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Initial Testing of Topotecan by the Pediatric Preclinical Testing Program

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Abstract

Background—Topotecan is a small molecule DNA topoisomerase I poison, that has been successful in clinical trials against pediatric solid tumors and leukemias. Topotecan was evaluated against the PPTP tumor panels as part of a validation process for these preclinical models.

Procedures—*In vivo* three measures of antitumor activity were used: 1) an objective response measure modeled after the clinical setting; 2) a treated to control (T/C) tumor volume measure; and 3) a time to event (4-fold increase in tumor volume for solid tumor models, or $\geq 25\%$ human CD45+ cells in the peripheral blood for ALL models) measure based on the median event-free survival (EFS) of treated and control animals for each xenograft.

Results—Topotecan inhibited cell growth *in vitro* with IC₅₀ values between 0.71 nM and 489 nM. Topotecan significantly increased EFS in 32 of 37 (87%) solid tumor xenografts and in all 8 of the ALL xenografts. Seventy five percent of solid tumors met EFS T/C activity criteria for intermediate (n=17) or high activity (n=7). Objective responses were noted in 8 solid tumor xenografts (Wilms, rhabdomyosarcoma, Ewing sarcoma, neuroblastoma). Among the 6 neuroblastomas, three achieved a PR. For the ALL panel, two maintained CRs, three CRs, and two PRs were observed.

Conclusions—Topotecan demonstrated broad activity *in vitro* and *in vivo* against both the solid tumor and ALL panels, with significant tumor growth delay generated in all the panels. These results further demonstrate the validity of the PPTP panel for preclinical testing of new drugs.

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Keywords

Preclinical Testing; Developmental Therapeutics; topotecan

Introduction

Topotecan is a water soluble semi-synthetic camptothecin analog that can be administered either parenterally or orally [1]. The anti-cancer activity of camptothecin was described in the late 1960's, but its use was precluded by broad toxicity. The identification of DNA topoisomerase I (topo I) as the target of this family of drugs stimulated renewed interest in developing less toxic analogs [2]. Topotecan was developed with the goal of reducing toxicity and improving bioavailability from those of the parent drug, and has shown remarkable activity against a range of cancers. In the mid 1990's the FDA granted approval for topotecan for the treatment of metastatic carcinoma of the ovary after failure of initial or subsequent chemotherapy. Topotecan has subsequently been approved as a single agent for recurrent small cell lung cancer as well as for use in combination with cisplatin for recurrent cervical cancer.

Topotecan acts as a poison of the enzyme topo I and retards reversal of the covalent topo I-DNA intermediate (the cleavable complex) and religation of DNA single-strand breaks. Its deleterious effects are more pronounced during S phase of the cell cycle, when topotecan-induced DNA double-strand breaks are generated, triggering cell cycle arrest and apoptosis through DNA damage sensory mechanisms.

Preclinical studies on the efficacy of topotecan showed very promising results against xenografts derived from brain tumors and neuroblastoma [3-5], sarcomas [6], osteosarcoma [7] and rhabdomyosarcoma [4,5], as well as ovarian cancer [6]. The effects obtained were maximal upon prolonged exposure at lower doses showing, in some cases, clearly improved results compared to those obtained at a higher dose on an intermittent schedule [5,8,9]. Thus, topotecan scheduling appears critical as a determinant of efficacy.

Topotecan has been reported to exhibit clinical activity against pediatric solid tumors, including neuroblastoma [10-13], rhabdomyosarcoma [14], medulloblastoma [15] and Wilms tumors [16]. Topotecan also has demonstrated significant, albeit modest, antileukemic activity against pediatric and adult acute lymphoblastic leukemia (ALL) [17-20], and it has been used in effective multi-agent reinduction regimens [19,21].

