Abstract: LB-317

Pediatric Preclinical Testing Program (PPTP) Evaluation of Volasertib (BI 6727), a Polo-Like Kinase (Plk) Inhibitor

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VOLASERTIB (BI 6727)

- Volasertib is a first in class, selective and potent cell cycle kinase inhibitor that induces mitotic arrest and apoptosis by targeting Plk.
- Genomic and small molecule kinase inhibitor screens have identified Plk1 as a potential therapeutic target for several pediatric cancers, including rhabdomyosarcoma, neuroblastoma, and osteosarcoma.
- Clinical development of volasertib is ongoing, with phase I/II trials investigating monotherapy and combination with chemotherapy or small molecule inhibitors in solid tumors and hematologic malignancies.

VOLASERTIB (BI 6727) *IN VITRO* ACTIVITY

- Volasertib potently inhibited PPTP cell lines with a median relative IC_{50} (rIC_{50}) of 14.1 nM, (range 6.0 nM – 135 nM).
- The median rIC_{50} values were lowest for the ALL cell line panel compared to the remaining cell lines (11.9 versus 16.0 nM, respectively), but this difference was not significant, and overall there were no differences in rIC_{50} by histotype.
- Volasertib induced Ymin values approaching 0% for most PPTP cell lines at the highest concentration tested (1.0 μM).

Cell Line	Histotype	rIC_{50} (nM)	Panel rIC_{50}/ Line rIC_{50}	Y_{min} (%) (Observed)	Y_{min} (%) (Model Based)
RD	Rhabdomyosarcoma	16.5	0.85	1.7	0.0
Rh41	Rhabdomyosarcoma	6.9	2.04	7.7	11.4
Rh18	Rhabdomyosarcoma	135.2	0.10	20.2	0.0
Rh30	Rhabdomyosarcoma	8.2	1.71	11.0	12.8
BT-12	Rhabdoid	55.7	0.25	13.1	15.2
CHLA-266	Rhabdoid	47.5	0.30	9.2	10.1
TC-71	Ewing sarcoma	13.8	1.02	0.1	0.0
CHLA-9	Ewing sarcoma	17.7	0.80	0.5	0.0
CHLA-10	Ewing sarcoma	17.3	0.82	1.9	0.0
CHLA-258	Ewing sarcoma	37.4	0.38	6.1	5.8
SJ-GBM2	Glioblastoma	11.8	1.19	1.9	0.0
NB-1643	Neuroblastoma	15.5	0.91	4.4	5.5
NB-EBc1	Neuroblastoma	34.5	0.41	0.7	0.0
CHLA-90	Neuroblastoma	24.6	0.58	14.9	15.4
CHLA-136	Neuroblastoma	6.0	2.35	1.9	3.3
NALM-6	ALL	12.7	1.11	0.0	0.0
COG-LL-317	ALL	11.3	1.25	0.0	0.0
RS4;11	ALL	7.8	1.82	0.8	0.0
MOLT-4	ALL	12.2	1.16	0.0	0.0
CCRF-CEM (1)	ALL	11.5	1.23	0.0	0.0
CCRF-CEM (2)	ALL	14.2	1.00	0.0	0.0
Kasumi-1	AML	14.1	1.00	1.2	3.5
Karpas-299	ALCL	14.9	0.95	0.5	0.0
Ramos-RA1	NHL	13.0	1.08	0.0	0.0
Median		**14.1**	**1.00**	**1.5**	**0.00**
Minimum		**6.0**	**0.10**	**0.0**	**0.00**
Maximum		**135.2**	**2.35**	**20.2**	**15.38**

