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Initial Testing (Stage 1) of the Kinesin Spindle Protein Inhibitor Ispinesib by the Pediatric Preclinical Testing Program

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Abstract

Background—Ispinesib is a highly specific inhibitor of kinesin spindle protein (KSP, HsEg5), a mitotic kinesin required for separation of the spindle poles. Here we report the activity of ispinesib against the *in vitro* and *in vivo* panels of the Pediatric Preclinical Testing Program (PPTP).

Procedures—Ispinesib was tested against the PPTP *in vitro* panel cell lines at concentrations from 0.1 nM to 1 μ M and against the *in vivo* tumor panel xenografts by intraperitoneal administration (5 or 10 mg/kg) every 4 days for 3 doses repeated at day 21.

Results—Ispinesib was highly potent against the PPTP's *in vitro* cell lines with a median IC₅₀ of 4.1 nM. Ispinesib (10 mg/kg) induced unexplained toxicity in mice bearing osteosarcoma xenografts and exceeded the MTD in 12 of 40 non-osteosarcoma xenografts. Ispinesib induced significant tumor growth delay in 88% (23/26) of evaluable xenografts. Using a time to event measure of efficacy, ispinesib had intermediate and high levels of activity against 4 (21%) and 5 (26%) of the 19 evaluable solid tumor xenografts, respectively. Ispinesib induced maintained complete responses (CR) in a rhabdoid tumor, a Wilms tumor and a Ewing sarcoma xenograft. Ispinesib (5 mg/kg) produced 2 complete and 2 partial responses among 6 evaluable xenografts in the ALL panel. The *in vivo* pattern of activity was distinctive from that previously reported for vincristine.

Conclusions—Ispinesib demonstrated broad *in vivo* antitumor activity, including maintained complete responses for several xenografts, although with high toxicity rates at the doses studied.

Keywords

Preclinical Testing; Developmental Therapeutics; Ispinesib

INTRODUCTION

Mitotic kinesins are members of the kinesin superfamily of motor proteins that play essential roles in mitotic spindle function, including spindle-pole organization, chromosome alignment and segregation, and the regulation of microtubule dynamics [1]. Kinesin spindle protein (KSP, HsEg5) is a mitotic kinesin that utilizes energy from adenosine triphosphate (ATP) hydrolysis to produce directed mechanical force along microtubules of the emerging mitotic spindle, thereby driving formation of a bipolar structure [2]. Thus, KSP is required for formation and maintenance of the bipolar spindle [3]. Highly specific small molecule inhibitors of KSP have been identified [1,4]. These small molecule inhibitors of KSP are not competitive with ATP for binding to KSP, but rather act as allosteric inhibitors by binding to the ADP-KSP complex and by inhibiting ADP release from KSP [5–8].

KSP inhibition produces a characteristic cellular phenotype with mitotically arrested cells showing a monopolar spindle with arrays of microtubules projecting from a pair of unseparated centrosomes surrounded by chromosomes. Following cell cycle arrest induced by KSP inhibition, some, but not all, cell lines undergo apoptosis [9]. KSP is highly expressed in proliferating cells during mitosis, but is absent in post-mitotic neurons [6], suggesting that targeting KSP offers the potential for lesser toxicity to normal tissues than the tubulin-binding antimitotic agents currently used for cancer treatment.

Small molecule inhibitors of KSP have entered clinical evaluation, including ispinesib (SB-715992) [10–14], SB-743921 [15], and MK-0741 [16]. Preclinical data for these inhibitors have shown high potency *in vitro*, and the ability to induce regressions in some preclinical models *in vivo* [1,4]. For example, ispinesib administered intraperitoneally (IP) on an intermittent schedule (every four days for three doses) was active against advanced human colon tumor xenografts, inducing complete regressions as well as tumor growth delay [17]. The related compound CK0106023 induced regressions against an ovarian cancer xenograft (SKOV3), and treated tumor cells showed circular mitotic figures, similar to the monopolar spindles seen in CK0106023-treated cells in culture [6]. Ispinesib was selected for testing by the PPTP because of the utility of conventional tubulin-binding antimitotic agents for many childhood cancers and because of the distinctive mechanism of action by which ispinesib blocks mitotic progression.