Phase I clinical studies of different administration regimens in adults [22,23] and in children [12,18,24] confirmed the preclinical findings supporting greater activity with longer exposure. Phase II studies in adults based on prolonged exposure (primarily daily $\times$ 5 administered every 3 weeks) achieved encouraging results for the treatment of ovarian carcinoma [25,26] and small cell lung cancer [27,28] and in myelodysplastic syndrome and chronic myelomonocytic leukemia [29]. Phase II studies in children confirmed the achievement of improved outcomes when topotecan was administered for 5 consecutive days both in neuroblastoma and rhabdomyosarcoma [10,13,14].

Topotecan was the fourth drug with a well established profile of activity against pediatric cancer tested by the Pediatric Preclinical Testing Program (PPTP) with the aim of validating the results obtained to prioritize new chemotherapeutic agents for clinical trials in children. Topotecan was selected for systematic testing by the PPTP based on its relevance for solid tumors and leukemia, for its capacity of inducing apoptosis as a consequence of DNA damage, and for the general lack of cross resistance with conventional chemotherapeutic

agents. This report describes testing of topotecan against the PPTP's *in vitro* panel of cell lines and *in vivo* xenograft solid tumor and ALL panels, at a clinically relevant dose of 0.6 mg/kg and an administration schedule that resembles the treatment regimens used in the clinic.

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 [30]. Cells were incubated in the presence of Topotecan for 96 hours at concentrations from 0.1 nM to 1.0 μ M and analyzed as previously described [31].

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 [32-34][30-32]. Human leukemia cells were propagated by intravenous inoculation in female non-obese diabetic (NOD)/*scid*^{-/-} mice as described previously [35]. Female mice were used irrespective of the patient gender from which the original tumor was derived. 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. Ten mice were used in each control or treatment group. Tumor volumes (cm³) [solid tumor xenografts] or percentages of human CD45-positive [hCD45] cells [ALL xenografts] were determined as previously described [36] and responses were determined using three activity measures as previously described [36]. 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 SAS®, 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.

Drugs and Formulation

Topotecan was provided to the PPTP by Glaxo Smith Kline, through the Cancer Therapy Evaluation Program (NCI). Topotecan was dissolved in sterile saline and administered i.p., using a 5 days on 2 days off for 2 weeks schedule, repeated at 21 days, at a dose of 0.6 mg/kg. Topotecan was provided to each consortium investigator in coded vials for blinded testing.

Results

Topotecan *in vitro* testing

Topotecan inhibited growth of the cell lines from the PPTP *in vitro* panel (Table I), with all 23 lines achieving greater than 50% growth inhibition. The median IC₅₀ for the entire panel was 9.13 nM, with a range of 0.71 nM to 489 nM. Topotecan activity is represented visually using a dot plot in Figure 1A and examples of typical response curves (Rh30 and Kasumi-1) are displayed in Figure 1B.

Topotecan *in vivo* testing

Topotecan was evaluated in 45 xenograft models. Eleven of 859 mice died during the study (1.3%), with 4 of 428 in the control arms (0.9%) and 7 of 431 in the topotecan treatment arms (1.6%). A complete summary of results is provided in Supplemental Table I, 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. Topotecan induced significant differences in EFS distributions compared to controls in 32/37 (86%) solid tumor models and 8/8 (100%) ALL models (Table II). Nine lines met the criteria for high activity with EFS T/C values greater than 2 and with final tumor volumes less than the initial volumes (Table II). An additional 23 of 40 evaluable solid tumor and ALL models met criteria for intermediate activity for the EFS T/C activity measure by having EFS T/C values exceeding 2.0 and significant differences in EFS distribution between treated and control groups.

The *in vivo* testing results for the objective response measure of activity are presented in Figure 2 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 were seen in 8 of 37 solid tumor models with maintained complete responses in Wilms, Ewing and rhabdomyosarcoma. Partial responses were achieved in the glioblastoma and neuroblastoma panels. Figure 3 illustrates the maintained complete responses of the xenografts KT-10 (Wilms) and Rh28 (rhabdomyosarcoma) as well as a xenograft achieving a partial response, NB-1643 (neuroblastoma). Objective responses were seen in 7 of 8 ALL models (two MCR, three CR and 2 PR). Figure 4 illustrates the maintained complete responses of the xenografts ALL-2 and ALL-16.

