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Initial Testing of NSC 750854, a novel purine analog, against pediatric tumor models by the Pediatric Preclinical Testing Program

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Abstract

Background—NSC 750854 is a purine analog with an antitumor activity profile distinctive from that of other anticancer purines. It has shown significant activity against adult cancer preclinical models.

Procedures—NSC 750854 was tested against the Pediatric Preclinical Testing Program (PPTP) *in vitro* cell line panel at concentrations from 1.0 nM to 10 μ M and against the PPTP *in vivo* xenograft panels administered intraperitoneally at a dose of 5 mg/kg, daily $\times$ 5 repeated at day 15.

Results—The median relative IC₅₀ (rIC₅₀) value for the PPTP cell lines was 32 nM, (range from 11 nM-124 nM), with consistent cytotoxicity across all cell lines. Acute lymphoblastic leukemia (ALL) cell lines were more sensitive to NSC 750854 than non-ALL cell lines. NSC 750854 induced significant differences in EFS distribution compared to control in 31 of 35 (89%) solid

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tumor xenografts. It induced tumor growth inhibition meeting criteria for intermediate or high EFS T/C activity in 17 of 32 (53%) evaluable solid tumor xenografts (most consistently in the rhabdomyosarcoma panel). Objective responses were observed in 15 of 37 (41%) solid tumor xenografts and in all 8 leukemia models with CR or MCR in 7 of 8.

Conclusions—NSC-750854 has a unique spectrum of antitumor activity compared with other agents tested by the PPTP as it induces regression in tumor models with limited sensitivity to most agents tested to date. Given the promising level of activity observed for NSC 750854 against PPTP preclinical models, further exploration of its mechanism of action is warranted.

Keywords

Preclinical Testing; Developmental Therapeutics; NSC 750854; purine analogs

INTRODUCTION

NSC 750854 [(9H-Purine, 9-[5-O-(aminosulfonyl)-.beta.-D-ribofuranosyl)]-[(2R,3S,4R, 5R)-3,4-Dihydroxy-5-(9H-purin-9-yl)tetrahydrofuran-2-yl]methyl sulfamate);

Supplemental Figure 1] is a purine analog with an activity profile substantially different from that of other anticancer purines [1]. Unlike purine analogs used as anticancer agents, it does not have any substituents on the purine base. Because of the 5'-sulfamate on its sugar component, it cannot be phosphorylated. Initial mechanistic studies have shown that it does not interact with the RNA/DNA pathways typically affected by purine analogs, and further work on target identification is underway.

5'-O-aminosulfonyl-adenosine (NSC 133114) was initially reported as a potential anticancer agent in the 1970s based on *in vitro* studies [2]. NSC 750854 is the 6-desamino derivative of NSC 133114. NSC 750854 was evaluated for activity in the NCI *in vitro* screen and was active against a broad range of the 60 cell lines with the melanoma panel being most sensitive. Testing of NSC 750854 using the Hollow Fiber Assay (HFA) produced scores of 38/48 IP and 14/48 subcutaneously (SC) for a total score of 52 with cell kill occurring in several of the cell lines tested [1]. NSC 750854 was nearly twice as active *in vivo* as NSC 133114 [1].

For subcutaneous xenograft studies, antitumor activity was assessed against 8 distinct human tumor xenografts (A498 renal, Colo 205 colon, HCT-15 colon, LOX-IMVI melanoma, PC-3 prostate, RPMI-8226 myeloma, U251 CNS, UACC-62 melanoma) [1]. Under the conditions evaluated the most sensitive xenograft was the A498 renal tumor line, which responded to NSC 750854 administered by the PO, IP or IV routes. Against the RPMI-8226 myeloma line, NSC 750854 caused durable tumor stasis following administration by all 3 routes. The least responsive tumors under the conditions evaluated were Colo 205, HCT-115 and UACC-62, for which there was some tumor growth suppression but no durable tumor stasis. The remaining 3 xenografts were responsive with tumor growth stasis of varying durations.

Because of its preclinical activity and novel spectrum of antitumor activity, NSC 750854 was evaluated against the *in vitro* and *in vivo* models of the PPTP. NSC 750854

demonstrated significant and broad-spectrum activity both *in vitro* and *in vivo* against a range of pediatric preclinical models.

