#2752 Pediatric Preclinical Testing Program (PPTP) Stage 1 Evaluation of NSC750854, a Sulfated Purine Analog with a Distinctive Anticancer Activity Profile

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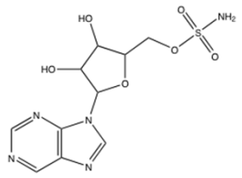
NSC750854

- 5'-O-aminosulfonyl-adenosine (NSC133114) was initially reported as a potential anticancer agent in the 1970s based on in vitro studies (A.Bloch, Biochemistry 10:4394, 1971).
- NCI found that NSC750854, the 6-desamino derivative of NSC133114 was equally potent as NSC133114 in vitro, and that it was nearly twice as active in vivo as NSC133114 (see Hollingshead, et al., Abstract # 4489).
- The structure of NSC750854 is shown below. Its 5'-sulfamate prevents phosphorylation. Studies are ongoing at NCI to elucidate its cellular target and mechanism of action.



NSC750854 [3,4-dihydroxy-5-purin-9-yloxolan-2-yl)methyl sulfamate]

NSC750854 IN VITRO ACTIVITY

- The median relative IC_{50} (rIC_{50}) for NSC750854 against the PPTP cell lines was 32 nM (range 11 nM to 124 nM).
- NSC750854 demonstrated potent cytotoxic activity, with Ymin values approaching 0% for all but one of the cell lines at the highest concentration tested.
- NSC-750854 showed no dependence on TP53 for its cytotoxic effect, with TP53 mutated cell lines showing nominally lower rIC_{50} values compared to wildtype lines.
- Leukemia cell lines were generally more sensitive than solid tumor cell lines (median rIC_{50} 19 nM vs 45 nM, respectively).

Relative IC_{50} values by histotype

	RMS	Non RMS	Ewing	Non Ewing	Neuro	Non Neuro	ALL	Non ALL	Panel rIC_{50}
Number of values	4	20	4	20	4	20	6	18	24
Median (nM)	38	32	43	29	49	27	19	45	32
P-value	0.82		0.51		0.09		0.01		

- NSC750854 was provided for testing by the Developmental Therapeutics Program (NCI). Testing was supported by NCI NO1CM42216.
- This poster will be available at: http://pptp.nchresearch.org/presentations.html.

PPTP IN VITRO & IN VIVO TESTING METHODS

In vitro: 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 (Kang MH, et al. Pediatr Blood Cancer 56:239-249, 2011). Testing was for 96 hours at concentrations from 1.0 nM to 10.0 μM with replicates of 6-12 per data point. Data were analyzed by fitting a non-linear regression model-sigmoidal dose-response model to the response-relative fluorescence values vs. the concentration.

In vivo: Standard PPTP methods for in vivo testing were employed (http://pptp.nchresearch.org/documents/detailedAnalysisMethods.pdf).

NSC750854 was tested in vivo using a dose of 5 mg/kg administered by the intraperitoneal (IP) route daily for 5 days repeated at day 15.

For each xenograft line, 10 mice bearing SC tumors initiated treatment when the tumors were between 0.2–0.5 cm^3. Two perpendicular tumor diameters were measured at either once or twice weekly intervals with digital vernier calipers Assuming tumors to be spherical, volumes were calculated from the formula $(\pi/6) \times d3$, where d represents the mean diameter.

The primary activity measures were the objective response measure (see legend to figure at right) and the EFS T/C measure. The EFS T/C value is defined by the ratio of the median time to event of the treatment group and the median time to event of the respective control group.

In vitro activity of NSC750854 against PPTP cell lines

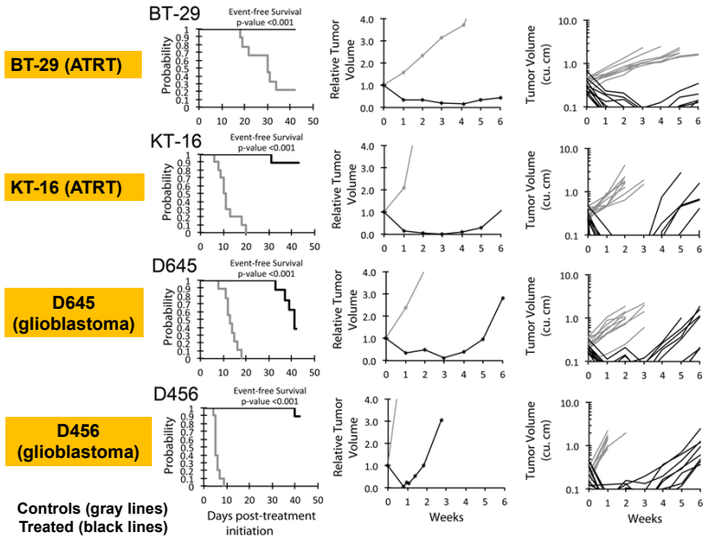
Cell Line	Histotype	p53 Status	rIC_{50} (nM)	Panel rIC_{50}/ Line rIC_{50}	Y_{min} (%) (Observed)	Relative In/Out (Observed Y_{min})
RD	Rhabdomyosarcoma	Mutant	48	0.7	0.0	-99%
Rh41	Rhabdomyosarcoma	Mutant	28	1.1	0.0	-100%
Rh18	Rhabdomyosarcoma	WT	62	0.5	0.1	-100%
Rh30	Rhabdomyosarcoma	Mutant	15	2.1	0.2	-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%**