MATERIALS AND METHODS

In vitro testing

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 [18]. Cells were incubated in the presence of ispinesib for 96 hours at concentrations from 0.1 nM to 1.0 μ M and analyzed as previously described [19]. Vincristine was tested in a similar manner across the same concentration range.

In vivo tumor growth inhibition studies

CB17SC-M *scid*^{-/-} female mice (Taconic Farms, Germantown NY), were used to propagate subcutaneously implanted kidney/rhabdoid tumors, sarcomas (Ewing, osteosarcoma, rhabdomyosarcoma), neuroblastoma, and non-glioblastoma brain tumors, while BALB/c nu/nu mice were used for glioma models, as previously described [20–22]. Human leukemia cells were propagated by intravenous inoculation in female non-obese diabetic (NOD)/*scid*^{-/-} mice as described previously [23]. All mice were maintained under barrier

conditions and experiments were conducted using protocols and conditions approved by the institutional animal care and use committee of the appropriate consortium member. Tumor volumes (cm^3) [solid tumor xenografts] or percentages of human CD45-positive [hCD45] cells [ALL xenografts] were determined as previously described [24]. Responses were determined using three activity measures as previously described [24]. An in-depth description of the analysis methods is included in the Supplemental Response Definitions section.

Statistical Methods

The exact log-rank test, as implemented using Proc StatXact for SASR, was used to compare event-free survival distributions between treatment and control groups. P-values were two-sided and were not adjusted for multiple comparisons given the exploratory nature of the studies. The Mann-Whitney test was used to test the difference of medians of *in vitro* parameters (e.g., EC_{50} and maximal effect) between the groups of lines with similar tumor types to the remaining lines of the panel. The relationship between *in vitro* parameters for ispinesib and vincristine were analyzed by linear regression analysis.

Drugs and Formulation

Ispinesib was provided to the Pediatric Preclinical Testing Program by Cytokinetics and GlaxoSmithKline through the Cancer Therapy Evaluation Program (NCI). Ispinesib was dissolved in a solution of Cremophor EL (2% v/v), dimethylacetamide (2% v/v), acidified water (pH 5), and administered intraperitoneally every 4 days for 3 doses, with the treatment course repeated at day 21. The dose of ispinesib was 10 mg/kg in the solid tumor models and 5 mg/kg in the ALL models and was based on toxicity testing in non-tumored animals. Ispinesib was provided to each consortium investigator in coded vials for blinded testing according the PPTP program standard operating procedures.

RESULTS

Ispinesib *in vitro* testing

Ispinesib was active against most of the cell lines of the PPTP *in vitro* panel, with only a single rhabdomyosarcoma line (Rh18) having an IC_{50} greater than 1 μM . The median IC_{50} value for all of the cell lines in the panel was 4.1 nM (Table I). With the exception of a higher median IC_{50} for the rhabdomyosarcoma panel, reflecting in part the lack of response of Rh18, there was no relationship between histotype and IC_{50} . T/C% values at the highest concentration tested (1 μM) were used as a measure of the maximal effect of ispinesib. Figure 1 illustrates the two primary patterns of response to ispinesib: one indicative of near complete cytotoxicity as typified by a Ewing cell line (TC-71) with a T/C% value at 1 μM of 0.3% and the other indicative of partial cytotoxicity or cytostasis as typified by a rhabdomyosarcoma cell line (Rh30) with a T/C% value at 1 μM of 22.8%. The four rhabdomyosarcoma lines evaluated had significantly higher T/C% values at 1 μM compared to the other lines in the panel (median of 21.1% versus 4.3%, $p=0.013$) (Table I). The ALL lines had T/C% values at 1 μM that were significantly lower than those of the remaining lines in the panel (0.1% versus 10.7%, $p=0.003$).