MATERIALS AND METHODS

In vitro testing

Testing was performed using DIMSCAN, as previously described in a characterized panel of 24 cell lines [3]. Cells were incubated in the presence of NSC 750854 for 96 hours at concentrations from 1.0 nM to 10 μ M and analyzed as previously described [4].

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 [5]. Human leukemia cells were propagated by intravenous inoculation in female non-obese diabetic (NOD)/*scid*^{-/-} mice as described previously [6]. 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. Eight to ten mice were used in each control or treatment group. Tumor volumes (cm³) were determined and responses were determined using three activity measures as previously described [5]. 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

NSC 750854 was provided to the Pediatric Preclinical Testing Program by the Developmental Therapeutics Program (DTP), NCI, through the Cancer Therapy Evaluation Program (NCI). NSC 750854 was formulated in 0.9% w/v saline and stored for up to 7 days at 4°C, protected from light. NSC 750854 was administered orally at 5 mg/kg daily $\times$ 5 (days 1-5) repeated at day 15 (15-19).with an observation period of 6 weeks. NSC 750854 was provided to each consortium investigator in coded vials for blinded testing.

RESULTS

In vitro testing

NSC 750854 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 relative IC₅₀ (rIC₅₀) value for the PPTP cell lines was 32 nM, with a range from 11 nM

(MOLT-4) to 124 nM (CHLA-258). A metric used to compare the relative responsiveness of the PPTP cell lines to NSC 750854 is the ratio of the median rIC_{50} of the entire panel to that of each cell line (see Table I and Figure 1). Higher ratios are indicative of greater sensitivity to NSC 750854 and are shown in Figure 1 by bars to the right of the midpoint line. Of note, all of the neuroblastoma cell lines (NB-1643 through CHLA-136) are less sensitive than the panel median, while all of the ALL cell lines (NALM-6 through CCRF-CEM) are more sensitive than the panel median. Examination of the rIC_{50} values by histotype shows that the ALL cell lines have lower rIC_{50} values than the non-ALL cell lines (19 nM versus 45 nM, $p=0.01$). Although rIC_{50} values are nominally higher for TP53 wildtype cell lines compared to mutant cell lines (46 nM versus 29 nM), this difference is not significant. The Relative In/Out (I/O)% values compare the relative difference in final cell number compared with the starting cell number for treated cells and for control cells calculated as follows: (Observed $Y_{min}-Y_0$)/(100- Y_0) if Observed $Y_{min}>Y_0$; and (Observed $Y_{min}-Y_0$)/(Y_0) if Observed $Y_{min}<Y_0$. Y_0 is an estimate of the starting cell number derived from determinations of the doubling time for each cell line. 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. At the highest concentration evaluated, NSC-750854 induced a Relative I/O of -100% for all cell lines tested.

In vivo testing

NSC 750854 was tested against the PPTP solid tumor xenografts using a dose of 5 mg/kg administered by the intraperitoneal (IP) route daily for 5 days repeated at day 15. The total planned treatment and observation period was 6 weeks. The dose was selected based on toxicity testing with doses of 3, 5, and 7.5 mg/kg administered to groups of 3 mice on the schedule above. Weight loss occurred quickly after week 1 dosing, with substantial recovery by day 15 (Supplemental Figure 2). Following the second treatment course that began at day 15, recovery to > 90% of baseline weight was delayed for the 7.5 mg/kg dose, while the 5 mg/kg dose group recovered more quickly. The 5 mg/kg dose was selected for efficacy testing. NSC 750854 was generally well tolerated at the 5 mg/kg dose, with only 1.6% drug related mortality in the treated groups (7 of 431), similar to that observed for control animals (5 of 422; 1.2%).

All 45 tested xenograft models were considered evaluable for efficacy. Complete details of testing are 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.

NSC 750854 induced significant differences in EFS distribution compared to control in 31 of 35 (89%) of the solid tumor xenografts evaluable for this measure (Table II). For those xenografts with a significant difference in EFS distribution between treated and control groups, the EFS T/C activity measure additionally requires an EFS T/C value of > 2.0 for intermediate activity and indicates a substantial agent effect in slowing tumor growth. High activity further requires a reduction in final tumor volume compared to the starting tumor volume. NSC 750854 induced tumor growth inhibition meeting criteria for intermediate EFS

T/C activity in 17 of 32 (53%) evaluable solid tumor xenografts. Intermediate or high activity for the EFS T/C metric occurred most consistently in the rhabdomyosarcoma panel (5 of 6). It was also observed for 1 of 1 rhabdoid xenografts evaluable for this metric, as well as for 3 of 3 Wilms tumor, 1 of 5 Ewing, 2 of 4 glioblastoma, 3 of 6 neuroblastoma, and 2 of 6 osteosarcoma xenografts. The two models with high EFS T/C activity were in the Wilms panel (KT-10) and the neuroblastoma panel (CHLA-79).