Discussion

This study provides the largest preclinical evaluation to date of topotecan as a single agent against both *in vitro* and *in vivo* models of pediatric malignancy. The cell lines tested *in vitro* were all inhibited by topotecan with IC₅₀ values within three orders of magnitude. The neuroblastoma cell lines showed the widest range of sensitivity to this drug. The IC₅₀ values of the group formed by the rhabdoid and rhabdomyosarcoma cell lines (all above the general median) are higher than those of the Ewing and leukemias grouped together (nine out of twelve under the median), although with a *p* value close to 0.05. The relative differences in sensitivity could point to biological differences among the histotypes, but since this distribution of susceptibility to growth inhibition *in vitro* found no correspondence for the solid tumors in the *in vivo* panels it is more likely to be due to particular features of the cell lines used, particularly in light of the proliferation dependence of topotecan-induced cytotoxicity. Similarly, Jonsson et al [37] reported differences in the activity of topotecan, irinotecan and SN-38 between cell lines and primary tumor cells.

The efficacy results obtained by the PPTP for topotecan as a single agent *in vivo* are similar to those that have been reported in children with the respective clinical diagnoses. In the experiments reported here the dose of topotecan was adjusted from the mouse MTD of 2.0 mg/kg on the daily $\times$ 5 for 2 weeks schedule to a dose of 0.6 mg/kg, based on pharmacokinetic data suggesting that this lower dose produced systemic exposures that are comparable to those achieved in humans for 21 day courses of topotecan [38]. Whenever

possible, this adjustment to clinically relevant doses for preclinical testing should be made to produce more meaningful results. At this dose none of the xenografts was excluded from reporting because of excessive toxicity.

Among the solid tumors the Wilms tumor panel was the only one with two xenografts reaching high activity for the EFS T/C measure, while the rhabdoid, the medulloblastoma and the ependymoma panels did not record any high activity for this parameter.

Topotecan induced significant differences in tumor volume between treated and control groups in 33 of 37 solid tumor xenografts (89%). High activity for this measure among the solid tumor panels (defined as T/C < 15%) was observed in 4 xenografts, including one xenograft each from the Wilms tumor, neuroblastoma, glioblastoma, and rhabdomyosarcoma panels (Table II).

In terms of objective response measure, two of three Wilms tumor xenografts achieved maintained complete responses as did a rhabdomyosarcoma xenograft (1 of 5) and a Ewing sarcoma xenograft (1 of 5). Among the 6 xenografts in the neuroblastoma panel, 3 achieved a partial response and 1 had stable disease while one glioblastoma (out of 4) achieved a partial response (see heat map in Figure 2).

In a phase II trial for children with newly diagnosed neuroblastoma, topotecan was administered daily for 5 days over 2 consecutive weeks for two cycles, with doses individualized to attain a single-day topotecan lactone area under the plasma concentration-time curve (AUC) of 80 to 120 ng/mL, which was comparable to that associated with responses in neuroblastoma xenografts [11]. The response rate achieved was 60% (95% CI, 41% to 77%); with one complete and 17 partial responses. Single agent activity was also observed for newly diagnosed neuroblastoma using the daily $\times$ 5 every 3 week schedule [13].

Clinical activity for topotecan was reported for patients with recurrent Wilms tumor using the same 10-day treatment schedule described above for neuroblastoma, with dosages adjusted to achieve a target AUC of 80 ng/mL*h [16]. Out of 25 assessable patients with favorable histology Wilms tumor, 12 had partial response (PR), six had stable disease (SD), and seven had progressive disease (PD), for an overall response rate of 48% (95% CI, 27.8% to 68.7%). Of 11 assessable patients with anaplastic histology Wilms tumor, two had PR, one had SD, and eight had PD.