VOLASERTIB *IN VIVO* ACTIVITY

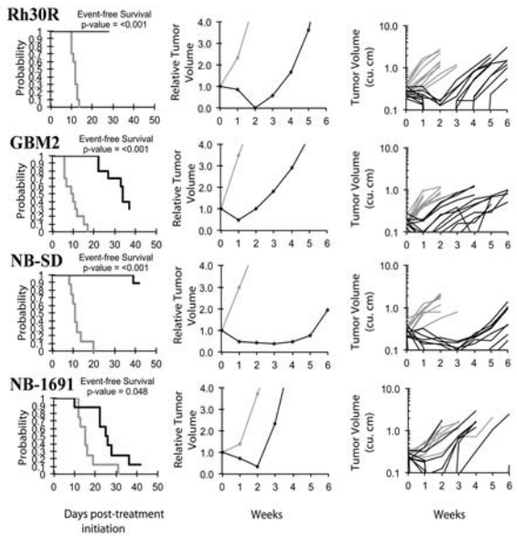

Figure 1. Volasertib activity *in vivo* at 30 mg/kg administered IV weekly x 3 against individual solid tumor xenografts. Responses of Rh30R (rhabdomyosarcoma), GBM2 (glioblastoma) and neuroblastomas (NB-SD and NB-1691) established as xenografts from previously treated patients. Kaplan-Meier curves for 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), significances of the difference between treated and control groups are included.

PPTP *IN VITRO* & *IN VIVO* TESTING METHODS

***In vitro*: *In vitro* testing was performed using DIMSCAN, a semiautomatic fluorescence-based digital image microscopy system (Kang MH, et al. Pediatr Blood Cancer 56:239-249, 2011). Testing was for 96 hours at concentrations from 1.0 nM to 100 μM with replicates of 6-12 per data point.**

***In vivo*: Standard PPTP methods for in vivo testing were employed (see http://pptp.nchresearch.org/documents/detailedAnalysisMethods.pdf). Volasertib was tested *in vivo* using a 30 mg/kg dose administered intravenously every 7 days x 3.**

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.

Acute lymphoblastic leukemia (ALL) 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.

Line	Tumor Type	Median Time to Event	P-value	EFS T/C	Tumor Volume T/C	EFS Activity	Response
BT-29	Rhabdoid	31.2	0.091	1.3	0.91	Low	PD1
KT-14	Rhabdoid	25.6	**<0.001**	1.2	0.70	Low	PD1
KT-12	Rhabdoid	> EP	**<0.001**	**> 2.3**	0.36	**Int**	PD2
KT-10	Wilms	19.2	**0.049**	1.2	0.58	Low	PD1
KT-13	Wilms	30.3	**<0.001**	**2.1**	0.26	**Int**	PD2
SK-NEP-1	Ewing	23.8	**<0.001**	**2.8**	0.29	**Int**	PD2
EW5	Ewing	8.0	0.198	1.2	0.86	Low	PD1
TC-71	Ewing	11.5	0.300	1.2	0.84	Low	PD1
CHLA258	Ewing	20.1	0.168	1.2	0.79	Low	PD1
Rh10	ALV RMS	20.3	0.066	1.1	0.76	Low	PD1
Rh28	ALV RMS	20.1	**0.006**	1.5	0.63	Low	PD1
Rh30	ALV RMS	19.4	0.052	1.7	0.73	Low	PD2
Rh30R	ALV RMS	36.9	**<0.001**	**3.1**	0.31	**Int**	CR
Rh18	EMB RMS	14.9	0.652	1.3	0.79	Low	PD1
BT-28	Medulloblastoma	5.9	0.513	0.9	1.09	Low	PD1
BT-45	Medulloblastoma	6.2	0.157	0.9	1.10	Low	PD1
BT-50	Medulloblastoma	> EP	0.474	.	0.38	*NE*	SD
GBM2	Glioblastoma	33.7	**<0.001**	**3.5**	0.17	**Int**	CR
BT-39	Glioblastoma	12.6	0.843	1.1	0.59	Low	PD1
D645	Glioblastoma	13.0	**<0.001**	**2.1**	0.26	**Int**	PD2
NB-SD	Neuroblastoma	> EP	**<0.001**	**> 3.8**	0.08	**Int**	CR
NB-1771	Neuroblastoma	16.3	**0.006**	**2.4**	0.47	**Int**	PD2
NB-1691	Neuroblastoma	25.4	**0.048**	1.7	0.40	Low	CR
NB-EBc1	Neuroblastoma	13.1	**<0.001**	**2.6**	0.22	**Int**	PD2
CHLA-79	Neuroblastoma	10.0	0.155	0.8	1.74	Low	PD1
NB-1643	Neuroblastoma	27.3	**<0.001**	**2.9**	0.30	**Int**	SD
OS-1	Osteosarcoma	> EP	**0.001**	> 1.1	0.52	*NE*	PD2
OS-2	Osteosarcoma	22.2	**0.026**	1.2	0.84	Low	PD1
OS-17	Osteosarcoma	21.5	0.277	1.0	0.93	Low	PD1
OS-9	Osteosarcoma	> EP	**<0.001**	**> 2.2**	0.53	**Int**	PD2
OS-33	Osteosarcoma	30.6	**0.014**	1.4	0.75	Low	PD1
OS-31	Osteosarcoma	27.7	**<0.001**	1.6	0.63	Low	PD2
ALL-2	ALL B-precursor	22.5	0.082	1.4	.	Low	PD1
ALL-7	ALL B-precursor	35.8	**<0.001**	**4.0**	.	**Int**	CR
ALL-8	ALL T-cell	34.8	**<0.001**	**4.9**	.	**Int**	CR
ALL-17	ALL B-precursor	17.1	**<0.001**	**2.4**	.	**Int**	PD2
ALL-19	ALL B-precursor	20.8	0.379	2.1	.	Low	PD2