Objective responses were observed in 15 of 37 solid tumor xenografts. The objective response results for solid tumor models are represented in Figure 2 using a 'COMPARE' format, based on the objective response scoring criteria centered around the midpoint score of 0 that represents stable disease. In the figure, xenografts with PD2 are indicated by a score of -3, and xenografts with regression (PR or CR) are indicated by bars to the right of the midpoint line. Red bars indicate xenografts with significant differences in EFS distribution between the treated and control groups. Figure 2 shows also the objective response activity in a "heat map" format. Objective responses were observed for 2 of 3 rhabdoid tumor models, 2 of 3 Wilms tumor models, and 4 of 6 rhabdomyosarcoma xenografts tested. Both the glioblastoma and neuroblastoma panels had two xenografts with objective responses. Results for rhabdoid tumors (BT-29, KT-16) and two glioblastoma models (D456, D645) are shown in Figure 3. NSC 750854 induced objective responses in all 8 leukemia models, with CR or MCR in 7 of 8 (Figure 4).

DISCUSSION

NSC 750854 is a purine nucleoside analog that cannot be phosphorylated at the 5' position of the sugar, and hence it is not incorporated into nucleic acids. The agent had broad-spectrum activity when tested against the NCI 60 *in vitro* panel with melanoma cell lines being somewhat more sensitive under conditions of testing. NSC 750854 showed activity in the Hollow Fiber *in vivo* assay and was advanced to testing against subcutaneous, cell line-derived xenograft models. Of these, RPMI-8226 (myeloma) and A498 (renal cell carcinoma) were the most sensitive showing partial regressions. NSC-750845 was not active against other models including Colo 205, HCT-115 (colon carcinoma), or U251 (glioblastoma). This differential sensitivity increases the likelihood that the antitumor activity is specific and not simply a non-specific effect due to species differences in tolerated drug exposures. However, the mechanism of action of NSC 750854 has not been elucidated, creating challenges for further development of the agent.

At the request of Developmental Therapeutics Program, NCI, the PPTP tested NSC 750854 against its *in vitro* and *in vivo* tumor panels. *In vitro* the median rIC_{50} was 32 nM, with the MOLT-4 leukemia line being most sensitive (11 nM) and with the Ewing sarcoma line CHLA258 being least sensitive (124 nM). At the highest concentrations tested NSC 750854 caused uniform cytotoxicity with at least 99 per cent reduction in cell number relative to the number of cells plated. As shown in Figure 1, the leukemic cell lines were slightly more sensitive than the cell line panel (leukemias and solid tumor cell lines) median sensitivity (~2-fold). We and others have reported that leukemia (or hematopoietic lineage) cell lines are generally more sensitive to cytotoxic agents [4]. However, our data suggest that other biological factors other than the cell cycle times also contribute to *in vitro* sensitivity of

chemotherapeutic agents. For example, some cell cycle specific drugs, cabazitaxel and docetaxel, have lesser activity against leukemia cells than against pediatric solid tumor cell lines [7]. In general the leukemia panel was not more sensitive than the cell line panel median sensitivity when 20 drugs randomly sampled from the PPTP testing database were examined. These agents included drug or biologics with a broad spectrum of mechanisms of action (alkylating agents, a replication competent virus, antimetabolites, receptor tyrosine kinase targeted antibodies, signaling inhibitors, topoisomerase poisons, etc). In 12 studies the leukemia cell line panel was no more sensitive than the median panel sensitivity, suggesting that the result for NSC-750854 was not merely a consequence of leukemic cells being sensitive to all agents, or a consequence of more rapid proliferation. For example, an mTOR inhibitor, MLN0128 was more active in solid tumor lines than in leukemia [8]. Of note, the greatest differential sensitivity of the leukemic cell line panel over the solid tumor panel was for cytosine arabinoside (cytarabine), a drug used extensively in treatment of childhood ALL [9].

