

# PEDIATRIC PRECLINICAL TESTING PROGRAM (PPTP) EVALUATION OF NEDD8-ACTIVATING ENZYME (NAE) INHIBITOR MLN4924



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## ABSTRACT

**Background:** MLN4924 is a potent and selective small molecule NAE inhibitor. In most cancer cells tested, inhibition of NAE leads to induction of DNA rereplication, resulting in DNA damage and cell death. NEDD8-activating enzyme (NAE) is an essential component of the NEDD8 conjugation pathway that controls the activity of the cullin-RING subtype of ubiquitin ligases, thereby regulating the turnover of a subset of proteins upstream of the proteasome. Substrates of cullin-RING ligases have important roles in cellular processes associated with cancer cell growth and survival pathways. The activity of MLN4924 was evaluated against the PPTP's *in vitro* and *in vivo* panels.

**Methods:** The PPTP includes a molecularly characterized *in vitro* panel of cell lines (n=27) and *in vivo* panel of xenografts (n=61) representing most of the common types of childhood solid tumors and childhood acute lymphoblastic leukemia (ALL). MLN4924 (provided by Millennium Pharmaceuticals) was tested *in vitro* at concentrations from 1.0 nM to 10.0 μM and was tested against the PPTP *in vivo* panel using a 100 mg/kg dose (66mg/kg for ALL panel) BID administered SQ daily x 5 for 3 weeks, with observation for 6 weeks. 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) measure based on the median event-free survival (EFS) of treated and control animals for each xenograft.

**Results:** The median relative IC<sub>50</sub> for MLN4924 against the PPTP cell lines was 143 nM, with a range from 15 nM to 678 nM. The median relative IC<sub>50</sub> value for the Ewing panel (31 nM) was significantly lower than that of the remaining PPTP cell lines. MLN4924 demonstrated an activity pattern consistent with cytotoxic activity. MLN4924 was well tolerated, with a toxicity rate of 4.6%. 42 of 45 xenograft models were considered evaluable for efficacy. MLN4924 induced significant differences in EFS distribution compared to control in 20 of 34 (59%) 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 EFS T/C measure. The maximum shift in time to event for treated animals was 3.8-fold (ALL-3), and two other xenografts had shifts in time to event of 3.0-fold (ALL-2 and ALL-17). MLN4924 did not induce objective responses in the PPTP solid tumor or ALL panels, with the best response being stable disease for 1 ALL xenograft.

**Conclusions:** MLN4924 potentially inhibits the cell lines of the PPTP *in vitro* panel and shows an interesting pattern of *in vivo* activity. Pharmacokinetic and pharmacodynamic studies are ongoing.

## 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 (see <http://pptp.nchresearch.org/documents/detailedAnalysisMethods.pdf>). MLN4924 was tested *in vivo* using a 100 mg/kg dose administered subcutaneously twice-daily for 5 days, repeated for 3 weeks.

**Solid tumor testing:** For each xenograft line, 10 mice bearing SC tumors initiated treatment when the tumors were between 0.2-0.5 cm<sup>3</sup>. 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 (π/6)×d<sup>3</sup>, where d represents the mean diameter.

**Acute lymphoblastic leukemia testing:** For each xenograft line, 8 mice were inoculated with 3-5 x 10<sup>6</sup> mononuclear cells purified from the spleens of secondary recipient mice. Engraftment was monitored weekly by flow cytometry, and treatment was initiated when the proportion of human CD45+ cells in the peripheral blood reached 1%. The proportion of human CD45+ cells in the peripheral blood was monitored weekly throughout the course of treatment.

## MLN4924 IN VITRO ACTIVITY

□ The median relative IC<sub>50</sub> (rIC<sub>50</sub>) for MLN4924 against the PPTP cell lines was 143 nM (range 15 nM to 678 nM).

□ The median rIC<sub>50</sub> value for the Ewing panel (31 nM) was significantly lower than the median for the other PPTP cell lines. The single GBM cell line also had a low rIC<sub>50</sub> value.

□ MLN4924 demonstrated potent cytotoxic activity, with Ymin values approaching 0% for most of the cell lines at the highest concentration tested.

□ 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).

