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Abstract

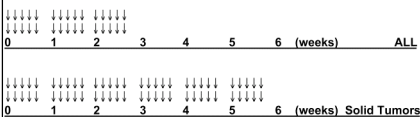
Background: MLN8237 is a small molecule inhibitor of Aurora A kinase that is in adult phase 1 testing. Aurora A kinase plays a pivotal role in centrosome maturation and spindle formation during mitosis. MLN8237 demonstrated activity in preclinical models of adult cancers, and previous PPTP testing identified broad *in vivo* activity for MLN8237 when tested at its MTD against neuroblastoma and acute lymphoblastic leukemia (ALL) xenografts.

Methods: MLN8237 was tested against selected responsive lines from the PPTP in vivo panels at doses of 20, 10, 5, and 2.5 mg/kg administered orally twice daily x 5 days repeated weekly and against 2 additional neuroblastoma models at 20 mg/kg. 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. Pharmacodynamic (PD) studies were performed on selected neuroblastoma lines to evaluate the effect of MLN8237 on mitotic index (determined by %MPM2), with %pHistH3 positive cells determined to support Aurora A rather than Aurora B kinase inhibition.

Results: Dose response testing showed 2/4 neuroblastoma and 3/3 ALL models achieving an objective response at 10 mg/kg (50% of MTD), with the most sensitive neuroblastoma model (NB-1643) achieving an objective response at 5 mg/kg and with 2/3 ALL models showing good leukemia growth control during treatment at 5 mg/kg. Two additional neuroblastomas were not responsive to MLN8237 at a dose of 20mg/kg. MLN8237 induced an increase in mitotic index and %pHistH3 positive cells following a single dose of agent that peaked at 12 hrs, returning to baseline levels within 24 hrs.

Conclusions: Dose response testing indicates MLN8237 efficacy at 50% of its MTD in a subset of responsive neuroblastoma and ALL models. PD studies are consistent with *in vivo* anti-neuroblastoma activity through inhibition of Aurora A kinase. Further preclinical studies of MLN8237 focusing on combinations with other agents are anticipated, and pediatric clinical development of MLN8237 is proceeding. (Supported by NCI NO1CM42216)

Treatment Schema: Twice Daily X 5 for 3 (ALL) or 6 weeks (Solid Tumors)



Methods for PPTP *In Vivo* Testing

MLN8237 stage 2 testing involves dose response testing in a subset of responsive models.

Solid tumor testing: For each xenograft line, 10 mice bearing SC tumors initiated treatment when the tumors were between 0.2–0.5 cm³. Two perpendicular tumor diameters were measured at once 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 10⁶ 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 (CTEP). MLN8237 was dissolved in a mixture of 10% 2-hydroxypropyl-β-cyclodextrin and 1% sodium bicarbonate in water, and administered twice daily by oral gavage for 5 consecutive days out of 7, repeated for 6 weeks in the solid tumor xenografts and 3 weeks in the ALL xenografts, at doses of 20, 10, 5 and 2.5 mg/kg.

Solid Tumor Response Criteria:

Response	Definition	Score
PD1 (Progressive Disease 1)	>25% ↑ in tumor volume, TGD value ≤1.5	0
PD2 (Progressive Disease 2)	>25% ↑ in tumor volume, TGD value >1.5	2
SD (Stable Disease)	<25% ↑ in tumor volume, <50% regression	4
PR (Partial Response)	≥50% regression, but no CR	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)	No PR & TGD value of ≤1.5 & events at EOS	0
PD2 (Progressive Disease 2)	No PR & TGD value >1.5 & events at EOS	2
SD (Stable Disease)	No PR and no events at EOS	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 last 3 weeks of study	10

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

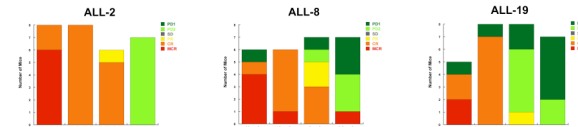
If Median Score (MS) from 1):	Overall Group Response
0 ≤ MS ≤1	PD1
1 < MS ≤3	PD2
3 < MS ≤5	SD
5 < MS ≤7	PR
7 < MS ≤9	CR
9 < MS	MCR

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.

