313 Pediatric Preclinical Testing Program (PPTP) Evaluation of the KSP Inhibitor Ispinesib (SB-715992)

Peter J. Houghton[1], John M. Maris[2], Henry S. Friedman[3], Stephen T. Keir[3], Richard B. Lock[4], Richard Gorlick[5], E. Anders Kolb[5], C. Patrick Reynolds[8], Christopher Morton[1], Malcolm A. Smith[7]. [1]St. Jude Children's Research Hospital, [2]Children's Hospital of Philadelphia, [3]Duke University, [4]Children's Cancer Inst., Australia, [5]Albert Einstein College, [6]Children's Hospital of Los Angeles, [7]CTEP/NCI.

Abstract*

Background: Ispinesib is a novel small molecule inhibitor of kinesin spindle protein (Eg5), a mitotic kinesin required for separation of the spindle poles. Ispinesib inhibits growth of a broad range of cancer cell lines at low nanomolar concentrations and induces regressions or tumor growth delay against adult cancer xenografts. The COG Phase 1 Consortium is initiating a phase 1 trial of ispinesib for children with refractory solid tumors.

Methods: The PPTP includes an *in vitro* panel (23 lines) as well as panels of xenografts (n=61) representing most of the common types of childhood solid tumors and childhood ALL. Ispinesib was administered IP (10 mg/kg or 5 mg/kg) to a representative subset of xenografts on a q 4d × 3 schedule repeated once at day 21. Three measures of antitumor activity were used: 1) response criteria modeled after the clinical setting [e.g., partial response (PR), complete response (CR), etc.]; 2) treated to control (T/C) tumor volume at day 21; and 3) a time to event measure based on the median EFS of treated and control lines (intermediate activity required EFS T/C > 2, and high activity additionally required a net reduction in median tumor volume at the end of the experiment).

Results: Ispinesib induced significant tumor growth delay in 88% (22/25) of evaluable xenografts. Using a time to event measure of efficacy, ispinesib had intermediate and high levels of activity against 5 (28%) and 4 (22%) of the 18 evaluable solid tumor xenografts, respectively. Intermediate or high activity for the EFS measure was observed for most diagnoses (e.g., Wilms, rhabdoid, EWS, RMS, GBM), but not for neuroblastoma. Ispinesib induced maintained CRs in 3 xenografts: 1 of 2 Wilms tumors, 1 Ewing sarcoma, and 1 of 2 rhabdoid tumors, and it induced a CR in 1 of 4 GBM. Ispinesib produced 2 CRs and 2 PRs among the 6 evaluable xenografts in the ALL panel. Ispinesib induced excessive toxicity in mice bearing osteosarcoma xenografts, and excessive toxicity precluded analysis of 6 xenografts for other diagnoses.

Conclusions: Ispinesib demonstrated broad activity against the PPTP's solid tumor and ALL xenografts. Antitumor activity manifested primarily as tumor growth delay, though tumor regressions were also observed for a range of histologies. Further preclinical work with ispinesib will include determining the dose-response relatinshiop for ispinesib for sensitive xenografts and determining the systemic exposures of ispinesib associated with activity in the PPTP's childhood cancer models.

*Updated from initial submission.

PPTP *In Vitro* Testing Methods

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

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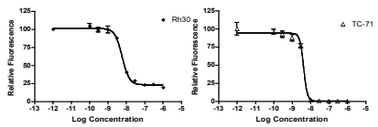
In Vitro Test Results for Ispinesib

- Ispinesib was active against all but one of the cell lines of the PPTP *in vitro* panel [a rhabdomyosarcoma line (Rh18) with an EC50 > 1 microM].
- The median EC50 for all of the lines in the panel was 4 nM. There was no significant difference between median EC50 values by tumor type, although the median EC50 values tended to be greater for the rhabdomyosarcoma lines compared to the other lines in the panel.
- Two patterns of dose-response curves were identified: one in which the maximal effect approached 100% (i.e., T/C ~ 0%) and the other in which the maximal effect was significantly below 100% (see dose response curves below for examples).
- The four rhabdomyosarcoma lines evaluated had significantly lower maximal effect values compared to the other lines in the panel (p=.007).
- The ALL lines had maximal effect values that were significantly greater than those of the remaining lines in the panel (p=.002).
- Three of four Ewing sarcoma lines had maximal effect values greater than the median for the in vitro panel.