In vivo, NSC 750854 was tested at 5 mg/kg administered IP daily for 5 days with the cycle repeated at day 15. This dose was selected based upon prior toxicity testing, and it is in the range of doses used previously against adult cancer models. Because dosing was limited to two cycles of treatment, it is not possible to assess cumulative toxicity for NSC-750854. Assessing cumulative toxicity is outside the scope of the PPTP testing strategy, and will be undertaken in formal IND enabling toxicity testing should further development of this agent occur. NSC 750854 caused objective responses (PR or greater) in 15 of 37 solid tumor xenograft models and in all 8 leukemia models with CR or MCR in 7 of 8. Of note, CNS tumors including rhabdoid tumors and glioblastoma, tumors not regarded as sensitive to many agents, were relatively responsive to treatment with NSC 750854. Other responders included rhabdomyosarcoma (4 of 6), and CHLA258 which was the least sensitive cell line *in vitro* yet showed tumor regression *in vivo*.

Whereas the *in vivo* activity of NSC 750854 is substantial, lack of understanding of its mechanism hinders its progression to the clinic. While the mechanism of action of NSC 750854 is not known, structurally related compounds (e.g., 5'-O-aminosulfonyl-adenosine and nucleocidin) inhibit protein synthesis [2,10], and selected adenosine sulfamate analogues (e.g., MLN4924) inhibit E1 enzymes thereby preventing neddylation and ubiquitination of proteins [11,12]. We reviewed previous PPTP testing data in the anticipation that the antitumor activity of NSC-750854 may be similar to an agent previously tested. A review of COMPARE graphs for all agents tested by PPTP (available at: <http://gccri.uthscsa.edu/pptp/comparison.asp>) showed that the spectrum of antitumor activity for NSC-750843 was unique. The most similar COMPARE profile is for topotecan, a DNA topoisomerase I poison, where there were 10 xenograft models that were sensitive to both agents, and 8 models where NSC-750854 induced regressions whereas topotecan did not. Of note, of 31 drugs/biologics where the BT-29 CNS rhabdoid model has been used in testing, NSC-750845 is the only agent to induce tumor regression in this model.

We next examined expression data and exome sequencing data for each of the tumor models evaluated for drug sensitivity. We were unable to identify exomic mutations or expression profiles (Agilent) that segregated with sensitivity or resistance (<http://target.nci.nih.gov/>

dataMatrix/), or Affymetrix U133 Plus 2 profiles (<http://gccri.uthscsa.edu/pptp/data.asp>). However, it is of note that of the 15 solid tumor models demonstrating objective regressions 12 are wild type for TP53, as are the six ALL models that were responsive to NSC-750843

The PPTP has usually tested agents that have IND status or are already in adult phase I trials. NSC-750854 is an exception as it is in early development, and no mechanism of action has been identified. Here we report the single agent activity against 45 xenograft models, although the relationship between response and drug exposure has not been determined. If clinical development of NSC-750854 does proceed, then establishing effective antitumor exposures to this agent will be important, particularly when allometric scaling in different species, or pharmacokinetic data from phase I trials is available. The significance of the data presented here will depend upon the extent to which drug exposures in mice can be replicated in humans.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

NSC 750854 *in vitro* activity: A. The median rIC_{50} ratio graph shows the relative rIC_{50} values for the cell lines of the PPTP panel. Bars with stripes represent Ewing sarcoma cell lines and bars with dots represent ALL cell lines. Each bar represents the ratio of the panel rIC_{50} to the rIC_{50} value of the indicated cell line. Bars to the right represent cell lines with higher sensitivity, while bars to the left indicate cell lines with lesser sensitivity; B. All cell lines have Relative I/O% values between -99% and -100% , consistent with a prominent cytotoxic effect.

Figure 2.

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 3.

NSC 750854 *in vivo* objective response activity against solid tumor and brain tumor models. KT-16 (kidney), BT-29 (brain) AT/RT models, D456 and D645 glioblastoma models. Kaplan-Meier curves for EFS (left), median relative tumor volume graphs (center), and individual tumor volume graphs (right) are shown. Treated (black lines), statistical significance (p values) of the difference between treated and control groups are included.