Because of previous reports suggesting that KSP inhibitors have *in vitro* activity profiles similar to those of antimetabolic tubulin binders [25,26], we compared the *in vitro* activity of ispinesib to that of vincristine focusing on the T/C% values of each agent at the highest concentration tested (1 μM) as a measure of the maximal effect of each agent (Supplemental Table 1). For both vincristine and ispinesib, Rh18 was the only cell line which had a T/C% value > 50% at the 1 μM concentration. There was a highly significant relationship between the T/C% values at 1 μM for ispinesib and vincristine ($p<0.0001$, $r^2 = 0.87$), with the

significant relationship being maintained even when Rh18 was omitted from the analysis ($p=0.0003$, $r^2 = 0.49$). Both ispinesib and vincristine were highly active against the cell lines of the ALL panel, with T/C% values at 1 μ M approaching zero for each of the cell lines, indicative of strong cytotoxic activity. For the rhabdomyosarcoma panel, however, T/C% values at 1 μ M for both agents were $> 10\%$, consistent with a cytostatic or at best a partial cytotoxic response.

Activity of ispinesib against the PPTP in vivo panel

Ispinesib was evaluated against 46 xenograft models using an every 4 day x 3 repeated at Day 21 schedule that was selected to model the weekly administration schedule evaluated in a pediatric phase 1 trial of ispinesib [27]. Of 1021 mice studied, 174 died during the study (17%), with 5 of 497 in the control arms (1.0%) and 169 of 524 in the ispinesib treatment arms (32%). Excessive toxicity was especially problematic for the osteosarcoma panel, with few animals surviving past day 7 of testing. The mice carrying osteosarcoma xenografts experienced early death even at a reduced dose of 5 mg/kg. For the non-osteosarcoma lines in the panel, toxicity was more manageable, although toxicity rates were still elevated [111/466 (24%)]. All six of the osteosarcoma xenografts, as well as 12 of 40 non-osteosarcoma xenografts, were excluded from analysis due to toxicity rates greater than 25 percent. A complete summary of results is provided in Supplemental Table II, including total numbers of mice, number of mice that died (or were otherwise excluded), numbers of mice with events and average times to event, tumor growth delay, as well as numbers of responses and T/C values.

Antitumor effects were evaluated using the PPTP activity measures for time to event (EFS T/C), tumor growth delay (tumor volume T/C), and objective response. Ispinesib induced significant differences in EFS distributions compared to controls in 17/20 (85%) evaluable solid tumor models and 6/6 (100%) ALL models (Table II). Four solid tumor xenografts had objective responses and also met the criteria for high activity for the EFS T/C activity measure, including one xenograft each from the rhabdoid tumor, Wilms tumor, Ewing sarcoma, and glioblastoma panels. An additional 5 of 19 evaluable solid tumor xenografts met criteria for intermediate activity for the EFS T/C activity measure. Among the 6 evaluable ALL xenografts, all met criteria for either intermediate ($n=4$) or high ($n=2$) activity using the EFS T/C activity measure. Figure 2 illustrates patterns of response to ispinesib for selected solid tumor models, and Figure 3 illustrates patterns of response of the ALL models.

The *in vivo* testing results for the objective response measure of activity are presented in Figure 4 in a 'heat-map' format as well as a 'COMPARE'-like format, based on the scoring criteria described in the Material and Methods and the Supplemental Response Definitions section. The latter analysis demonstrates relative tumor sensitivities around the midpoint score of 5 (stable disease). Objective responses [either complete response (CR) or maintained CR] were seen in 4 of 20 solid tumor models. Objective responses were observed in 4 of 6 ALL models, with two PRs (ALL-3 and ALL-19) and two CRs (ALL-2 and ALL-16).

To evaluate ispinesib's activity profile at lower, better tolerated doses, three responsive lines (KT-11, SK-NEP-1, and Rh28) were evaluated at 4, 5, or 7.5 mg/kg. The former two xenografts achieved maintained CRs at 10 mg/kg, while Rh28 achieved PR at 10 mg/kg (but with excessive toxicity). There were no deaths among animals treated at these lower doses. SK-NEP-1 responded similarly at the lower doses with maintained CR responses at 4 and 7.5 mg/kg. The other two lines exhibited progressive disease at the reduced doses. Complete results from the dose-response testing are provided in Supplemental Table III.

