#LB-354

Pediatric Preclinical Testing Program (PPTP) Stage 1 Evaluation of the XPO1/CRM1 Inhibitor KPT-330

Peter J. Houghton[1], Min Kang[2], C. Patrick Reynolds[2], Richard Gorlick[3], E. Anders Kolb[4], John M. Maris[5], Stephen T. Keir[6], Hernan Carol[7], Richard B. Lock[7], Catherine A. Billups[8], Raushan T. Kurmasheva[1], Yosef Landesman[9], Sharon Shacham[9], Michael Kauffman[9], Malcolm A. Smith[10]

[1]Nationwide Children's Hospital, [2]Texas Tech University Health Science Center, [3]Children's Hospital at Montefiore, [4]A.I. duPont Hospital, [5]Children's Hospital of Philadelphia, [6]Duke University, [7]Children's Cancer Inst., Australia, [8]St. Jude Children's Research Hospital, [9]Karyopharm Therapeutics, [10] CTEP/NCI

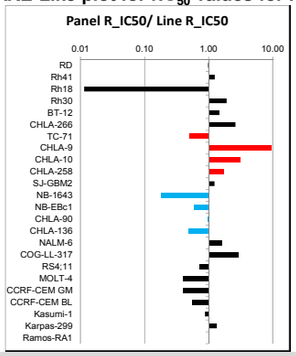
KPT-330

- KPT-330 is an orally bioavailable Selective Inhibitor of Nuclear Export (SINE).
- KPT-330 binds covalently to the nuclear export protein XPO1 at Cys528 resulting in irreversible inactivation.
- XPO1 exports over 200 proteins with specific nuclear export sequences.
- Cargo proteins include cancer-relevant proteins such as FOXO, IκB, pRB, p53, p73, p21 and p27.
- KPT-330 is in clinical trials for adults with cancer.

KPT-330 *IN VITRO* ACTIVITY

- The median relative IC_{50} (rIC_{50}) for KPT-330 against PPTP cell lines was 125 nM (range 13 nM to > 10 μM).
- KPT-330 induced Relative In/Out% values between -75% and -100% for most cell lines, consistent with a prominent cytotoxic effect.
- There were no significant differences in rIC_{50} values by histotype, although there was a trend for greater sensitivity for the Ewing sarcoma cell lines (median rIC_{50} = 57 nM) and lesser sensitivity for the neuroblastoma cell lines (median rIC_{50} = 235 nM).
- The COMPARE-like plot illustrates the relative sensitivity of Ewing sarcoma cell lines (red bars) and lesser sensitivity of the neuroblastoma cell lines (blue bars).

COMPARE-Like plot for rIC_{50} values for KPT-330



This poster will be available at: http://pptp.nchresearch.org/presentations.html

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

In vitro: ***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 (Kang MH, et al. Pediatr Blood Cancer 56:239-249, 2011). Testing was for 96 hours at concentrations from 1.0 nM to 10.0 μM with replicates of 6-12 per data point. Data were analyzed by fitting a non-linear regression model-sigmoidal dose-response model to the response-relative fluorescence values vs. the concentration.

In vivo: Standard PPTP methods for in vivo testing were employed (see http://pptp.nchresearch.org/documents/detailedAnalysisMethods.pdf). KPT-330 was tested *in vivo* using a dose of 10 mg/kg administered PO 3 times weekly (M,W,F) for a total of 4 consecutive wks. The total period of treatment and observation was 6 wks.

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 highly engrafted 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.