Line	Diagnosis	EC50*	Maximal Effect*	HILL SLOPE	R^2
RD	EMB RMS	6.9	86.9	-3.8	0.98
Rh41	ALV RMS	8.5	74.5	-2.1	0.99
Rh18	EMB RMS	> 1000	< 50	-0.3	NA
Rh30	ALV RMS	6.0	77.1	-2.2	0.97
BT-12	Rhabdoid	2.1	93.3	-3.0	0.98
CHLA-266	Rhabdoid	0.5	71.0	-1.3	0.96
TC-71	Ewing sarcoma	4.2	99.7	-4.3	0.96
CHLA-9	Ewing sarcoma	2.0	98.8	-1.6	0.94
CHLA-10	Ewing sarcoma	2.5	99.0	-1.8	0.94
CHLA-258	Ewing sarcoma	4.3	85.5	-3.2	0.98
SJ-GBM2	Ewing sarcoma	0.3	88.0	-0.7	0.98
NB-1643	Glioblastoma	3.0	89.5	-23.5	0.98
NB-EBc1	Neuroblastoma	10.4	93.1	-2.3	0.99
CHLA-90	Neuroblastoma	3.3	88.5	-2.9	0.99
CHLA-136	Neuroblastoma	6.4	90.7	-1.7	0.99
NALM-6	ALL	2.0	99.8	-5.1	0.98
COG-LL-317	ALL	2.4	100.0	-2.7	0.98
RS4;11	ALL	0.7	99.9	-2.5	0.99
MOLT-4	ALL	7.6	100.0	-2.7	0.97
CCRF-CEM	ALL	2.6	100.0	-2.5	0.96
KASUMI-1	AML	10.6	89.3	-3.5	0.98
KARPAS-299	ALCL	8.1	94.6	-6.8	0.97
RAMOS	NHL	5.1	100.0	-7.5	0.99
MEDIAN		3.8	93.2	-2.7	0.98
MINIMUM		0.3	< 50	-23.5	0.94
MAXIMUM		> 1000	100.0	-0.3	0.99

* Red shading indicates lines with EC50 < median or with maximal effect > median.

Examples of Dose Response Curves



PPTP *In Vivo* Testing Methods

- 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.
- **Ispinesib** was provided by GlaxoSmithKline and Cytokinetics through the Cancer Therapy Evaluation Program (NCI). Ispinesib was dissolved in 2% Cremophor EL / 2%dimethylacetamide in acidified water (pH 5) and administered i.p. q4 days x 3 (repeated at day 21) at a dose of 10 mg/kg (solid tumor) or 5 mg/kg (ALL). Ispinesib was provided to each consortium investigator in coded vials for blinded testing according the PPTP program standard operating procedures.
- **Solid Tumor Response Criteria:**

Response		Definition	Score
PD 1	Progressive Disease 1	<50% regression at all measurements and >25% increase in tumor volume at the end of the study period, TGD value of ≤ 1 .5	0
PD 2	Progressive Disease 2	<50% regression at all measurements and >25% increase in tumor volume at the end of the study period, TGD value of > 1 .5	2
S D	Stable Disease	<50% regression at all measurements and ≤25% increase at the end of the study	4
P R	Partial Response	≥50% regression but with tumor volume ≥0.1 cm^3 during study	6
C R	Complete Response	Tumor volume <0.1 cm^3 for at least one study measureme n t	8
M C R	Maintained Complete Response	Tumor volume <0.1 cm^3 at the end of study	1 0

- **Leukemia Response Criteria:**

Response		Definition	Score
PD 1	Progressive Disease 1	CD45% never dr ops below 1%, event s before end of study, TGD value of ≤ 1 .5	0
PD 2	Progressive Disease 2	CD45% never dr ops below 1%, eve nts before end of study, TGD value of > 1 .5	2
S D	Stable Disease	CD45% never drops below 1%, n o events before end of study	4
P R	Partial Response	CD45% drops below 1% for only 1 week	6
C R	Complete Response	CD45% drops be low 1% for 2 consecut ive weeks	8
M C R	Maintained Complete Response	CD45% drops be low 1% for last 3 consecutive measurements of the study	1 0

- **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

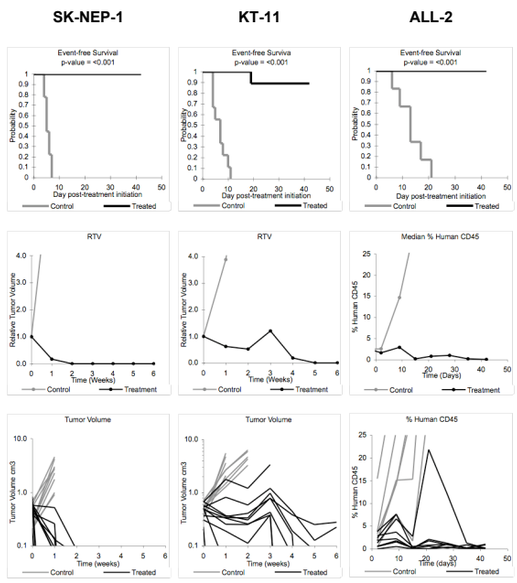
- **Statistical Methods:** Event-free survival (EFS) distributions of each treatment group were compared to the EFS distribution of the respective control group using the exact log rank test. P-values were 2-sided & were not adjusted for multiple comparisons given the exploratory nature of this study. P-values < 0.05 were considered to be significant. Relative tumor volumes (RTV) for control (C) and treatment (T) mice were calculated at day 21 or when all mice in the control and treated groups still had measurable tumor volumes (if less than 21 days). The mean relative tumor volumes for control and treatment mice for each study were then calculated and the T/C value was the mean RTV for the treatment group divided by the mean RTV for the control group.