KSP (KIF11) gene expression for the PPTP cell lines and xenografts is shown in Figure 5. KSP was expressed at significantly higher levels in the ALL xenografts and cell lines compared to the solid tumor xenografts (Supplemental Table IV). Among the solid tumor panels, KSP expression was highest for the rhabdomyosarcoma xenografts and lowest for the osteosarcoma xenografts. There was no correlation between the objective response score and KSP expression levels ($r^2 = 0.02$). KSP expression was highly correlated with the expression of other genes whose expression is associated with mitosis and the mitotic checkpoint machinery (e.g., CDC2, BUBR1, and CENPF) [28].

DISCUSSION

Ispinesib is a potent, highly specific inhibitor of KSP. The *in vitro* activity profile of ispinesib was characterized by nanomolar range IC_{50} values for almost all of the PPTP cell lines. The maximal effect of ispinesib, as measured by its T/C% value at the highest concentration tested (1 μ M), ranged from near complete cytotoxicity (T/C% values approaching 0% at 1 μ M) for the ALL cell lines and most of the Ewing sarcoma cell lines to partial cytotoxic or cytostatic activity (T/C% values > 10% at 1 μ M) for the rhabdomyosarcoma cell lines. For small molecule KSP inhibitors, the determinants of cellular response (cell cycle arrest versus apoptosis induction) are not yet well defined [9]. The activity pattern for ispinesib against the PPTP's *in vitro* panel is similar to that observed for the antimetabolic agent vincristine. This observation is consistent with NCI-60 cell line panel testing results, for which similar activity profiles for antitubulin agents such as vincristine and the KSP inhibitor S-trityl-L-cysteine (STLC) were also observed [25,26].

In vivo activity for ispinesib was noted across a diverse range of histotypes, with CR or maintained CRs observed in single xenografts from the rhabdoid tumor, Ewing sarcoma, glioblastoma, and Wilms tumor panels. While ispinesib's *in vitro* activity profile is similar to that of vincristine, its *in vivo* activity profile appears to be distinctive. Overall for the PPTP solid tumor panels, vincristine demonstrated high activity using the objective response activity measure in 6 of 24 xenografts [24], compared to high activity for ispinesib in 4 of 20 evaluable xenografts. However, two lines with maintained CRs to ispinesib (KT-16 and SK-NEP-1) had progressive disease (PD2) in response to vincristine. Conversely, KT-13 (Wilms) and BT-39 (GBM), which had maintained CRs to vincristine, had progressive disease (PD2) following treatment with ispinesib. The six ALL xenografts were generally responsive to both vincristine and ispinesib, although the response categories were higher for vincristine (4 maintained CRs, 2CRs, and 1PR) than for ispinesib (2CRs, 2PRs, 1SD, and 1PD2) [24].

Ispinesib produced a high level of toxicity in tumor-bearing mice at the 10 mg/kg dose. Lower doses (4 – 7.5 mg/kg) were better tolerated, but activity was substantially reduced for two of three responsive solid tumor xenografts for which dose-response testing was performed. For the ALL panel, toxicity at the 5 mg/kg dose precluded analysis of results in two of eight xenograft models. Preliminary testing at a dose of 2.5 mg/kg in the ALL-panel (data not shown) point to reduced efficacy, with 6 of 7 xenografts achieving a lower response score than at the 5 mg/kg dose and with only one objective response at the lower dose as compared to 4 for the higher dose. These results suggest a relatively narrow therapeutic range, which is typical of many cytotoxic agents. Ispinesib-associated toxicity was especially severe in the osteosarcoma panel, an observation for which there is no obvious explanation. There is to our knowledge no report of a similar “tumor specific” toxicity profile, and thus the clinical significance of this observation is not known.