- **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**

Volasertib (BI 6727) was provided for testing by Boehringer Ingelheim . Testing was supported by NCI NO1CM42216. (AACR 2012)

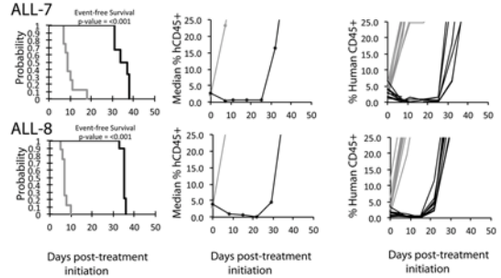

Figure 2. Volasertib activity *in vivo* against individual ALL xenografts. Kaplan-Meier curves for EFS (left), median relative tumor volume graphs (center), and individual tumor volume graphs (right) are shown for selected lines. Controls (gray lines); Treated (black lines), significances of the difference between treated and control groups are included. NOTE: ALL-8 was treated at 15 mg/kg weekly x 3, while ALL-7 was initially treated at 30 mg/kg but was dose-reduced to 15 mg/kg for week 2 and week 3 treatment because of toxicity.

IN VIVO RESULTS AND CONCLUSIONS

- Volasertib was well tolerated at 30 mg/kg administered IV weekly for 3 consecutive weeks for the solid tumor models.
- Volasertib inhibited tumor growth in most PPTP solid tumor xenografts as shown by significant differences in EFS distribution compared to control in 19 of 32 (59%) evaluable xenografts.
- Eleven of 30 (37%) evaluable solid tumor xenografts met criteria for intermediate EFS T/C activity (EFS T/C> 2).
- Objective responses(CR) were achieved in 2 of 6 neuroblastoma, 1 of 3 GBM and 1 of 5 rhabdomyosarcoma xenografts.
- For the ALL panel, excessive toxicity was noted at 30 mg/kg and the dose was reduced to 15 mg/kg. 1 of 4 xenografts treated exclusively at 15 mg/kg achieved CR. Among the 4 xenografts treated initially at 30 mg/kg, the one evaluable model (ALL-7) achieved a CR.
- Although volasertib demonstrated significant antitumor activity, available pharmacokinetic data indicate that mice tolerate higher systemic exposure to volasertib compared to humans raising the possibility that the PPTP results may overestimate the clinical activity of volasertib for the childhood cancers studied.
- Plk1 expression at the RNA level did not correlate with the occurrence of objective responses, and there are ongoing efforts to identify gene expression signatures associated with response to volasertib for the PPTP models.