Figure 4. NSC 750854 *in vivo* objective response activity against leukemia models. ALL-4, ALL-7 (ALL B-precursor), ALL-8 (T-cell), MLL-7 (Tertiary, Infant, Precursor B-ALL). Kaplan-Meier curves for EFS (left), median relative tumor volume graphs (center), and individual tumor volume graphs (right) are shown. Treated (black lines), statistical significance (p values) of the difference between treated and control groups are included.

Table I*In vitro* activity of NSC 750854 against PPTP cell lines.

Cell Line	Histotype	p53 Status	rIC ₅₀ (nM)	Panel rIC ₅₀ /Line rIC ₅₀	Y _{min} (%) (Observed)	Relative In/Out (Observed Y _{min})
RD	Rhabdomyosarcoma	Mutant	48	0.7	0.0	–99%
Rh41	Rhabdomyosarcoma	Mutant	28	1.1	0.0	–100%
Rh18	Rhabdomyosarcoma	WT	62	0.5	0.1	–100%
Rh30	Rhabdomyosarcoma	Mutant	15	2.1	0.2	–99%
BT-12	Rhabdoid	WT	98	0.3	0.0	–100%
CHLA-266	Rhabdoid	WT	48	0.7	0.3	–99%
TC-71	Ewing sarcoma	Mutant	49	0.7	0.0	–100%
CHLA-9	Ewing sarcoma	WT	12	2.6	0.0	–100%
CHLA-10	Ewing sarcoma	Mutant	37	0.9	0.0	–100%
CHLA-258	Ewing sarcoma	WT	124	0.3	0.0	–100%
SJ-GBM2	Glioblastoma	Mutant	31	1.0	0.0	–100%
NB-1643	Neuroblastoma	WT	43	0.7	0.0	–100%
NB-EBc1	Neuroblastoma	WT	51	0.6	0.0	–100%
CHLA-90	Neuroblastoma	Mutant	46	0.7	0.0	–100%
CHLA-136	Neuroblastoma	WT	62	0.5	0.3	–99%
NALM-6	ALL	WT	23	1.4	0.0	–100%
COG-LL-317	ALL	WT	14	2.2	0.0	–100%
RS4;11	ALL	WT	27	1.2	0.0	–100%
MOLT-4	ALL	WT	11	3.0	0.1	–99%
CCRF-CEM (1)	ALL	Mutant	20	1.6	0.0	–100%
CCRF-CEM (2)	ALL	Mutant	17	1.8	0.0	–100%
Kasumi-1	AML	Mutant	25	1.2	0.0	–100%
Karpas-299	ALCL	Mutant	22	1.5	0.0	–100%
Ramos-RA1	NHL	Mutant	33	1.0	0.0	–100%
Median			32	1.0	0.0	–100%
Minimum			11	0.3	0.0	–100%
Maximum			124	3.0	0.3	–99%

Table IISummary of *in Vivo* Activity of NSC 750854.

