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Initial Testing of the Investigational NEDD8 Activating Enzyme Inhibitor MLN4924 by the Pediatric Preclinical Testing Program

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Abstract

Background—MLN4924 is an investigational first-in-class small molecule inhibitor of NEDD8-activating enzyme (NAE). NAE is an essential component of the NEDD8 conjugation pathway, controlling the activity of a subset of ubiquitin-proteasome system (UPS) E3 ligases, multiprotein complexes that transfer ubiquitin molecules to substrate proteins.

Procedures—MLN4924 was tested against the PPTP *in vitro* panel using 96 hour exposure time at concentrations ranging from 1.0 nM to 10 μ M. It was tested *in vivo* at a dose of 100 mg/kg [66 mg/kg for the acute lymphoblastic leukemia (ALL) xenografts] administered orally twice daily $\times$ 5 days. Treatment duration was 3 weeks.

Results—The median relative IC₅₀ for MLN4924 against the PPTP cell lines was 143 nM, (range 15 nM to 678 nM) with that for the Ewing panel being significantly lower (31 nM). MLN4924 induced significant differences in EFS distribution compared to control in 20 of 34 (59%) evaluable solid tumor xenografts. MLN4924 induced intermediate activity (EFS T/C values $>$ 2) in 9 of the 33 evaluable xenografts (27%), including 4 of 4 glioblastoma xenografts, 2 of 3 Wilms tumor xenografts, 2 of 5 rhabdomyosarcoma xenografts, and 1 of 4 neuroblastoma xenografts. For the ALL panel, 5 of 8 evaluable xenografts showed intermediate activity for the

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EFS T/C measure. MLN4924 did not induce objective responses in the PPTP solid tumor or ALL panels.

Conclusions—MLN4924 showed potent activity *in vitro* and *in vivo* showed tumor growth inhibitory activity against a subset of the PPTP solid tumor and ALL xenografts.

Keywords

Preclinical Testing; Developmental Therapeutics; MLN4924

INTRODUCTION

MLN4924 is an investigational first-in-class small molecule inhibitor of NEDD8-activating enzyme (NAE). NAE is an essential component of the NEDD8 conjugation pathway, controlling the activity of a subset of ubiquitin-proteasome system (UPS) E3 ligases, multiprotein complexes that transfer ubiquitin molecules to substrate proteins. Specifically, MLN4924 inhibits the activity of cullin-RING dependent ubiquitin E3 ligases (CRLs), which for activation require conjugation of NEDD8 (i.e., neddylation) to their cullin subunit. In the absence of cullin neddylation, CRL activity is blocked and CRL substrates that are normally targeted for 26S proteasome degradation accumulate. These CRL substrates are proteins that play important roles in cell cycle progression and signal transduction, cellular processes that are integral to tumor cell growth, proliferation, and survival. Hence, NAE inhibition is an attractive strategy to pursue as a novel anticancer therapy.

NEDD8 activation by NAE involves ATP hydrolysis resulting in NEDD8 adenylation and subsequent transfer of NEDD8 to NAE and eventually its transfer through a multistep process to the C-terminal of a cullin protein. MLN4924 inhibits this pathway by inducing NAE to produce a covalent NEDD8-MLN4924 adduct that resembles NEDD8 adenylate [1–3]. The NEDD8-MLN4924 adduct is stable within the NAE active site and blocks enzyme activity [1]. MLN4924 is a potent inhibitor of NAE (half-maximal inhibitory concentration 50 (IC₅₀) of 4.7 nM), and is selective relative to the closely related activating enzymes [4]. MLN4924 shows cytotoxicity through at least two distinctive mechanisms that both result from increased levels of CRL substrate proteins. In one mechanism, accumulation of Cdt-1, a factor required for licensing origins of DNA replication, leads to DNA re-replication, resulting in S-phase accumulation, DNA-damage responses, and cell death [4]. A second mechanism of action has been demonstrated for activated B-cell-like (ABC) diffuse large B-cell lymphoma (DLBCL) that involves NF-kappaB pathway inhibition. Treatment of ABC DLBCL cells with MLN4924 results in rapid accumulation of pI κ B α (pI B), decrease in nuclear p65 content, reduction of NF-kappaB transcriptional activity, G1 arrest, and ultimately apoptosis induction [5]. MLN4924 shows *in vitro* cytotoxicity with EC₅₀ values in the 10 to 200 nM range [5], which is the concentration range over which MLN4924 induces accumulation of CRL substrate proteins such as Cdt-1 and pI B [4,5].