Ispinesib has completed its phase 1 evaluation in children with recurrent solid tumors using a weekly for 3 weeks repeated every 28 day schedule [27]. In adults, ispinesib has been

evaluated using a variety of schedules [10–12], with the primary focus of clinical development being the every three week schedule. Objective responses were not observed for ispinesib in phase II trials using the every three week schedule for head and neck squamous cell carcinoma [30], colorectal cancer [13], hepatocellular carcinoma [31], and melanoma [32]. For a cohort of patients with metastatic breast cancer previously treated with both an anthracycline and a taxane, partial responses were observed in 4 of 45 evaluable patients using the every 21 day schedule (K. Wood, personal communication), and an every two week schedule is under evaluation in chemotherapy-naïve breast cancer patients [14].

The PPTP *in vivo* testing results suggest that ispinesib may be an active agent for several pediatric cancers, although the biological characteristics associated with responsiveness are not apparent and there appears to be a relatively narrow therapeutic range for responsive pediatric models. Further clinical evaluations will be required to determine whether the potential therapeutic benefits associated with a novel antimetabolic agent like ispinesib that causes minimal toxicity to non-proliferating cells can be realized in the clinical setting.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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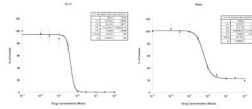


Figure 1. Ispinesib *in vitro* activity

The figures illustrate typical cytotoxicity/growth inhibition dose-response curves for cell lines with T/C% values at 1 μ M approaching 0% (TC-71) and for cell lines with T/C% values at 1 μ M indicating a plateau agent effect of 10% or greater (Rh30). Error bars represent standard deviations for each concentration tested.