Topotecan demonstrated activity in children with previously untreated high-risk medulloblastoma when administered daily $\times$ 5 days, with a second course administered at day 21 and targeting a plasma AUC of 120 to 160 ng/mL*h [15]. Of 36 assessable patients, four patients (11.1%) had a complete response and six (16.6%) showed a partial response, and disease was stable in 17 patients (47.2%). Topotecan was also effective in the treatment of newly diagnosed rhabdomyosarcoma using the daily $\times$ 5 every three week schedule, with a reported objective response rate of 46% [14].

The ALL panel achieved the highest frequency of objective responses (seven out of eight), with three complete responses and two maintained complete responses. These results confirm previous reports and point to topotecan as a potentially relevant drug for the treatment of ALL refractory to conventional drugs. Topotecan has been studied in a phase I trial of children with leukemia, which included children with relapsed or refractory ALL. Within this heavily pretreated population, evidence of anti-leukemia activity was observed when the drug was administered for 9 to 12 days, with one CR and two PRs observed among 25 patients in this phase I study [17]. A recent report on a phase II study for relapsed ALL described the use of topotecan in combination with the conventional platform

dexamethasone, L-asparaginase and vincristine. The administration of topotecan at 2.4 mg/m² daily for 5 days preceded the regular induction schedule [19]. With 28 evaluable patients, 89.3% had responses (defined as a $\geq 25\%$ decrease in circulating blast count on day 6 or 7) to the treatment with topotecan alone, while at the end of induction 74.2% had a complete response, 3.2% a partial response and 16.1% had no response.

It is worth noting that the only xenograft that did not to achieve an objective response in the ALL panel was ALL-4, which was derived from a patient with Bcr-Abl expressing Ph+ ALL. A more detailed analysis of this and other BCR-ABL ALL xenografts could lead to identifying underlining mechanisms of resistance to this drug in leukemia with this genetic alteration.

Our results also indicate that gene expression levels of topo I do not give an indication of the response/resistance to topotecan (data not shown), although a recent report suggests that protein levels and enzyme activity may prove more relevant parameters for relating to topotecan activity [39]. Resistance to topo I inhibitors has been associated with a variety of factors [40] including reduction of topo I enzyme activity and consequent reduced generation of drug-induced cleavable complexes, with mutations of topo I, with changes in drug transport and efflux (mainly ABCG2 and Pgp) [41], and with changes in DNA repair mechanisms and apoptosis regulation in cell lines (reviewed in [42]). Also events downstream from DNA damage sensors could influence resistance since both p53-dependent and -independent mechanisms may operate to induce death as a consequence of topotecan exposure.

Cross-resistance with other chemotherapeutic agents to topo I inhibitors is limited, making topotecan and other members of this family of drugs appealing candidates for the treatment of resistant forms of cancer. Combination of topotecan with other agents has led to the establishment of effective treatment regimens, particularly with cyclophosphamide [43-45], and platinum based drugs [46]. Exposure to topotecan also potentiates the anti-cancer effects of radiotherapy [47] extending the potential range of applications of this drug.

The reported activities of cyclophosphamide [36] and cisplatin [48] against the PPTP panel differ quite clearly from those reported here for topotecan. Although all three drugs cause death through DNA damage, the patterns of activity delineated from the xenografts for the different histotypes are quite divergent pointing to different mechanisms of action.

In summary, the reported activities of topotecan against a broad range of cell lines *in vitro* and xenografts *in vivo* at a clinically relevant dose from multiple PPTP panels situate this drug among the most effective anticancer agents available. The activities observed using the PPTP's models have generally been replicated in clinical trials of topotecan against a range of childhood cancer histotypes further validating the results obtained by the PPTP and confirming the usefulness of this strategy to prioritize drugs for clinical trials.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Topotecan *in vitro* activity. Figure 1A is a dot plot chart that illustrates the relative sensitivity of the cell lines using the IC₅₀ values displayed by histology. The black line indicates the median IC₅₀ (9.13 nM) for the panel. Figure 1B illustrates typical growth inhibition curves for Rh30 and Kasumi-1. Error bars represent standard deviations for each concentration tested.

Figure 2.

Topotecan *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 (objective responses), and to the left are tumor models that are less sensitive (non-objective 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 3.

