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Pediatric Preclinical Testing Program (PPTP) evaluation of the Aurora A Kinase Inhibitor MLN8237

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Abstract

Background: MLN8237 is a small molecule inhibitor of Aurora A kinase that is currently in phase 1 testing in adults with cancer. Aurora A kinase plays a pivotal role in centrosome maturation and spindle formation during mitosis, and inhibitors of this kinase have shown activity in preclinical models of adult cancers.
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 ALL. MLN8237 was tested against the PPTP *in vitro* panel at concentrations ranging from 1 nM to 10 μM and was tested against the PPTP *in vivo* panel at a dose of 20 mg/kg administered orally twice daily x 5 days repeated weekly. Treatment duration was 6 weeks for solid tumor xenografts and 3 weeks for ALL xenografts, with a total treatment/observation period of 6 weeks for all xenografts. 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: MLN8237 had an IC_{50} of 61 nM against the PPTP *in vitro* panel. The ALL cell lines were more sensitive and the rhabdomyosarcoma cell lines less sensitive than the remaining PPTP cell lines. The neuroblastoma cell lines showed intermediate sensitivity to MLN8237. MLN8237 was satisfactorily tolerated with toxicity in 6.4% of treated animals compared to 0.7% of control animals. 37 of 38 tested solid tumor xenografts and 6 of 8 tested ALL xenografts were evaluable for efficacy. MLN8237 significantly increased EFS in 30 of 37 (81%) evaluable solid tumor xenografts and 6 of 6 ALL xenografts. For the 30 solid tumor xenografts evaluable for the EFS T/C activity measure, 19 met criteria for either intermediate (n=11) or high activity (n=8). Maintained complete responses (CRs) were observed in 3 of 4 neuroblastoma xenografts, and all 6 evaluable ALL xenografts achieved CR (n=4) or maintained CR (n=2) status. Maintained CRs were observed among single xenografts in other panels, including the Wilms tumor, rhabdoid tumor, rhabdomyosarcoma, Ewing sarcoma, osteosarcoma, and medulloblastoma panels.
Conclusions: The *in vitro* activity of MLN8237 was most pronounced against the PPTP's ALL cell line panel. The very high level of *in vivo* activity observed against the neuroblastoma panel far exceeds that observed for standard agents evaluated by the PPTP that are used in the treatment of neuroblastoma (e.g., vincristine, cisplatin, and cyclophosphamide). High levels of *in vivo* activity were also observed against the ALL panel. Further PPTP testing of MLN8237 is evaluating the dose-response relationship of MLN8237 in responsive xenografts and the pharmacodynamic correlates of response.
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In Vitro Test Results for MLN8237

__Methods__: *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 (Keshelava, et al. Methods Mol.Med., *110*: 139-153, 2005). Testing was for 96 hours at concentrations from 1.0 nM to 10 μM with replicates of 6 per data point. Data were analyzed using Kaleidagraph (Synergy), fitting a non-linear regression model-sigmoidal dose-response model to the response-relative fluorescence values *vs.* the concentration.

Cell Line	Status	Histology	Minimum T/C	EC_{50} (nM)	IC_{50} (nM)
RD		Rhabdomyosarcoma	20.5	150	228
Rh41	Post-Therapy	Rhabdomyosarcoma	22.7	57	90
Rh18	Diagnosis	Rhabdomyosarcoma	60.5	>10,000	>10,000
Rh30	Diagnosis	Rhabdomyosarcoma	22.6	89	230
BT-12	Diagnosis	Rhabdoid	29.3	1	58
CHLA-266	Diagnosis	Rhabdoid	28.4	27	72
TC-71	Post-Therapy	Ewing	10.0	100	102
CHLA-9	Diagnosis	Ewing	4.2	16	18
CHLA-10	Post-Therapy	Ewing	4.7	56	60
CHLA-258	Post-Bone Marrow Transplant	Ewing	18.8	82	132
SJ-GBM2	Post-Therapy	Glioblastoma	8.0	45	50
NB-1643	Diagnosis	Neuroblastoma	10.2	32	37
NB-EBc1	Post-Therapy	Neuroblastoma	3.6	49	50
CHLA-90	Post-Bone Marrow Transplant	Neuroblastoma	16.3	48	61
CHLA-136	Post-Bone Marrow Transplant	Neuroblastoma	10.4	36	39
COG-LL-317	Post-Therapy	ALL T-cell	0.5	58	62
NALM-6	Post-Therapy	ALL B-precursor	0.0	47	47
RS4;11[1]	Post-Therapy	ALL B-precursor	0.3	18	18
MOLT-4	Post-Therapy	ALL T-cell	0.2	26	26
CCRF-CEM		ALL T-cell	4.1	85	94
Kasumi-1	Post-Bone Marrow Transplant	AML	3.4	98	103
Karpas-299	Post-Therapy	ALCL	2.5	40	38
Ramos-RA1		NHL	2.5	120	127
Median			8.0	49	61
Minimum			60.5	1	18
Maximum			0.0	>10,000	>10,000

- ❑ The MLN8237 median IC_{50} was 61 nM (range from 18 nM to > 10 mM).
- ❑ MLN8237 was most active against the ALL cell lines and least active against cell lines from the rhabdomyosarcoma panel (all of which had IC_{50} values > panel median). Neuroblastoma cell lines showed intermediate sensitivity.
- ❑ MLN8237's cytotoxic effect (as assessed by T/C values approaching 0) was most notable for the ALL panel, with 4 of 5 cell lines showing T/C values < 1%.