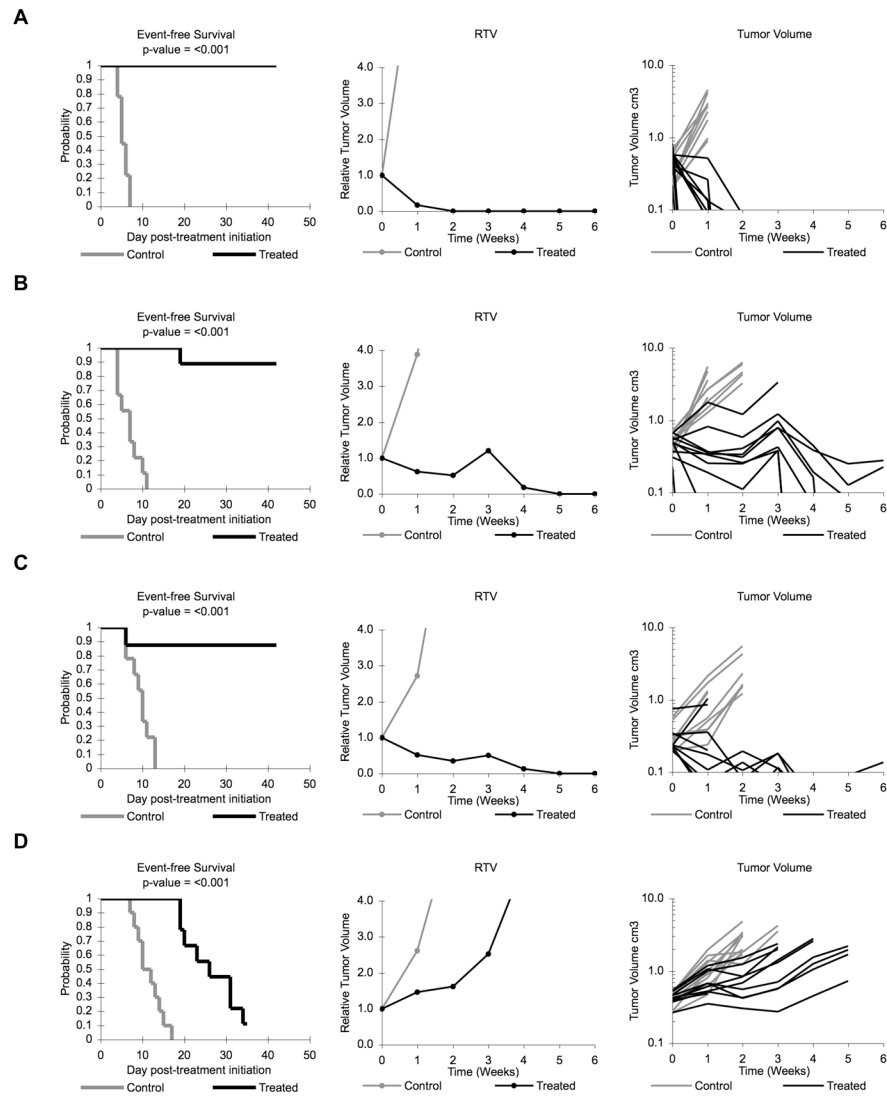


Figure 2. Ispinesib activity against individual solid tumor xenografts
 Kaplan-Meier curves for EFS, median relative tumor volume graphs, and individual tumor volume graphs are shown for selected lines: (A) SK-NEP-1 (B) KT-11, (C) KT-16, and (D) Rh30. Controls (gray lines); Treated (black lines).

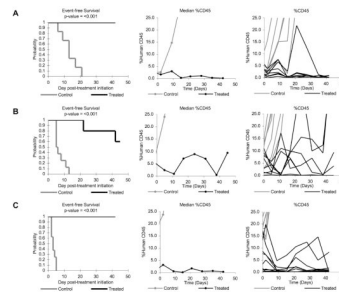


Figure 3. Ispinesib activity against individual ALL xenografts
 Kaplan-Meier curves for EFS, median %hCD45 graphs, and individual %hCD45 graphs are shown for selected lines: (A) ALL-2, (B) ALL-3, and (C) ALL-16. Controls (gray lines); Treated (black lines).

Xenograft Line	Histology	Median Score	Midpoint Difference	Overall Group Response	Heat Map
BT-29	Rhabdoid	2.0	-3.0	PD2	Green
KT-16	Rhabdoid	10.0	5.0	MCR	Red
KT-10	Wilms	2.0	-3.0	PD2	Green
KT-11	Wilms	10.0	5.0	MCR	Red
KT-13	Wilms	2.0	-3.0	PD2	Green
SK-NEP-1	Ewing	10.0	5.0	MCR	Red
EW8	Ewing	2.0	-3.0	PD2	Green
Rh30	ALV RMS	2.0	-3.0	PD2	Green
Rh30R	ALV RMS	2.0	-3.0	PD2	Green
Rh41	ALV RMS	2.0	-3.0	PD2	Green
BT-28	Medulloblastoma	0.0	-5.0	PD1	Green
BT-46	Medulloblastoma	0.0	-5.0	PD1	Green
BT-41	Ependymoma	4.0	-1.0	SD	Grey
GBM2	Glioblastoma	2.0	-3.0	PD2	Green
BT-39	Glioblastoma	2.0	-3.0	PD2	Green
D645	Glioblastoma	1.0	-4.0	PD1	Green
D456	Glioblastoma	8.0	3.0	CR	Orange
NB-1771	Neuroblastoma	2.0	-3.0	PD2	Green
NB-1691	Neuroblastoma	0.0	-5.0	PD1	Green
CHLA-79	Neuroblastoma	2.0	-3.0	PD2	Green
ALL-2	ALL B-precursor	8.0	3.0	CR	Orange
ALL-3	ALL B-precursor	6.0	1.0	PR	Yellow
ALL-4	ALL B-precursor	2.0	-3.0	PD2	Green
ALL-8	ALL T-cell	5.0	0.0	SD	Grey
ALL-16	ALL T-cell	8.0	3.0	CR	Orange
ALL-19	ALL B-precursor	6.0	1.0	PR	Yellow
	Median	2.0			