Cell line	Histotype	Relative IC <sub>50</sub> (nM)	Ymin (%)
RD	RMS	112	6.8
Rh41	RMS	179	9.7
Rh18	RMS	266	18.5
Rh30	RMS	235	10.9
BT-12	Rhabdoid	119	7.2
CHLA-266	Rhabdoid	175	7.1
TC-71	Ewing	33	0.0
CHLA-9	Ewing	29	0.8
CHLA-10	Ewing	98	1.5
CHLA-258	Ewing	15	0.4
GBM2	GBM	42	2.6
NB-1643	NB	143	9.3
NB-EBc1	NB	430	1.2
CHLA-90	NB	110	22.0
CHLA-136	NB	678	47.7
NALM-6	ALL	165	0.0
COG-LL-317	ALL	254	0.0
RS4;11	ALL	138	0.1
MOLT-4	ALL	144	0.2
CCRF-CEM	ALL	251	0.1
Kasumi-1	AML	122	0.9
Karpas-299	ALCL	257	0.0
Ramos-RA1	NHL	105	0.0
Median		143	1.2
Minimum		15	0.0
Maximum		678	47.7

Testing was supported by NCI NO1CM42216

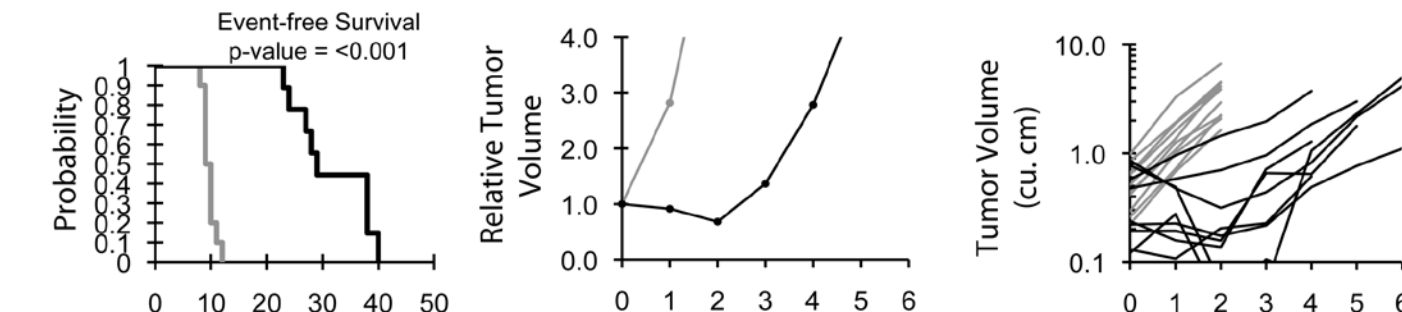
## MLN4924 IN VIVO ACTIVITY

Xenograft Line	Histology	EFS T/C	P-value	T/C	P-value	Response
BT-29	Rhabdoid	1.3	0.047	1.08	0.971	PD1
KT-14	Rhabdoid	> 1.3	0.012	0.65	0.065	PD2
KT-12	Rhabdoid	1.5	0.005	0.58	0.002	PD1
KT-10	Wilms	3.2	<0.001	0.21	<0.001	PD2
KT-11	Wilms	2.2	<0.001	0.55	0.038	PD2
KT-13	Wilms	1.8	0.003	0.51	0.008	PD2
SK-NEP-1	Ewing	1.4	0.206	0.75	0.161	PD1
EW5	Ewing	1.5	0.007	0.91	0.739	PD1
EW8	Ewing	1.4	<0.001	0.72	0.003	PD1
TC-71	Ewing	0.9	0.695	1.15	0.481	PD1
CHLA258	Ewing	1.1	0.084	0.73	0.218	PD1
Rh28	ALV RMS	1.2	0.200	0.74	0.353	PD1
Rh30	ALV RMS	.	<0.001	2.19	<0.001	PD1
Rh30R	ALV RMS	0.9	0.445	1.49	0.022	PD1
Rh41	ALV RMS	2.4	0.012	0.63	0.052	PD2
Rh18	EMB RMS	2.2	<0.001	0.46	<0.001	PD2
BT-28	Medulloblastoma	1.1	0.519	0.77	0.393	PD1
BT-45	Medulloblastoma	1.8	0.061	0.73	0.075	PD2
BT-50	Medulloblastoma	0.8	0.090	0.58	0.052	PD1
BT-44	Ependymoma	1.1	0.733	0.79	0.645	PD1
GBM2	Glioblastoma	3.0	<0.001	0.14	<0.001	PD2
BT-39	Glioblastoma	2.6	<0.001	0.32	<0.001	PD2
D645	Glioblastoma	3.4	<0.001	0.46	<0.001	PD2
D456	Glioblastoma	2.3	<0.001	0.36	<0.001	PD2
NB-SD	Neuroblastoma	1.9	<0.001	0.63	0.002	PD2
NB-1771	Neuroblastoma	3.6	<0.001	0.59	<0.001	PD2
NB-1691	Neuroblastoma	1.5	0.003	0.6	0.005	PD1
NB-1643	Neuroblastoma	1.7	<0.001	0.57	0.001	PD2
OS-1	Osteosarcoma	1.4	0.437	0.74	0.053	PD1
OS-2	Osteosarcoma	1.2	0.020	0.76	0.052	PD1
OS-17	Osteosarcoma	1.1	0.468	0.97	0.631	PD1
OS-9	Osteosarcoma	1.2	0.309	0.94	0.579	PD1
OS-33	Osteosarcoma	1.1	0.037	0.64	0.001	PD1
OS-31	Osteosarcoma	1.1	0.741	1.05	0.436	PD1
ALL-2	ALL B-precursor	3.0	<0.001	.	.	PD2
ALL-3	ALL B-precursor	3.8	0.036	.	.	SD
ALL-4	ALL B-precursor	1.5	0.112	.	.	PD2
ALL-7	ALL B-precursor	1.2	0.653	.	.	PD1
ALL-8	ALL T-cell	2.8	<0.001	.	.	PD2
ALL-16	ALL T-cell	1.8	0.176	.	.	PD2
ALL-17	ALL B-precursor	3.0	<0.001	.	.	PD2
ALL-19	ALL B-precursor	2.2	0.035	.	.	PD2