Given the unique mechanism of action of MLN4924 compared to agents currently under evaluation for children with cancer and given its promising preclinical activity against adult cancer models, the PPTP initiated an evaluation of the agent against its *in vitro* and *in vivo* models of pediatric cancers.

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 [6]. Cells were incubated in the presence of

MLN4924 for 96 hours at concentrations from 1 nM to 10 μ M and analyzed as previously described [7]. Absolute IC₅₀ values represent the concentration of MLN4924 that reduces cell survival to 50% of the control value, while relative IC₅₀ values represent the MLN4924 concentration that reduces cell survival by 50% of the maximum MLN4924 effect [8]. Relative In/Out (I/O)% values represent the percentage difference between the Y_{min} value and the estimated starting cell number and either the control cell number (for agents with Y_{min} > starting cell number) or 0 (for agents with Y_{min} < estimated starting cell number). Relative I/O% values range between 100% (no treatment effect) to -100% (complete cytotoxic effect), with a Relative I/O% value of 0 being observed for a completely effective cytostatic agent.

In vivo tumor growth inhibition studies

CB17SC *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 [9–11]. Human leukemia cells were propagated by intravenous inoculation in female non-obese diabetic (NOD)/*scid*^{-/-} mice as described previously [12]. 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. Female mice were used irrespective of the gender from which the tumor was derived. Tumor volumes (cm³) [solid tumor xenografts] or percentages of human CD45-positive [hCD45] cells [ALL xenografts] were determined as previously described [13]. Responses were determined using three activity measures as previously described [13]. Ten mice (solid tumors) or 8 mice (leukemias) were used per group. 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.

Pharmacodynamic Studies

Tumor bearing mice were treated with MLN4924 (100 mg/kg S.C.), and overall levels of neddylated cullin proteins were determined over 24 hours by immunoblotting, as described previously [4]. Neddylated cullin protein levels were normalized to α -tubulin.

Compound Information and Formulation

Millennium Pharmaceuticals, Inc., through the Cancer Therapy Evaluation Program (NCI), provided MLN4924 to the Pediatric Preclinical Testing Program. MLN4924 was formulated in 10% hydroxy-propyl- β -cyclodextrin, in 1X PBS (adjusted to pH 5.0–5.5), and administered subcutaneously twice daily for 5 days repeated each week for a total of 3 weeks. Solid tumor models were tested at a dose of 100 mg/kg, while the ALL xenografts were tested at a dose of 66 mg/kg/dose. MLN4924 was provided to each consortium investigator in coded vials for blinded testing against a placebo control consisting of vehicle only.

RESULTS

MLN4924 *in vitro* testing

MLN4924 was tested against the PPTP's *in vitro* cell line panel at concentrations ranging from 1.0 nM to 10.0 μ M using the PPTP's standard 96 hour exposure period. The median MLN4924 relative IC₅₀ (rIC₅₀) value for the PPTP cell lines was 143 nM, with a range between a minimum of 15 nM and a maximum of 678 nM (Table I). The median rIC₅₀ values varied by histotype, from 31 nM for the Ewing panel to 287 nM for the neuroblastoma panel. The median rIC₅₀ value for the Ewing panel (31 nM; shown as hatched bars) was significantly lower than that of the remaining PPTP cell lines evaluated ($p=.003$), Supplemental Figure 1A. MLN4924 demonstrated an activity pattern consistent with cytotoxic activity for many of the PPTP cell lines with minimum T/C% values (Ymin) approaching 0% for these cell lines (e.g., CCRF-CEM, Figure 1B). Even those cell lines with Ymin T/C% values not approaching 0% (e.g., Rh18, Supplemental Figure 1B) showed some level of cytotoxic effect, as evidenced by Relative I/O values below 0% for all but two cell lines. The median observed Ymin T/C% for the entire panel was 1.1%, but there was variation by histotype. The rhabdomyosarcoma and neuroblastoma cell lines had greater observed Ymin values (10.3% and 15.7%, respectively) than the Ewing and ALL cell lines (0.6% and 0.1%, respectively).