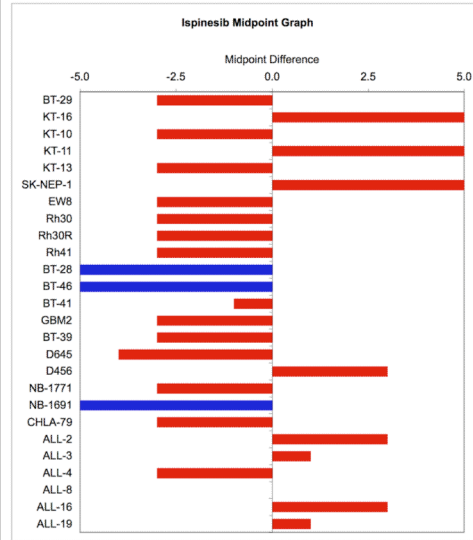


Figure 4. Ispinesib *in vivo* objective response activity

Left: The colored ‘heat map’ depicts group response scores. A high level of activity is indicated by a score of 6 or more, intermediate activity by a score of ≥ 2 but < 6 , and low activity by a score of < 2 . Right: representation of tumor sensitivity based on the difference of individual tumor lines from the midpoint response (stable disease). Bars to the right of the median represent lines that are more sensitive and that achieve regression as a best response. Bars to the left are tumor models that are less sensitive and that achieve tumor progression with growth delay or no treatment effect as their best response. Red bars indicate lines with a significant difference in EFS distribution between treatment and control groups, while blue bars indicate lines for which the EFS distributions were not significantly different.

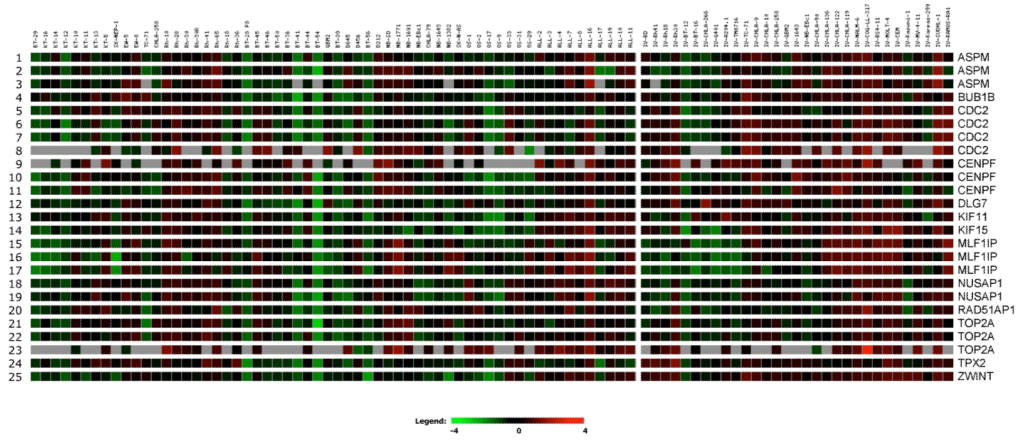


Figure 5. Gene expression for KSP (KIF11) and genes with correlated expression patterns
 Relative expression of KSP (KIF11) based on Affymetrix U133 Plus 2.0 expression profiles for PPTP xenografts (left panel) and cell lines (right panel), as visualized using GeneSifter software (VizX Labs, Seattle, WA). Gray indicates an absent call from Affymetrix quality control. The expression profiles for genes whose expression is highly correlated with that of KSP are also shown.

Table I

Activity of Ispinesib against the PPTP in Vitro Panel

Cell Line	Status	Histology	T/C% at1 μ M	IC ₅₀ (nM)
RD		Rhabdomyosarcoma	11.4	5.8
Rh41	Post-Therapy	Rhabdomyosarcoma	22.8	11.1
Rh18	Diagnosis	Rhabdomyosarcoma	71.2	>1000
Rh30	Diagnosis	Rhabdomyosarcoma	19.4	8.0
BT-12	Diagnosis	Rhabdoid	6.2	2.1
CHLA-266	Diagnosis	Rhabdoid	25.8	1.1
TC-71	Post-Therapy	Ewing sarcoma	0.3	4.1
CHLA-9	Diagnosis	Ewing sarcoma	0.9	2.8
CHLA-10	Post-Therapy	Ewing sarcoma	2.2	3.6
CHLA-258	Post-Bone Marrow Transplant	Ewing sarcoma	12.8	4.8
SJ-GBM2	Post-Therapy	Glioblastoma	13.5	0.5
NB-1643	Diagnosis	Neuroblastoma	9.9	3.0
NB-EBc1	Post-Therapy	Neuroblastoma	6.5	11.4
CHLA-90	Post-Bone Marrow Transplant	Neuroblastoma	11.6	3.6
CHLA-136	Post-Bone Marrow Transplant	Neuroblastoma	14.9	7.0
NALM-6	Post-Therapy	ALL	0.0	1.6
COG-LL-317	Post-Therapy	ALL	0.1	2.5
RS4; 11	Post-Therapy	ALL	0.2	0.6
MOLT-4	Post-Therapy	ALL	0.1	7.9
CCRF-CEM		ALL	0.1	3.0
Kasumi-1	Post-Bone Marrow Transplant	AML	9.4	11.9
Karpas-299	Post-Therapy	ALCL	4.3	8.9
Ramos-RA1		NHL	0.0	8.0
Median			6.5	4.1
Minimum			0.0	0.5
Maximum			71.2	>1000