Topotecan 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) KT-10 (B) Rh28, and (C) NB-1643. Controls (gray lines); Treated (black lines).

Figure 4. Topotecan activity against individual ALL xenografts. Kaplan-Meier curves for EFS and graphs of median and individual percentages of hCD45 cells, are shown for selected lines: (A) ALL-2, and (B) ALL-16. Controls (gray lines); Treated (black lines).

Table I
Activity of topotecan against cell lines in the PPTP *in Vitro* panel

Cell Line	Status	Histology	EC ₅₀ (nM)	IC ₅₀ (nM)
RD		Rhabdomyosarcoma	7.78	13.29
Rh41	Post-Therapy	Rhabdomyosarcoma	13.20	15.61
Rh18	Diagnosis	Rhabdomyosarcoma	488.92	489.28
Rh30	Diagnosis	Rhabdomyosarcoma	8.64	9.76
BT-12	Diagnosis	Rhabdoid	13.02	15.18
CHLA-266	Diagnosis	Rhabdoid	28.78	45.43
TC-71¹	Post-Therapy	Ewing	2.20	2.20
CHLA-9	Diagnosis	Ewing	1.06	1.06
CHLA-10	Post-Therapy	Ewing	11.49	11.50
CHLA-258	Post-Bone Marrow Transplant	Ewing	6.52	7.23
SJ-GBM2	Post-Therapy	Glioblastoma	4.57	4.76
NB-1643	Diagnosis	Neuroblastoma	0.71	0.71
NB-EBc1	Post-Therapy	Neuroblastoma	8.09	7.85
CHLA-90	Post-Bone Marrow Transplant	Neuroblastoma	49.01	112.19
CHLA-136	Post-Bone Marrow Transplant	Neuroblastoma	15.68	17.75
COG-LL-317	Post-Therapy	ALL T-cell	6.36	7.10
NALM-6	Post-Therapy	ALL B-precursor	2.86	2.98
RS4;11¹	Post-Therapy	ALL B-precursor	8.75	9.13
MOLT-4	Post-Therapy	ALL T-cell	3.10	3.09
CCRF-CEM		ALL T-cell	3.51	3.51
Kasumi-1	Post-Bone Marrow Transplant	AML	15.75	17.34
Karpas-299	Post-Therapy	ALCL	16.13	16.41
Ramos-RA1		NHL	4.25	4.25
Median			8.09	9.13
Minimum			0.71	0.71
Maximum			488.92	489.28

Table II

Activity for topotecan 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	P-value	T/C Volume Activity	EFS Activity	Response Activity
BT-29	Rhabdoid	37.3	<0.001	2.3	>4	0.23	<0.001	Int	Int	Int
KT-14	Rhabdoid	> EP	<0.001	> 3.1	1.3	0.55	0.004	Low	Int	Int
KT-10	Wilms	> EP	<0.001	> 4.4	0.1	0.12	<0.001	High	High	High
KT-11	Wilms	19.7	<0.001	3.6	>4	0.35	<0.001	Int	Int	Int
KT-13	Wilms	> EP	<0.001	> 7.0	0	0.35	<0.001	Int	High	High
SK-NEP-1	Ewing	18.2	<0.001	3.5	>4	0.15	<0.001	Int	Int	Int
EW5	Ewing	14.2	<0.001	1.2	>4	0.61	<0.001	Low	Low	Low
EW8	Ewing	11	0.879	0.9	>4	1.09	0.853	Low	Low	Low
TC-71	Ewing	9.8	0.036	1.5	>4	0.54	0.156	Low	Low	Int
CHLA258	Ewing	> EP	<0.001	> 6.4	0	0.16	<0.001	Int	High	High
Rh28	ALV RMS	> EP	<0.001	> 3.2	0	0.03	<0.001	High	High	High
Rh30	ALV RMS	21.7	<0.001	1.7	>4	0.34	<0.001	Int	Low	Int
Rh30R	ALV RMS	37.9	<0.001	2.5	>4	0.21	<0.001	Int	Int	Int
Rh41	ALV RMS	17.6	<0.001	2.9	>4	0.38	<0.001	Int	Int	Int
Rh18	EMB RMS	13.4	0.012	1.1	>4	0.63	0.007	Low	Low	Low
BT-28	Medulloblastoma	5	0.481	1.1	>4	0.84	0.720	Low	Low	Low
BT-45	Medulloblastoma	> EP	<0.001	> 3.3	1.1	0.25	<0.001	Int	Int	Int
BT-46	Medulloblastoma	13.7	<0.001	2.4	>4	0.28	<0.001	Int	Int	Int
BT-50	Medulloblastoma	> EP	0.211	.	0.9	0.81	0.043	Low	NE	Int
BT-36	Ependymoma	> EP	0.474	.	1.3	0.5	<0.001	Low	NE	Int
BT-44	Ependymoma	7.4	0.463	0.8	>4	1.24	0.123	Low	Low	Low
GBM2	Glioblastoma	> EP	<0.001	> 3.3	2.8	0.35	<0.001	Int	Int	Int
BT-39	Glioblastoma	12	0.019	1.2	>4	0.78	0.019	Low	Low	Low
D645	Glioblastoma	> EP	<0.001	> 3.3	0.9	0.1	<0.001	High	High	High