MLN4924 *in vivo* testing

MLN4924 was tested *in vivo* using a 100 mg/kg dose (66 mg/kg for the ALL xenografts) administered subcutaneously twice-daily for 5 days, repeated for 3 weeks. The total planned treatment and observation period was 6 weeks. Initially, the treatment duration had been planned for 6 weeks, but skin hardening at the site of injection prevented additional compound administration and thus the treatment was restricted to 3 weeks. MLN4924 was evaluated in 45 xenograft models. Twenty-three of 834 mice died during the study (2.8%), with 3 of 410 in the control arms (0.7%) and 20 of 424 in the MLN4924 treatment arms (4.7%). Three xenograft lines (Rh10, NB-EBc1, and CHLA-79) were excluded from analysis due to toxicity greater than 25 percent. 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.

MLN4924 induced significant differences in EFS distribution compared to control in 20 of 33 (61%) evaluable solid tumor xenografts and 5 of 8 (63%) evaluable ALL xenografts (Table II). MLN4924 was also assessed using the PPTP "time to event" activity measure (EFS T/C). Intermediate activity requires EFS T/C values > 2 , and high activity additionally requires a net reduction in median tumor volume at the end of the experiment. MLN4924 did not induce high activity in any of 31 solid tumor xenografts evaluable for this measure of activity, while it induced intermediate activity in 9 of the 31 evaluable xenografts (29%), including 4 of 4 glioblastoma xenografts, 2 of 3 Wilms tumor xenografts, 2 of 4 rhabdomyosarcoma xenografts, and 1 of 4 neuroblastoma xenografts. Because treatment was stopped at day 21 due to skin hardening at the site of compound injection, growth of solid tumors was analyzed during the period of treatment (Supplemental Table II). Seventeen of 33 studies evented prior to day 21. Of the 13 studies for which treated and control tumor volumes were available at day 21, all except KT-10 showed progressive disease.

For the ALL panel, high activity for the EFS T/C measure was not observed, while 5 of 8 evaluable xenografts showed intermediate activity for the EFS T/C measure. The maximum shift in time to event for treated animals was 3.8-fold (ALL-3), and two other leukemia xenografts had shifts in time to event of 3.0-fold (ALL-2 and ALL-17).

The *in vivo* testing results for the objective response measure of activity are presented in Figure 1 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). MLN4924 did not induce objective responses in the PPTP solid tumor panels. The best response in the solid tumor panel was PD2 (progressive disease with growth delay), which was observed in 14 of 33 (42%) xenografts, being most commonly observed in the glioblastoma (4 of 4), Wilms (3 of 3), neuroblastoma (3 of 4), and rhabdomyosarcoma (2 of 5) panels. Examples of PD2 responses are shown in Figure 2. In the ALL panel, the best response was stable disease, which was observed in a single xenograft (ALL-3) (Figure 3). The PD2 response with EFS T/C of 3.0 for ALL-2 is also shown in Figure 4.

Inhibition of NEDD8 was determined by measuring the total levels of neddylated cullin proteins in control (vehicle treated) or MLN4924 treated mice. Tumors were excised 0.5 to 24 hr after MLN4924 administration, and neddylated cullin proteins normalized against tubulin in immunoblots (Figure 4). Two tumors that demonstrated intermediate sensitivity (EFS T/C 3.0, KT-10 and GBM2) and two tumors demonstrating PD1 responses (EFS T/C 1.4 and 1.5 for EW5 and EW8 xenografts, respectively) were selected. As shown in Figure 5, there was a similar marked decrease in neddylated cullin protein levels after treatment irrespective of tumor sensitivity.