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Methods for PPTP *In Vivo* Testing

Stage 1 testing involves testing an agent across the entire PPTP panel of childhood cancer xenograft lines at its MTD (or at a dose selected based on PK/PD studies using adult preclinical models).

➢ __Solid tumor testing__: 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 (π/6)×d3, where d represents the mean diameter.

➢ __Acute lymphoblastic leukemia testing__: For each xenograft line, 8 mice were inoculated with 3-5 x 10^6 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.

➢ Drug: MLN8237 was provided to the Pediatric Preclinical Testing Program by Millennium Pharmaceuticals through the Cancer Therapy Evaluation Program (NCI). MLN8237 was dissolved in a mixture of 10% 2-hydroxypropyl-ß-cyclodextrin and 1% sodium bicarbonate in water, and administered twice daily by oral gavage on a daily X 5 schedule for 6 weeks in the solid tumor xenografts and 3 weeks in the ALL xenografts, at a dose of 20 mg/kg. MLN8237 was provided to each testing site in coded vials for blinded testing according the PPTP's standard operating procedures.

➢ __Solid Tumor Response Criteria__:

Response		Definition	Score
PD1	Progressive Disease 1	>25% increase in tumor volume, TGD value of ≤1.5	0
PD2	Progressive Disease 2	>25% increase in tumor volume, TGD value of >1.5	2
SD	Stable Disease	≤25% increase, <50% regression	4
PR	Partial Response	≥50% regression	6
CR	Complete Response	<0.1 cm^3 tumor volume	8
MCR	Maintained CR	<0.1 cm^3 tumor volume at the end of study	10

➢ __Leukemia Response Criteria__:

Response		Definition	Score
PD1	Progressive Disease 1	CD45% never drops below 1%, events before end of study, TGD value of ≤1.5	0
PD2	Progressive Disease 2	CD45% never drops below 1%, events before end of study, TGD value of >1.5	2
SD	Stable Disease	CD45% never drops below 1%, no events before end of study	4
PR	Partial Response	CD45% <1% for only 1 week	6
CR	Complete Response	CD45% <1% for 2 consecutive weeks	8
MCR	Maintained CR	CD45% <1% for 2 consecutive weeks and at end of study CD45% <1%	10

➢ __Median Group Response__: Each individual mouse in the treatment group was assigned a response score (see Tables above) and an median score for the treatment group was calculated and then each treatment group was assigned an overall response according to the table below.

If Average Score (AS) from (1):	Overall Group Response
0 ≤ AS ≤1	PD1
1 < AS ≤3	PD2
3 < AS ≤5	SD
5 < AS ≤7	PR
7 < AS ≤9	CR
9 < AS	MCR

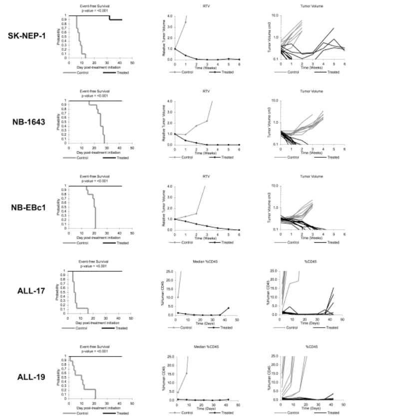
➢ __Statistical Methods__: Event-free survival (EFS) distributions of each treatment group were compared to the EFS distribution of the respective control group using the exact log rank test. P-values were 2-sided & were not adjusted for multiple comparisons given the exploratory nature of this study. P-values < 0.05 were considered to be significant.