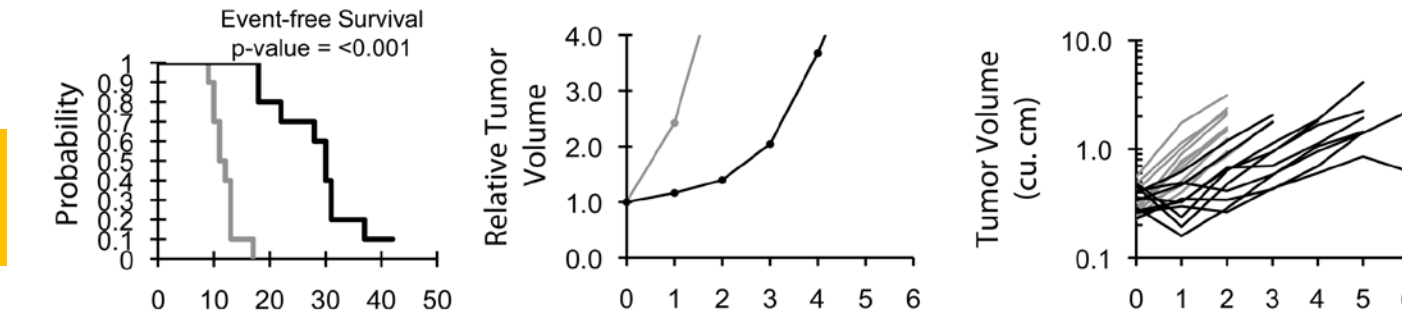
- Red shading in the p-value columns indicates a significant difference in EFS distribution or Tumor Volume T/C between treated and control groups.
- Shading in the EFS columns indicates xenografts that have either high (dark blue), intermediate (light blue), low (gray), or indeterminate (white) activity.
- PD1 (Progressive Disease 1): >25% ↑ in tumor volume, TGD value ≤1.5;
- PD2 (Progressive Disease 2): >25% ↑ in tumor volume, TGD value >1.5;
- SD (Stable Disease): <25% ↑ in tumor volume, <50% regression

MLN4924 was provided for testing by Millennium Pharmaceuticals

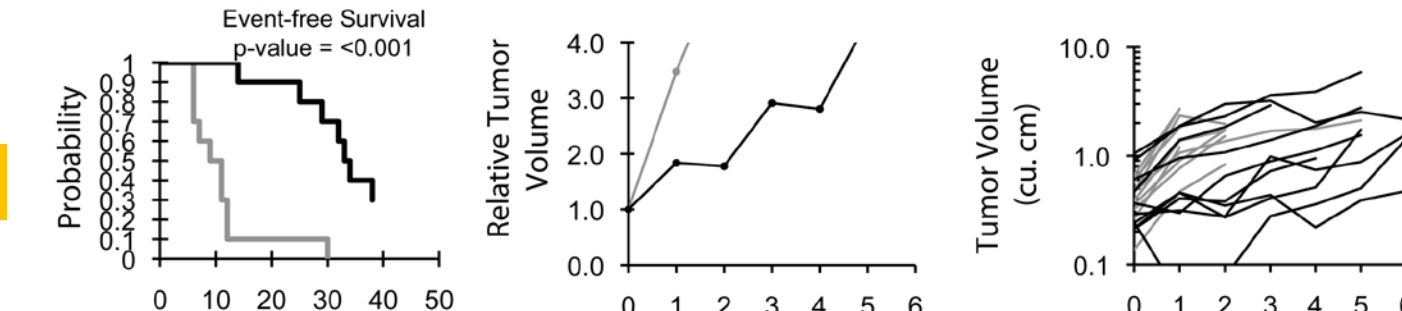
### GBM2 (glioblastoma)