DISCUSSION

MLN4924 showed potent *in vitro* activity against the PPTP cell line panel, with IC₅₀ values generally less than 200 nM. The range of IC₅₀ values observed for the PPTP cell lines are comparable to those observed for adult cancer cell lines [4,5,14]. There were clear differences in response to MLN4924 by histotype, with the Ewing sarcoma and ALL cell lines showing more complete cytotoxic responses compared with the rhabdomyosarcoma and neuroblastoma cell lines. There was not a clear relationship between *in vitro* sensitivity and *in vivo* activity for those xenografts that are represented by cell lines in the *in vitro* panel. The *in vitro* sensitivity of the Ewing cell lines was not replicated *in vivo* for the Ewing xenografts, which showed a disappointingly low level of activity. The GBM2 cell line did show relative *in vitro* sensitivity to MLN4924 and also showed good tumor growth inhibition *in vivo* to MLN4924, as did the other glioblastoma xenografts, although tumor regressions were not observed in this panel. The ALL cell lines showed the clearest cytotoxic response to MLN4924 *in vitro*, while *in vivo* several of the ALL xenografts showed clear evidence of treatment effect, albeit no remissions, during the 3 weeks that MLN4924 was administered.

MLN4924 appears to be particularly effective against ABC-DLBCL cell lines and xenografts, which are known to have NF- κ B pathway activation [5]. The mechanism of action responsible for this impressive activity appears to be inhibition of NF- κ B signaling by stabilization of I κ B [5]. In the pediatric setting, histologies showing this same level of survival dependence on NF- κ B signaling have not been described. NF- κ B activation has been described for ALL [15,16], although the PPTP ALL xenografts did not show the robust responses to MLN4924 that were previously demonstrated for ABC-DLBCL xenografts [5]. The most consistent evidence of tumor growth inhibition was observed for the glioblastoma panel, for which 4 of 4 xenografts showed EFS T/C values greater than two. Previous reports have described NF- κ B activation in glioblastomas, although discrepant results have been reported regarding whether inhibition results in inhibition of cell growth [17–21]. Interpretation of the PPTP glioblastoma results must be made in light of the subcutaneous

location of the tumors, and further evaluation of MLN4924 in orthotopic models may be warranted.

Target inhibition, determined by decreased levels of neddylated cullin proteins, was similar in each tumor model evaluated regardless of the level of tumor growth inhibition to MLN4924. Inhibition was maximal 2 hr after MLN4924 administration, and had recovered partially by 24 hr. It is likely that the accumulation of certain CRL substrates that are required for antitumor activity differs in models with greater versus lesser tumor growth inhibition; the accumulation of these substrates may depend on the activation of oncogenic pathways important to tumor survival. It will be important to identify the pathways that make tumors more sensitive to NAE inhibition.

MLN4924 has entered clinical evaluation in adults using a range of schedules administered at 21 day intervals (e.g., days 1 to 5; days 1, 2, 8, and 9; days 1, 3, and 5; days 1, 4, 8, and 11) [22–25]. Dose-limiting toxicities have primarily been non-hematological, including transaminase elevation, hyperbilirubinemia, muscle cramps/myalgia, and elevated serum creatinine. Objective responses in MLN4924 phase 1 trials have been noted for patients with AML, MDS, Hodgkin lymphoma, and melanoma [22–25]. In considering potential pediatric clinical applications of MLN4924, one focus could be on tumors that have a requirement for NF- κ B activation. A number of mutations have been identified in adult cancers that result in NF- κ B pathway activation, including somatic mutations in genes that positively (e.g., CARD11, CD79A, and CD79B) and negatively (A20/TNFAIP3) regulate the NF- κ B pathway [26–30]. Recurring somatic mutations in these genes are not known to occur in the pediatric solid tumor or ALL setting. Among the lymphomas known to show NF- κ B activation in the adult setting, Hodgkin lymphoma and primary mediastinal B-cell lymphoma occur in the pediatric age range [31,32]. Further preclinical evaluations of MLN4924 will depend upon emerging data from adult preclinical and clinical testing and on future advances in the understanding of the molecular basis of pediatric cancers.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1. MLN4924 *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 to the left are tumor models that are less sensitive. 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 2. MLN4924 activity against individual solid tumor xenografts, Kaplan-Meier curves for EFS, median relative tumor volume graphs, and individual tumor volume graphs (controls, gray and treated, black lines) are shown for selected lines: Wilms tumor KT-10, glioblastomas BT-39 and GBM2, and neuroblastoma NB-1771.