MLN8237 in Vivo Activity

Xenograft Line	Histology	P-value	EFS T/C	Median Final RTV	Tumor Volume T/C	P-Value	Heat Map
BT-29	Rhabdoid	<0.001	> 3.6	0.8	0.47	0.002	SD
KT-14	Rhabdoid	<0.001	> 2.6	1.3	0.39	<0.001	PD2
KT-12	Rhabdoid	<0.001	> 3.6	0.0	0.15	<0.001	MCR
KT-10	Wilms	<0.001	> 4.7	0.0	0.18	<0.001	MCR
KT-11	Wilms	<0.001	3.5	>4	0.32	<0.001	PD2
KT-13	Wilms	<0.001	> 5.7	0.9	0.41	<0.001	SD
SK-NEP-1	Ewing	<0.001	> 5.3	0.0	0.14	<0.001	MCR
EW5	Ewing	<0.001	1.3	>4	0.77	<0.001	PD1
EW8	Ewing	<0.001	1.2	>4	0.78	0.001	PD1
TC-71	Ewing	0.013	1.7	>4	0.63	0.105	PD2
CHLA258	Ewing	0.351	1.3	>4	0.93	0.393	PD1
Rh28	ALV RMS	<0.001	> 2.2	2.3	0.32	<0.001	PD2
Rh30	ALV RMS	0.553	0.6	>4	1.28	0.280	PD1
Rh30R	ALV RMS	0.006	1.6	>4	0.54	<0.001	PD1
Rh41	ALV RMS	<0.001	1.4	>4	0.73	<0.001	PD1
Rh65	ALV RMS	<0.001	> 2.4	0.0	0.06	<0.001	MCR
Rh18	EMB RMS	<0.001	3.1	>4	0.24	<0.001	PD2
BT-28	Medulloblastoma	0.175	1.1	>4	0.88	0.912	PD1
BT-45	Medulloblastoma	<0.001	2.5	>4	0.57	0.013	PD2
BT-46	Medulloblastoma	0.087	.	0.0	0.10	<0.001	MCR
BT-50	Medulloblastoma	0.474	.	1.0	0.94	0.912	SD
BT-41	Ependymoma	0.531	.	1.9	0.93	0.243	PD2
BT-44	Ependymoma	0.002	1.8	>4	0.79	0.481	PD2
GBM2	Glioblastoma	<0.001	1.6	>4	0.69	0.001	PD2
BT-39	Glioblastoma	<0.001	> 3.3	1.9	0.39	<0.001	PD2
D645	Glioblastoma	<0.001	4.7	>4	0.32	<0.001	PD2
D456	Glioblastoma	<0.001	> 6.2	0.7	0.18	<0.001	PR
NB-1771	Neuroblastoma	<0.001	> 1.5	0.0	0.13	<0.001	MCR
NB-1691	Neuroblastoma	<0.001	3.4	>4	0.60	0.019	PD2
NB-EBc1	Neuroblastoma	<0.001	> 2.1	0.0	0.06	<0.001	MCR
NB-1643	Neuroblastoma	<0.001	> 1.7	0.0	0.02	<0.001	MCR
OS-1	Osteosarcoma	<0.001	> 1.6	0.1	0.23	<0.001	MCR
OS-2	Osteosarcoma	<0.001	1.9	>4	0.38	<0.001	PD2
OS-17	Osteosarcoma	<0.001	2.2	>4	0.40	<0.001	PD2
OS-9	Osteosarcoma	0.206	.	0.9	0.58	<0.001	SD
OS-33	Osteosarcoma	<0.001	3.2	>4	0.24	<0.001	PD2
OS-31	Osteosarcoma	<0.001	2.0	>4	0.51	<0.001	PD2
ALL-2	ALL B-precursor	<0.001	> 2.7	2.3	.		CR
ALL-3	ALL B-precursor	<0.001	>11.8	11.2	.		CR
ALL-4	ALL B-precursor	0.005	36.2	>25	.		CR
ALL-16	ALL T-cell	0.002	> 4.1	0.4	.		MCR
ALL-17	ALL B-precursor	<0.001	> 8.0	4.2	.		CR
ALL-19	ALL B-precursor	<0.001	> 4.2	0.5	.		MCR

Red shading in the p-value column 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), or indeterminate (gray) activity.

MLN8237 *In Vivo* Activity



CONCLUSIONS

- MLN8237, an orally active Aurora A kinase small molecule inhibitor, showed selective activity against the PPTP's *in vitro* and *in vivo* panels.
- The ALL cell lines were the most sensitive histology to MLN8237 among the histotypes evaluated in the *in vitro* panel.
- MLN8237 induced objective responses in one or more xenografts in the rhabdoid, Wilms, Ewing, rhabdomyosarcoma, medulloblastoma, glioblastoma, neuroblastoma, osteosarcoma and ALL *in vivo* tumor panels.
- MLN8237 showed high *in vivo* activity (3 of 4 maintained CRs) in the neuroblastoma panel, exceeding the activity of standard agents previously tested against the panel (vincristine, cisplatin, and cyclophosphamide) .
- MLN8237 also demonstrated high *in vivo* activity (4 CR's, 2 maintained CR's) against the ALL panel, despite dosing for only 3 weeks.
- Further preclinical work studying the *in vivo* dose response of MLN8237 is ongoing in conjunction with studies to relate the agent's pharmacokinetic and pharmacodynamic behavior to its *in vivo* antitumor activity.

MLN8237 was provided to the PPTP by Millennium Pharmaceuticals Supported by NCI NO1CM42216