In Vivo Test Results for Ispinesib

- Broad antitumor activity was observed for ispinesib, with significant differences in EFS distribution observed for 88% of lines.
- Toxicity was problematic despite a reduction in dose from the planned 15 mg/kg dose to a dose of 10 mg/kg. Excessive toxicity was especially an issue for the osteosarcoma panel, with no animals surviving despite a reduction in dose to 5 mg/kg. For the non-osteosarcoma xenografts, toxicity rates were more manageable, although still elevated: 5/357 (1.4%) for control animals versus 88/366 (24%) for treated animals.
- Tumor regression was observed for 4 of 19 xenografts in the solid tumor panels, and these same lines had high levels of activity using the PPTP time to event measure of activity (EFS T/C) (highlighted in dark blue in the table below).
- 4 of 6 ALL xenografts achieved CR or PR, with 2 also showing a high level of activity for the time to event activity measure.
- The graphs below show two solid tumor xenografts with maintained CR's (SK-NEP-1 and KT-11) and an ALL xenograft with CR (ALL-2).

Xenograft Line	Histology	P-value*	EFS T/C*	Median Final RTV	Tumor Volume T/C	Overall Group Response
BT-29	Rhabdoid	<0.001	> 4.0	3.8	0.33	PD2
KT-16	Rhabdoid	<0.001	> 4.2	0.0	0.35	MCR
KT-10	Wilms	<0.001	1.7	>4	0.37	PD2
KT-11	Wilms	<0.001	> 5.8	0.0	0.13	MCR
KT-13	Wilms	<0.001	2.4	>4	0.46	PD2
SK-NEP-1	Ew ing	<0.001	> 8.9	0.0	0.03	MCR
Rh30	ALV RMS	<0.001	2.3	>4	0.25	PD2
Rh30R	ALV RMS	0.019	1.8	>4	0.64	PD2
Rh41	ALV RMS	<0.001	1.7	>4	0.35	PD2
BT-28	Medulloblastoma	0.127	1.3	>4	0.67	PD1
BT-46	Medulloblastoma	0.388	1.2	>4	0.81	PD1
BT-41	Ependymoma	0.003	> 1.3	1.1	0.52	SD
GBM2	Glioblastoma	<0.001	2.7	>4	0.31	PD2
BT-39	Glioblastoma	<0.001	4.0	>4	0.53	PD2
D645	Glioblastoma	<0.001	1.5	>4	0.68	PD1
D456	Glioblastoma	<0.001	> 8.3	1.0	0.10	CR
NB-1771	Neuroblastoma	<0.001	1.9	>4	0.40	PD2
NB-1691	Neuroblastoma	0.163	1.1	>4	0.79	PD1
CHLA-79	Neuroblastoma	<0.001	1.8	>4	0.32	PD2
ALL-2	ALL B-precursor	<0.001	> 3.2	0.1	.	CR
ALL-3	ALL B-precursor	<0.001	> 8.4	9.4	.	PR
ALL-4	ALL B-precursor	<0.001	4.4	>25	.	PD2
ALL-8	ALL T-cell	0.041	> 9.8	10.6	.	SD
ALL-16	ALL T-cell	<0.001	>16.1	0.3	.	CR
ALL-19	ALL B-precursor	0.011	6.8	>25	.	PR

* Red shading indicates significant difference in EFS distribution, and blue shading indicates either high (dark blue) or intermediate (light blue) activity using the time to event EFS T/C) measure of activity.



CONCLUSIONS

- The kinesin spindle protein inhibitor ispinesib was active at nanomolar concentrations against the lines in the PPTP's in vitro panel, with ispinesib showing the greatest effect against the ALL cell lines and the least effect against the rhabdomyosarcoma cell lines.
- Ispinesib demonstrates broad activity against the PPTP *in vivo* tumor panels, although with substantial toxicity rates.
- High level activity was observed for Ewing, Wilms, GBM, rhabdoid, and ALL xenografts.
- Planned additional testing includes determining the dose-response relationship for ispinesib against sensitive xenograft lines and determining the ispinesib systemic exposures associated with activity in the PPTP's xenograft models.
- Ispinesib is currently being evaluated in a pediatric phase 1 clinical trial by the Children's Oncology Group Phase 1 Consortium.

Supported by NCI NO1CM42216