Figure 3.

MLN4924 activity against individual ALL xenografts, Kaplan-Meier curves for EFS and graphs of median and individual percentages of hCD45 cells (controls, gray and treated, black lines), are shown for selected lines: (A) ALL-2, and (B) ALL-3.

Figure 4. MLN4924 decreases total neddylated cullin proteins *in vivo*. Mice were treated with a single administration of MLN4924 (100 mg/kg S.C.), or compound vehicle (Veh). Tumors were excised from 0.5 to 24 hr after MLN4924 administration. A. Total neddylated cullin proteins; B. Relative levels of neddylated cullin proteins normalized to α -tubulin.

Table 1Activity of MLN4924 against Cell Lines in the PPTP *in Vitro* Panel

	Tumor Type	Relative IC ₅₀ (nM)	Absolute IC ₅₀ (nM)	Observed Ymin	Median EC ₅₀ Ratio	Relative In/Out
RD	Rhabdomyosarcoma	112	137	6.8	1.27	1%
Rh41	Rhabdomyosarcoma	179	176	9.7	0.80	-56%
Rh18	Rhabdomyosarcoma	266	304	18.5	0.54	-59%
Rh30	Rhabdomyosarcoma	235	265	10.9	0.61	-34%
BT-12	Rhabdoid	119	138	7.2	1.20	-12%
CHLA-266	Rhabdoid	175	192	7.1	0.82	-73%
TC-71	Ewing	33	33	0.0	4.36	-99%
CHLA-9	Ewing	29	29	0.8	4.90	-78%
CHLA-10	Ewing	98	87	1.5	1.46	-75%
CHLA-258	Ewing	15	18	0.4	9.71	-99%
GBM2	Glioblastoma	42	46	2.6	3.43	-74%
NB-1643	Neuroblastoma	143	179	9.3	1.00	-56%
NB-EBcl	Neuroblastoma	430	468	1.2	0.33	-95%
CHLA-90	Neuroblastoma	110	230	22.0	1.31	-21%
CHLA-136	Neuroblastoma	678	>10000	47.7	0.21	27%
NALM-6	ALL	165	182	0.0	0.87	-99%
COG-LL-317	ALL	254	255	0.0	0.56	-100%
RS4;11	ALL	138	143	0.1	1.04	-99%
MOLT-4	ALL	144	160	0.2	0.99	-98%
CCRF-CEM	ALL	251	255	0.1	0.57	-98%
Kasumi-1	AML	122	127	0.9	1.18	-97%
Karpas-299	NHL	257	256	0.0	0.56	-99%
Ramos-RA1	NHL	105	105	0.0	1.36	-100%
Median		143	176	1.2	1.00	-78%
Minimum		15	18	0.0	0.21	-100%
Maximum		678	>10000	47.7	9.71	27%