In vitro activity of KPT-330 against PPTP cell lines

Cell Line	Histotype	rIC_{50} (nM)	Panel rIC_{50}/ Line rIC_{50}	Ymin % (Observed)	Relative In/Out (Observed Ymin)
RD	Rhabdomyosarcoma	128	0.98	1.70	-69%
Rh41	Rhabdomyosarcoma	100	1.25	0.64	-97%
Rh18	Rhabdomyosarcoma	>10,000	0.01	69.83	46%
Rh30	Rhabdomyosarcoma	65	1.92	1.68	-90%
BT-12	Rhabdoid	85	1.47	3.47	-58%
CHLA-266	Rhabdoid	48	2.62	2.79	-89%
TC-71	Ewing sarcoma	247	0.51	0.94	-28%
CHLA-9	Ewing sarcoma	13	9.53	0.02	-99%
CHLA-10	Ewing sarcoma	40	3.13	2.51	-60%
CHLA-258	Ewing sarcoma	73	1.72	0.47	-99%
SJ-GBM2	Glioblastoma	101	1.24	0.21	-98%
NB-1643	Neuroblastoma	683	0.18	6.59	-69%
NB-EBc1	Neuroblastoma	214	0.59	1.56	-93%
CHLA-90	Neuroblastoma	131	0.96	3.45	-88%
CHLA-136	Neuroblastoma	255	0.49	1.98	-93%
NALM-6	ALL	77	1.63	0.02	-99%
COG-LL-317	ALL	42	2.97	0.01	-100%
RS4;11	ALL	174	0.72	1.05	-93%
MOLT-4	ALL	317	0.40	0.03	-100%
CCRF-CEM (1)	ALL	317	0.40	0.22	-97%
CCRF-CEM (2)	ALL	222	0.57	0.10	-98%
Kasumi-1	AML	143	0.88	5.80	-80%
Karpas-299	ALCL	93	1.34	0.21	-97%
Ramos-RA1	NHL	123	1.02	0.00	-100%
Median		125	**1.00**	**1.00**	**-93%**
Minimum		13	**0.01**	**0.00**	**-100%**
Maximum		>10,000	**9.53**	**69.83**	**46%**

KPT-330 *IN VIVO* ACTIVITY

Line	Tumor Type	Median Time to Event	EFS T/C	P-value	Tumor Volume T/C	P-value	Median Group Response
BT-29	Rhabdoid	> EP	> 1.8	<0.001	0.29	<0.001	PD2
KT-16	Rhabdoid	34.8	4.1	<0.001	0.39	0.002	PD2
KT-14	Rhabdoid	> EP	> 1.7	<0.001	0.48	<0.001	PD2
KT-10	Wilms	> EP	> 4.0	<0.001	0.00	<0.001	MCR
KT-11	Wilms	20.2	1.3	0.072	0.69	0.053	PD1
KT-13	Wilms	18.0	2.0	<0.001	0.35	<0.001	PD2
SK-NEP-1	Ewing	36.2	3.9	<0.001	0.34	<0.001	PD2
EW5	Ewing	9.9	1.1	0.083	0.83	0.436	PD1
EW8	Ewing	22.1	2.7	<0.001	0.67	0.003	PD2
TC-71	Ewing	25.0	3.1	0.001	0.79	0.123	PD2
CHLA258	Ewing	31.8	3.3	<0.001	0.22	<0.001	PD2
Rh10	Alveolar RMS	34.5	1.4	0.004	0.55	0.004	PD1
Rh28	Alveolar RMS	32.4	1.5	0.006	0.48	0.004	PD2
Rh30	Alveolar RMS	28.4	3.2	<0.001	0.55	<0.001	PD2
Rh30R	Alveolar RMS	24.2	1.7	<0.001	0.42	<0.001	PD2
Rh41	Alveolar RMS	26.2	1.9	<0.001	0.44	<0.001	PD2
Rh18	Embryonal RMS	11.1	1.6	<0.001	0.78	<0.001	PD2
BT-28	Medulloblastoma	30.8	3.1	<0.001	0.69	0.005	PD2
BT-50	Medulloblastoma	> EP	> 1.2	<0.001	0.17	<0.001	CR
BT-36	Ependymoma	> EP	> 1.1	<0.001	0.37	<0.001	PD2
BT-41	Ependymoma	> EP	.	1.000	0.49	<0.001	CR
GBM2	Glioblastoma	28.5	2.2	0.020	0.62	0.063	PD2
BT-39	Glioblastoma	17.4	2.0	0.165	0.70	0.043	PD2
D645	Glioblastoma	11.4	2.0	0.003	0.44	0.003	PD2
D456	Glioblastoma	9.0	1.5	0.288	0.60	0.063	PD1
NB-SD	Neuroblastoma	10.5	1.1	0.300	0.97	0.853	PD1
NB-1771	Neuroblastoma	20.8	2.0	<0.001	0.44	<0.001	PD2
NB-1691	Neuroblastoma	6.6	1.2	0.165	0.80	0.143	PD1
NB-EBc1	Neuroblastoma	7.7	1.4	0.046	0.64	0.105	PD1
CHLA-79	Neuroblastoma	39.1	4.3	<0.001	0.58	0.002	PD2
NB-1643	Neuroblastoma	6.7	1.2	0.010	0.64	0.015	PD1
OS-1	Osteosarcoma	30.4	1.3	<0.001	0.57	<0.001	PD1
OS-2	Osteosarcoma	18.0	1.1	<0.001	0.91	0.007	PD1
OS-17	Osteosarcoma	26.5	1.4	0.003	0.74	0.005	PD1
OS-9	Osteosarcoma	30.5	1.3	<0.001	0.73	0.002	PD1
OS-33	Osteosarcoma	18.3	1.4	<0.001	0.71	<0.001	PD1
OS-31	Osteosarcoma	17.6	1.0	0.315	0.93	0.340	PD1
ALL-2	ALL B-precursor	36.8	1.9	0.003	.		PD2
ALL-4	ALL B-precursor	6.0	1.0	0.647	.		PD1
ALL-7	ALL B-precursor	6.0	1.0	0.647	.		PD1
ALL-8	ALL T-cell	> EP	> 7.5	<0.001	.		CR
ALL-17	ALL B-precursor	29.8	4.0	0.001	.		PD2
ALL-19	ALL B-precursor	> EP	> 6.0	0.001	.		MCR
ALL-31	T-cell ALL	14.5	1.8	0.006	.		PD2
MLL-7	ALL B-precursor	12.5	2.1	0.077	.		PD1