Table II

Activity for Ispinesib against the PPTP *in Vivo* Panel

Xenograft Line	Histology	KM Estimate of Median Time to Event	P- value	EFS T/C	Median Final RTV	Tumor Volume T/C	T/C Activity	EFS Activity	Response Activity
BT-29	Rhabdoid	> EpI	<0.001	> 4.0	3.8	0.33	Int	Int	Int
KT-16	Rhabdoid	> EpI	<0.001	> 4.2	0.0	0.35	Int	High	High
KT-10	Wilms	17.5	<0.001	1.7	>4	0.37	Int	Low	Int
KT-11	Wilms	> EpI	<0.001	> 5.8	0.0	0.13	High	High	High
KT-13	Wilms	16.9	<0.001	2.4	>4	0.46	Low	Int	Int
SK-NEP-1	Ewing	> EpI	<0.001	> 8.9	0.0	0.03	High	High	High
EW8	Ewing	18.7	0.005	1.6	>4	0.44	Int	Low	Int
Rh30	ALV RMS	25.7	<0.001	2.3	>4	0.25	Int	Int	Int
Rh30R	ALV RMS	14.2	0.019	1.8	>4	0.64	Low	Low	Int
Rh41	ALV RMS	15.8	<0.001	1.7	>4	0.35	Int	Low	Int
BT-28	Medulloblastoma	3.8	0.127	1.3	>4	0.67	Low	Low	Low
BT-46	Medulloblastoma	8.2	0.388	1.2	>4	0.81	Low	Low	Low
BT-41	Ependymoma	> EpI	0.003	> 1.3	1.1	0.52	Low	NE2	Int
GBM2	Glioblastoma	37.2	<0.001	2.7	>4	0.31	Int	Int	Int
BT-39	Glioblastoma	31.7	<0.001	4.0	>4	0.53	Low	Int	Int
D645	Glioblastoma	12.2	<0.001	1.5	>4	0.68	Low	Low	Low
D456	Glioblastoma	> EpI	<0.001	> 8.3	1.0	0.10	High	High	High
NB-1771	Neuroblastoma	17.4	<0.001	1.9	>4	0.40	Int	Low	Int
NB-1691	Neuroblastoma	4.8	0.163	1.1	>4	0.79	Low	Low	Low
CHLA-79	Neuroblastoma	19.2	<0.001	1.8	>4	0.32	Int	Low	Int
ALL-2	ALL B-precursor	> EpI	<0.001	> 3.2	0.1	.		High	High
ALL-3	ALL B-precursor	> EpI	<0.001	> 8.4	9.4	.		Int	High
ALL-4	ALL B-precursor	17.9	<0.001	4.4	>25	.		Int	Int
ALL-8	ALL T-cell	> EpI	0.041	> 9.8	10.6	.		Int	Int
ALL-16	ALL T-cell	> EpI	<0.001	>16.1	0.3	.		High	High

Xenograft Line	Histology	KM Estimate of Median Time to Event	P- value	EFS T/C	Median Final RTV	Tumor Volume T/C	T/C Activity	EFS Activity	Response Activity
ALL-19	ALL B-precursor	42.5	0.011	6.8	>25	.		Int	High

¹ Greater than the Evaluation Period;

² Not evaluable