Xenograft Line	Histology	KM Estimate of Median Time to Event	P-value	EFS T/C	Median Final RTV	Tumor Volume T/C	P-value	T/C Volume Activity	EFS Activity	Response Activity
D456	Glioblastoma	18.7	<0.001	3.6	>4	0.31	0.001	Int	Int	Int
NB-SD	Neuroblastoma	> EP	<0.001	> 1.6	0.8	0.35	<0.001	Int	NE	High
NB-1771	Neuroblastoma	> EP	<0.001	> 2.1	0.9	0.23	<0.001	Int	High	Int
NB-1691	Neuroblastoma	40	<0.001	3.4	>4	0.17	<0.001	Int	Int	Int
NB-EBc1	Neuroblastoma	29.1	<0.001	4.7	>4	0.29	<0.001	Int	Int	Int
CHLA-79	Neuroblastoma	> EP	<0.001	> 1.6	0.7	0.29	<0.001	Int	NE	High
NB-1643	Neuroblastoma	> EP	<0.001	> 4.1	1.5	0.1	<0.001	High	Int	High
OS-1	Osteosarcoma	> EP	<0.001	> 2.1	1.3	0.3	<0.001	Int	Int	Int
OS-2	Osteosarcoma	> EP	<0.001	> 2.3	4	0.54	<0.001	Low	Int	Int
OS-17	Osteosarcoma	> EP	<0.001	> 2.4	2.8	0.51	0.002	Low	Int	Int
OS-9	Osteosarcoma	> EP	<0.001	> 1.2	0.6	0.49	<0.001	Low	NE	Int
OS-33	Osteosarcoma	> EP	<0.001	> 3.0	1.6	0.4	<0.001	Int	Int	Int
OS-31	Osteosarcoma	> EP	<0.001	> 2.2	0.7	0.24	<0.001	Int	High	Int
ALL-2	ALL B-precursor	> EP	<0.001	> 4.5	0.9	.		.	High	High
ALL-3	ALL B-precursor	> EP	<0.001	> 6.1	1	.		.	Int	High
ALL-4	ALL B-precursor	18.6	<0.001	9.3	>25	.		.	Int	Int
ALL-7	ALL B-precursor	40	<0.001	14.4	>25	.		.	Int	High
ALL-8	ALL T-cell	> EP	<0.001	> 8.5	1.5	.		.	Int	High
ALL-16	ALL T-cell	> EP	<0.001	> 6.4	0.3	.		.	High	High
ALL-17	ALL B-precursor	> EP	<0.001	> 17.7	1.1	.		.	Int	High
ALL-19	ALL B-precursor	20.9	0.033	20.9	>25	.		.	Int	High