7	0.063	1.5	Low	PD1
D456	Glioblastoma	13.2	0.003	2.1	Int	PD2
NB-SD	Neuroblastoma	23.8	0.010	1.9	Low	PD2
NB-1771	Neuroblastoma	18.6	<0.001	2.1	Int	PD2
NB-1691	Neuroblastoma	10.3	0.036	1.8	Low	PD2
NB-1643	Neuroblastoma	25.9	<0.001	2.1	Int	PD2
OS-1	Osteosarcoma	40.8	<0.001	1.4	Low	PD1
OS-2	Osteosarcoma	26.6	<0.001	1.5	Low	PD1
OS-17	Osteosarcoma	30.0	<0.001	1.5	Low	PD2
OS-9	Osteosarcoma	24.2	0.007	1.3	Low	PD1
OS-33	Osteosarcoma	20.4	<0.001	1.3	Low	PD1
OS-31	Osteosarcoma	28.8	<0.001	1.4	Low	PD1
Karpas-299	ALCL	10.0	0.164	1.1	Low	PD1
ALL-4	ALL B-precursor	7.7	0.951	1.1	Low	PD1
ALL-10	ALL B-precursor	8.4	0.163	1.7	Low	PD2
ALL-31	T-Cell ALL	7.1	0.214	0.9	Low	PD1
MLL-5	ALL B-precursor	4.3	0.837	1.1	Low	PD1
TGT-020	ALL JAK2 R867Q	23.6	<0.001	2.2	Int	PD2
TGT-047	ALL JAK2 R683G	8.0	0.471	1.0	Low	PD1
TGT-052	ALL JAK1 V658F	7.9	0.028	0.4	Low	PD1
TGT-144	ALL JAK1 L624_R629>W	14.9	0.090	0.8	Low	PD1
TGT-174	ALL JAK2 P933R	2.3	0.682	0.8	Low	PD1

- **PD1 (Progressive Disease 1):** >25% ↑ in tumor volume, TGD value ≤1.5; **PD2 (Progressive Disease 2):** >25% ↑ in tumor volume, TGD value >1.5; **SD (Stable Disease):** <25% ↑ in tumor volume, <50% regression
- **PR (Partial response):** a tumor volume regression ≥50% for at least one time point but with measurable tumor (≥ 0.10 cm^3). **CR (Complete response):** disappearance of measurable tumor mass (< 0.10 cm^3) for at least one time point. A complete response was considered **maintained (MCR)** if the tumor volume was <0.10 cm^3 at the end of the study period.
- **Red shading in the p-value columns indicates a significant difference in EFS distribution between treated and control groups. Blue shading highlights xenografts that have EFS T/C > 2.0**

pSTAT3 EXPRESSION FOR PPTP XENOGRAFTS



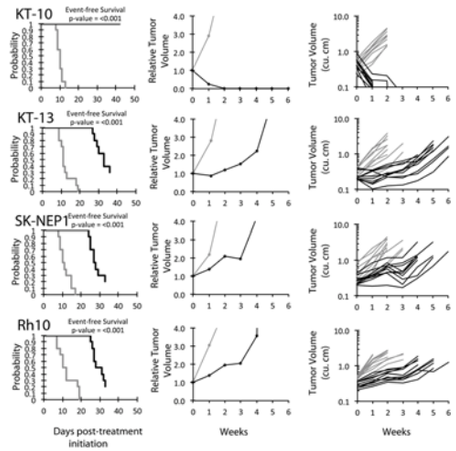


Figure 1. AZD1480 activity *in vivo* against individual solid tumor xenografts using a dose of 60 mg/kg dose administered by oral gavage daily x 5 for 3 weeks. Kaplan-Meier curves for EFS (left), median relative tumor volume graphs (center), and individual tumor volume graphs (right) are shown for selected lines. Controls (gray lines); Treated (black lines).

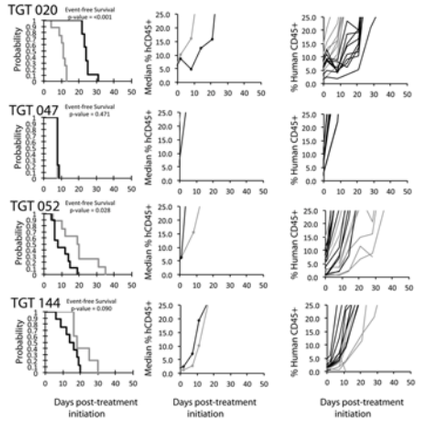


Figure 2. AZD1480 activity *in vivo* against individual JAK2 mutated ALL xenografts at 10 mg/kg administered twice daily (with a single daily dose of 15 mg/kg on weekends). Kaplan-Meier curves for EFS (left), median relative tumor volume graphs (center), and individual tumor volume graphs (right) are shown for selected lines. Controls (gray lines); Treated (black lines).

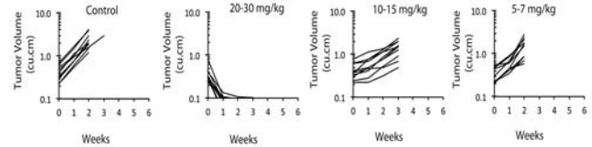


Figure 3. Dose response to AZD1480 in KT-10 Wilms tumor xenografts tumor xenografts. Mice received AZD1480 at 20 mg/kg twice daily (BID) x 5 and 30 mg/kg SID at weekends (20-30), 10 mg/kg BID and 15 mg/kg SID (weekends; 10-15) or 5 mg/kg BID and 7 mg/kg SID weekends; 5-7), or vehicle (Control) by oral gavage

IN VIVO RESULTS AND CONCLUSIONS

- ❑ AZD1480 induced significant differences in EFS distribution compared to control in 25 of 27 (93%) evaluable solid tumor models using a 60 mg/kg dose administered by oral gavage daily x 5 x 3 wks.
- ❑ AZD1480 induced tumor growth inhibition with EFS T/C > 2 in 11 of 26 (42%) solid tumor xenografts.
- ❑ AZD1480 induced a maintained CR in the KT-10 Wilms tumor xenograft. There was a sharp dose response curve for KT-10 with tumor regressing activity lost at doses below 40 mg/kg/day.
- ❑ For the ALL panel (using NOD-SCID mice), the MTD was lower, and a twice daily schedule was utilized: 10 mg/kg administered twice daily with a single daily dose of 15 mg/kg on weekends.
- ❑ Only 1 of 9 (11%) of the evaluable ALL xenografts showed significant delay in time to event.
- ❑ JAK mutated ALL xenografts were prioritized for testing to evaluate whether AZD1480 would show high activity in leukemia models in which the JAK-STAT pathway is activated by mutation. These models show phospho-STAT5 as evidence of JAK-STAT signaling. However, the only xenograft with EFS T/C > 2 was a JAK2 mutant (R867Q) model (TGT-020).
- ❑ Phospho-STAT3 (pSTAT3) is present in many PPTP solid tumor xenografts. However, there is no relationship between pSTAT3 expression and response to AZD1480. For example, NB-1643 shows low expression of pSTAT3, but has EFS T/C >2, while most of the osteosarcoma xenografts show marked pSTAT3 expression but have EFS T/C < 2. pSTAT3 is expressed by KT-10, but the expression is not prominent for KT-10 in comparison to other PPTP xenografts.
- ❑ Whole genome sequencing is in process for the PPTP xenografts and may identify the genomic basis for the complete response of KT-10 to AZD1480.