Table II

Activity for MLN4924 against the PPTP *in Vivo* Panel

Xenograft Line	Histology	Median Time to Event	P-value	EFS T/C	Median Final RTV	T/C	P-value	T/C Activity	EFS Activity	Response Activity
BT-29	Rhabdoid	24.1	0.047	1.3	>4	1.08	0.971	Low	Low	Low
KT-14	Rhabdoid	> EP *	0.012	> 1.3	3.0	0.65	0.065	Low	NE	Int
KT-12	Rhabdoid	12.2	0.005	1.5	>4	0.58	0.002	Low	Low	Low
KT-10	Wilms	37.2	<0.001	3.2	>4	0.21	<0.001	Int	Int	Int
KT-11	Wilms	24.6	<0.001	2.2	>4	0.55	0.038	Low	Int	Int
KT-13	Wilms	26.9	0.003	1.8	>4	0.51	0.008	Low	Low	Int
SK-NEP-1	Ewing	9.1	0.206	1.4	>4	0.75	0.161	Low	Low	Low
EW5	Ewing	14.0	0.007	1.5	>4	0.91	0.739	Low	Low	Low
EW8	Ewing	12.6	<0.001	1.4	>4	0.72	0.003	Low	Low	Low
TC-71	Ewing	6.7	0.695	0.9	>4	1.15	0.481	Low	Low	Low
CHLA258	Ewing	6.9	0.084	1.1	>4	0.73	0.218	Low	Low	Low
Rh28	ALV RMS	20.4	0.200	1.2	>4	0.74	0.353	Low	Low	Low
Rh30	ALV RMS	15.9	<0.001	.	>4	2.19	<0.001	Low	Low	Low
Rh30R	ALV RMS	18.4	0.445	0.9	>4	1.49	0.022	Low	Low	Low
Rh41	ALV RMS	33.4	0.012	2.4	>4	0.63	0.052	Low	Int	Int
Rh18	EMB RMS	27.2	<0.001	2.2	>4	0.46	<0.001	Low	Int	Int
BT-45	Medulloblastoma	20.4	0.061	1.8	>4	0.73	0.075	Low	Low	Int
BT-50	Medulloblastoma	26.2	0.090	0.8	>4	0.58	0.052	Low	Low	Low
BT-44	Ependymoma	8.7	0.733	1.1	>4	0.79	0.645	Low	Low	Low
GBM2	Glioblastoma	29.2	<0.001	3.0	>4	0.14	<0.001	High	Int	Int
BT-39	Glioblastoma	30.0	<0.001	2.6	>4	0.32	<0.001	Int	Int	Int
D645	Glioblastoma	33.2	<0.001	3.4	>4	0.46	<0.001	Low	Int	Int
D456	Glioblastoma	14.4	<0.001	2.3	>4	0.36	<0.001	Int	Int	Int
NB-SD	Neuroblastoma	10.4	<0.001	1.9	>4	0.63	0.002	Low	Low	Int
NB-1771	Neuroblastoma	21.8	<0.001	3.6	>4	0.59	<0.001	Low	Int	Int
NB-1691	Neuroblastoma	9.5	0.003	1.5	>4	0.6	0.005	Low	Low	Low
NB-1643	Neuroblastoma	11.1	<0.001	1.7	>4	0.57	0.001	Low	Low	Int

Xenograft Line	Histology	Median Time to Event	P-value	EFS T/C	Median Final RTV	T/C	P-value	T/C Activity	EFS Activity	Response Activity
OS-1	Osteosarcoma	28.4	0.437	1.4	>4	0.74	0.053	Low	Low	Low
OS-2	Osteosarcoma	22.9	0.020	1.2	>4	0.76	0.052	Low	Low	Low
OS-17	Osteosarcoma	17.0	0.468	1.1	>4	0.97	0.631	Low	Low	Low
OS-9	Osteosarcoma	28.6	0.309	1.2	>4	0.94	0.579	Low	Low	Low
OS-33	Osteosarcoma	21.7	0.037	1.1	>4	0.64	0.001	Low	Low	Low
OS-31	Osteosarcoma	21.3	0.741	1.1	>4	1.05	0.436	Low	Low	Low
ALL-2	ALL B-precursor	25.1	<0.001	3.0	>25	.			Int	Int
ALL-3	ALL B-precursor	38.6	0.036	3.8	>25	.			Int	Int
ALL-4	ALL B-precursor	12.4	0.112	1.5	>25	.			Low	Int
ALL-7	ALL B-precursor	4.1	0.653	1.2	>25	.			Low	Low
ALL-8	ALL T-cell	13.4	<0.001	2.8	>25	.			Int	Int
ALL-16	ALL T-cell	23.9	0.176	1.8	>25	.			Low	Int
ALL-17	ALL B-precursor	16.0	<0.001	3.0	>25	.			Int	Int
ALL-19	ALL B-precursor	11.8	0.035	2.2	>25	.			Int	Int

* EP=evaluation period (42 days)