- **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 and T/C columns indicates xenografts that have either high (dark blue) or intermediate (light blue) activity for these measures.**
- **PD1 (Progressive Disease 1, dark green):** >25% ↑ in tumor volume, TGD value ≤1.5
- **PD2 (Progressive Disease 2, light green):** >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%
- **CR (Complete response):** disappearance of measurable tumor mass (< 0.10 cm^3)
- **MCR (Maintained CR):** absence of measurable tumor mass at day 42.

KPT-330 was provided by Karyopharm Therapeutics. Testing was supported by NCI NO1CM42216. Children's Cancer Institute Australia is affiliated with the University of New South Wales and the Sydney Children's Hospitals Network.

KT-10 (Wilms tumor)

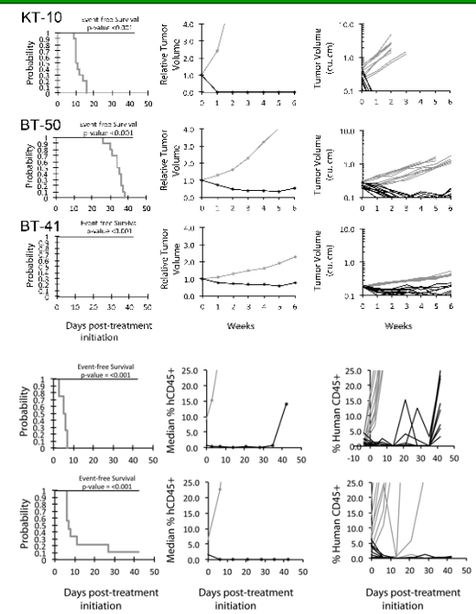
BT-50 (Medulloblastoma)

BT-41 (Ependymoma)

ALL-8 (T cell)

ALL-19 (B-precursor)

Controls (gray lines)
Treated (black lines)



IN VIVO RESULTS AND CONCLUSIONS

- **KPT-330 was well tolerated (0.9% mortality) at the dose (10 mg/kg PO) and schedule (M-W-F for 4 consecutive weeks) evaluated.**
- **KPT-330 induced tumor growth inhibition meeting criteria for intermediate or high EFS T/C activity (EFS T/C > 2) in 11 of 32 (34%) of solid tumor xenografts, most frequently for the Wilms tumor (2 of 3) and the Ewing sarcoma (4 of 5) panels. Three of 8 ALL xenografts met all criteria for intermediate or high EFS T/C activity.**
- **KPT-330 induced objective responses in 3 of 37 (8%) of solid tumor models, including two brain tumor xenografts, BT-50 (medulloblastoma) and BT-41 (ependymoma), as well as in 2 of 8 ALL xenografts (one B-cell and one T-cell ALL).**
- **Conclusions: KPT-330 shows tumor regressing activity against selected PPTP solid tumor and ALL xenografts, and shows tumor growth inhibition for a larger number of models. Defining the relationship between KPT-330 systemic exposures in mice and humans will be important in assessing the clinical relevance of the PPTP in vivo results. Planned pharmacodynamic testing may provide insight into biological factors associated with responsiveness to KPT-330.**