Line	Tumor Type	Median Time to Event	P-value	EFS T/C	Median Final RTV/CD45	Tumor Volume T/C	EFS Activity	Response
BT-29	Rhabdoid	> EP	<0.001	> 1.4	0.4	0.07	NE	CR
KT-16	Rhabdoid	> EP	<0.001	> 4.0	1.1	0.06	Int	CR
KT-14	Rhabdoid	> EP	<0.001	> 1.7	3.0	0.43	NE	PD2
KT-10	Wilms	> EP	<0.001	> 3.4	0.0	0.00	High	MCR
KT-11	Wilms	> EP	<0.001	> 2.3	2.2	0.23	Int	PR
KT-13	Wilms	30.6	<0.001	2.0	>4	0.45	Int	PD2
SK-NEP-1	Ewing	10.9	0.388	0.9	>4	1.18	Low	PD1
EW5	Ewing	10.2	0.272	1.3	>4	0.75	Low	PD1
EW8	Ewing	13.8	0.953	1.9	>4	0.59	Low	PD2
TC-71	Ewing	12.7	<0.001	1.9	>4	0.45	Low	PD2
CHLA258	Ewing	30.1	<0.001	3.4	>4	0.10	Int	CR
Rh10	Alveolar RMD	> EP	<0.001	> 3.0	2.3	0.08	Int	CR
Rh28	Alveolar RMS	31.8	<0.001	2.4	>4	0.28	Int	PR
Rh30	Alveolar RMS	20.0	<0.001	1.6	>4	0.37	Low	PD2
Rh30R	Alveolar RMS	23.2	<0.001	3.4	>4	0.20	Int	PD2
Rh41	Alveolar RMS	31.3	<0.001	3.3	>4	0.14	Int	PR
Rh18	Embryonal RMS	26.1	<0.001	3.3	>4	0.16	Int	PR
BT-28	Medulloblastoma	21.3	0.006	1.7	>4	0.58	Low	PD2
BT-50	Medulloblastoma	> EP	<0.001	> 1.2	1.7	0.23	NE	PR
BT-36	Ependymoma	> EP	1.000	.	0.7	0.38	NE	CR
BT-41	Ependymoma	> EP	1.000	.	1.4	0.57	NE	PD2
GBM2	Glioblastoma	24.4	0.006	1.7	>4	0.65	Low	PD2
BT-39	Glioblastoma	17.8	0.006	1.7	>4	0.28	Low	PD2
D645	Glioblastoma	41.1	<0.001	3.1	2.8	0.14	Int	CR
D456	Glioblastoma	> EP	<0.001	> 8.0	3.0	0.02	Int	CR
NB-SD	Neuroblastoma	17.2	0.011	1.6	>4	0.29	Low	PD2
NB-1771	Neuroblastoma	18.9	<0.001	2.2	>4	0.17	Int	SD
NB-1691	Neuroblastoma	10.2	<0.001	1.9	>4	0.39	Low	PD2
NB-EBc1	Neuroblastoma	13.0	<0.001	2.6	>4	0.05	Int	PR
CHLA-79	Neuroblastoma	> EP	<0.001	> 6.8	0.8	0.00	High	CR
NB-1643	Neuroblastoma	24.5	<0.001	1.9	>4	0.32	Low	PD2
OS-1	Osteosarcoma	29.8	<0.001	1.4	>4	0.67	Low	PD1
OS-2	Osteosarcoma	30.7	<0.001	1.9	>4	0.54	Low	PD2
OS-17	Osteosarcoma	38.0	<0.001	2.1	>4	0.36	Int	PD2
OS-9	Osteosarcoma	19.6	0.090	1.1	>4	0.85	Low	PD1

Line	Tumor Type	Median Time to Event	P-value	EFS T/C	Median Final RTV/CD45	Tumor Volume T/C	EFS Activity	Response
OS-33	Osteosarcoma	33.5	<0.001	2.5	>4	0.39	Int	PD2
OS-31	Osteosarcoma	33.4	0.005	1.3	>4	0.65	Low	PD1
ALL-2	ALL B-precursor	> EP	<0.001	> 2.4	18.3	.	Int	CR
ALL-4	ALL B-precursor	34.0	<0.001	5.7	>25	.	Int	CR
ALL-7	ALL B-precursor	40.3	<0.001	6.1	>25	.	Int	CR
ALL-8	ALL T-cell	> EP	<0.001	> 6.5	0.7	.	High	MCR
ALL-17	ALL B-precursor	> EP	<0.001	> 5.7	20.5	.	Int	CR
ALL-19	ALL B-precursor	> EP	<0.001	> 7.0	16.5	.	Int	CR
ALL-31	T-cell ALL	36.9	0.002	6.2	>25	.	Int	PR
MLL-7	ALL, MLL	38.6	<0.001	8.4	>25	.	Int	CR

¹ *Tumor Volume T/C value:* 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 T/C value is the mean RTV for the treatment group divided by the mean RTV for the control group. High activity = T/C ≥ 0.15 ; Intermediate activity = T/C ≥ 0.45 but < 0.15 ; and Low activity = T/C < 0.45 .

² Objective response measures are described in detail in the Supplemental Response Definitions. PD1 = progressive disease with EFS T/C ≥ 1.5 , and PD2 = progressive disease with EFS T/C > 1.5 .

³ EFS T/C values = the ratio of the median time to event of the treatment group and the median time to event of the respective control group. High activity requires: a) an EFS T/C > 2 ; b) a significant difference in EFS distributions, and c) a net reduction in median tumor volume for animals in the treated group at the end of treatment as compared to at treatment initiation. Intermediate activity = criteria a) and b) above, but not having a net reduction in median tumor volume for treated animals at the end of the study. Low activity = EFS T/C < 2 .