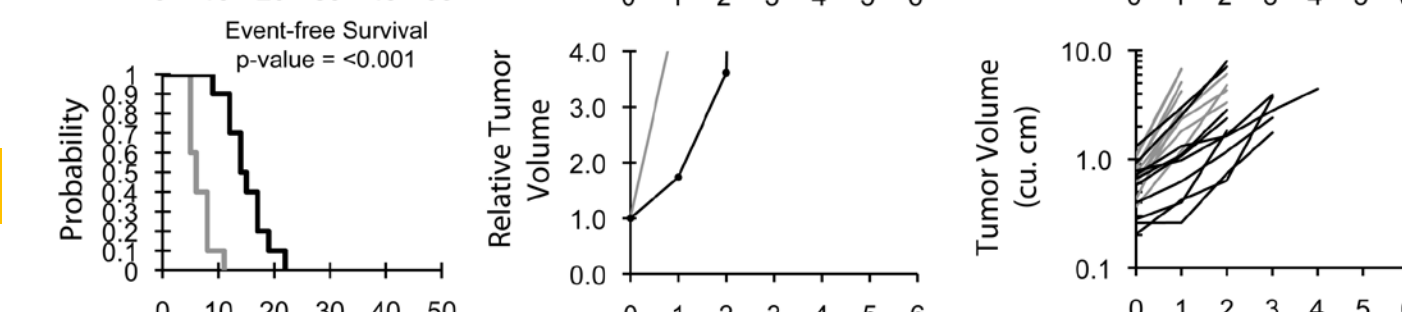
### BT-39 (glioblastoma)



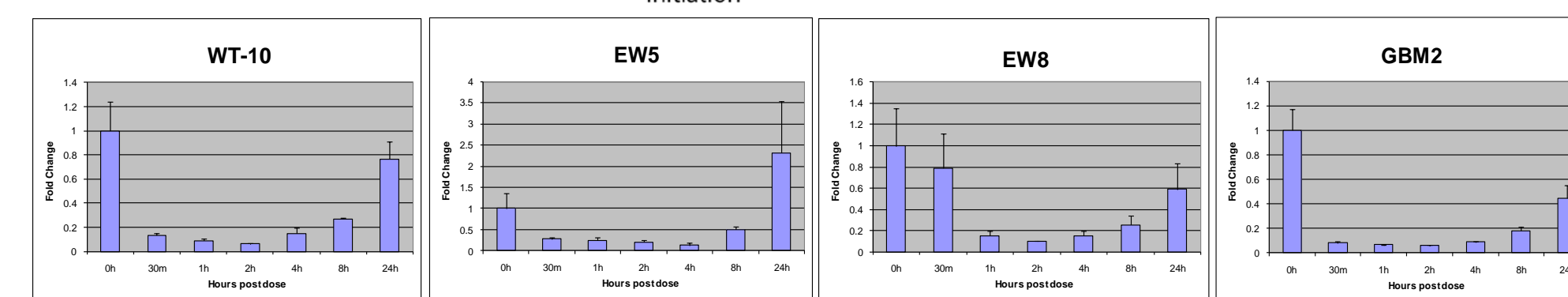
### D645 (glioblastoma)



### D456 (glioblastoma)



Controls (gray lines)  
Treated (black lines)



MLN4924 reduces the overall levels of neddylation in solid tumor xenografts: A single dose of MLN4924 (100 mg/kg S.C.) was administered to mice and tumors harvested at the times shown. Neddylation protein levels were determined by immunoblotting & normalized against β-tubulin as previously described (Soucy, et al. *Nature* 458:732-37, 2010).

## IN VIVO RESULTS AND CONCLUSIONS

- MLN4924 was well tolerated (4.6% mortality) at the dose (100 mg/kg SQ) and schedule (twice-daily x5 days x 3 weeks) evaluated, and 42 of 45 xenograft models were considered evaluable for efficacy.
- MLN4924 induced significant differences in EFS distribution compared to control in 20 of 34 (59%) evaluable solid tumor xenografts and 5 of 8 (63%) evaluable ALL xenografts.
- MLN4924 induced tumor growth inhibition meeting criteria for intermediate EFS T/C activity (EFS T/C > 2) in 9 of 33 (27%) evaluable solid tumor xenografts, including 4 of 4 GBM xenografts and 2 of 3 Wilms tumors.
- MLN4924 induced intermediate EFS T/C activity in 5 of 8 ALL xenografts.
- No objective responses were observed, although stable disease (SD) was observed in one ALL model.
- MLN4924 effectively reduced neddylation protein levels in each of the solid tumor models studied for this marker of treatment effect.

The poster will be available at [pptp.nchresearch.org](http://pptp.nchresearch.org)