NSC750854 IN VIVO ACTIVITY

In vivo activity of NSC750854 against PPTP solid tumor xenograft models

Xenograft Line	Histology	EFS T/C	P-value	T/C	P-value	Response
BT-29	Rhabdoid	> 1.4	<0.001	0.07	<0.001	CR
KT-16	Rhabdoid	> 4.0	<0.001	0.06	<0.001	CR
KT-10	Wilms	> 3.4	<0.001	0.00	<0.001	MCR
KT-13	Wilms	2.0	<0.001	0.45	<0.001	PD2
SK-NEP-1	Ewing	0.9	0.388	1.18	1.000	PD1
EW5	Ewing	1.3	0.272	0.75	0.075	PD1
EW8	Ewing	1.9	0.953	0.59	0.218	PD2
TC-71	Ewing	1.9	<0.001	0.45	<0.001	PD2
CHLA258	Ewing	3.4	<0.001	0.10	<0.001	CR
Rh10	Alveolar RMS	> 3.0	<0.001	0.08	<0.001	CR
Rh30R	Alveolar RMS	3.4	<0.001	0.20	0.002	PD2
Rh41	Alveolar RMS	3.3	<0.001	0.14	<0.001	PR
Rh18	Embryonal RMS	3.3	<0.001	0.16	<0.001	PR
BT-28	Medulloblastoma	1.7	0.006	0.58	0.002	PD2
GBM2	Glioblastoma	1.7	0.006	0.65	0.009	PD2
BT-39	Glioblastoma	1.7	0.006	0.28	<0.001	PD2
D645	Glioblastoma	3.1	<0.001	0.14	<0.001	CR
D456	Glioblastoma	> 8.0	<0.001	0.02	<0.001	CR
NB-SD	Neuroblastoma	1.6	0.011	0.29	<0.001	PD2
NB-1771	Neuroblastoma	2.2	<0.001	0.17	<0.001	SD
NB-1691	Neuroblastoma	1.9	<0.001	0.39	<0.001	PD2
NB-EBc1	Neuroblastoma	2.6	<0.001	0.05	<0.001	PR
CHLA-79	Neuroblastoma	> 6.8	<0.001	0.00	<0.001	CR
NB-1643	Neuroblastoma	1.9	<0.001	0.32	<0.001	PD2
OS-1	Osteosarcoma	1.4	<0.001	0.67	<0.001	PD1
OS-2	Osteosarcoma	1.9	<0.001	0.54	<0.001	PD2
OS-17	Osteosarcoma	2.1	<0.001	0.36	<0.001	PD2
OS-9	Osteosarcoma	1.1	0.090	0.85	0.043	PD1
OS-33	Osteosarcoma	2.5	<0.001	0.39	<0.001	PD2
OS-31	Osteosarcoma	1.3	0.005	0.65	<0.001	PD1

- Red shading in the p-value columns indicates a significant difference in EFS distribution or Tumor Volume T/C between treated and control groups.
- Shading in the EFS and T/C columns indicates xenografts that have either high (dark blue) or intermediate (light blue) activity for these measures.
- **PD1 (Progressive Disease 1, dark green):** >25% ↑ in tumor volume, TGD value ≤1.5
- **PD2 (Progressive Disease 2, light green):** >25% ↑ in tumor volume, TGD value >1.5
- **SD (Stable Disease):** <25% ↑ in tumor volume, <50% regression
- **PR (Partial response):** a tumor volume regression ≥50%
- **CR (Complete response):** disappearance of measurable tumor mass (< 0.10 cm^3)
- **MCR (Maintained CR):** absence of measurable tumor mass at day 42.



IN VIVO RESULTS AND CONCLUSIONS

- NSC750854 was well tolerated (1.7% mortality) at the dose (5 mg/kg IP) and schedule (daily x 5 repeated at day 15) evaluated, and 30 of 30 xenograft models were considered evaluable for efficacy.
- NSC750854 induced significant differences in EFS distribution compared to control in 26 of 30 (87%) evaluable solid tumor xenografts.
- NSC750784 induced tumor growth inhibition meeting criteria for intermediate or high EFS T/C activity (requiring EFS T/C > 2) in 14 of 29 xenografts (48%), with this level of activity most consistently observed in the rhabdomyosarcoma panel (4 of 4).
- NSC750854 induced objective responses in 11 of 30 (37%) of solid tumor models, including two glioblastoma models.
- While some of the xenografts that were responsive to NSC-750854 have also shown responsiveness to other cytotoxic agents (e.g., KT-10 and CHLA-258), other responsive models have shown limited sensitivity to most agents against which they've been tested (e.g., BT-29 and CHLA-79).
- NSC750854 demonstrated a wide spectrum of antitumor activity. However, as with other agents, accurate translation of these results to the clinic will depend upon the tolerance of patients to the agent and upon how the drug exposures causing tumor regression in this study compare to those achievable in humans.