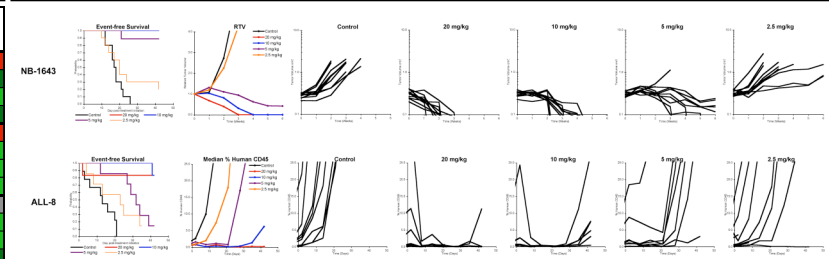
MLN8237 *In Vivo* Activity

Xenograft Line	Histology	Treatment	p-value	EFS T/C	Median Final RTV	T/C	p-value	Heat Map
KT-10	Wilms	20 mg/kg	0.0001	> 4.4	0.0	0.35	0.0004	MCR
		10 mg/kg	0.0090	1.5	>4	0.53	0.00520	PD1
		5 mg/kg	0.11025	1.7	>4	0.71	0.24745	PD2
		2.5 mg/kg	0.76783	1.0	>4	0.84	0.63053	PD1
Rh65	ALV RMS	20 mg/kg	0.0001	> 3.9	0.0	0.01	0.0002	MCR
		10 mg/kg	0.0005	> 3.9	3.2	0.11	0.0002	PD2
		5 mg/kg	0.0005	3.7	>4	0.19	0.0002	PD2
		2.5 mg/kg	0.0005	2.1	>4	0.31	0.0002	PD2
NB-SD	Neuroblastoma	20 mg/kg	0.0001	> 4.4	0.9	0.47	0.0002	SD
		10 mg/kg	0.0001	3.9	>4	0.50	0.00021	PD2
		5 mg/kg	0.0009	2.2	>4	0.60	0.00105	PD2
		2.5 mg/kg	0.0026	1.6	>4	0.59	0.00049	PD1
NB-1771	Neuroblastoma	20 mg/kg	0.0002	> 2.1	0.0	0.02	0.0005	MCR
		10 mg/kg	0.0001	> 2.1	0.0	0.08	0.0002	MCR
		5 mg/kg	0.0025	> 2.1	2.2	0.38	0.00151	PD2
		2.5 mg/kg	0.0086	1.9	>4	0.38	0.00105	PD2
NB-EBc1	Neuroblastoma	20 mg/kg	0.0001	> 7.2	0.3	0.15	0.0002	MCR
		10 mg/kg	0.0001	3.1	>4	0.22	0.0001	PD2
		5 mg/kg	0.0035	2.0	>4	0.44	0.00049	PD2
		2.5 mg/kg	0.0136	1.7	>4	0.57	0.01854	PD2
NB-1643	Neuroblastoma	20 mg/kg	0.0001	> 2.4	0.0	0.14	0.0002	MCR
		10 mg/kg	0.0001	> 2.4	0.0	0.30	0.0004	MCR
		5 mg/kg	0.0005	1.2	0.4	0.38	0.00041	PR
		2.5 mg/kg	0.20336	2.4	>4	1.01	0.73936	PD1
NB-1382	Neuroblastoma	20 mg/kg	0.0020	> 1.7	2.1	0.31	0.0002	PD2
		SK-N-AS	20 mg/kg	0.07717	1.4	>4	0.71	0.13879
OS-1	Osteosarcoma	20 mg/kg	0.00071	> 1.5	0.8	0.45	0.0004	SD
		10 mg/kg	0.00071	> 1.5	2.0	0.56	0.00105	PD2
		5 mg/kg	0.02202	> 1.5	3.4	0.70	0.02881	PD2
		2.5 mg/kg	0.02613	> 1.5	3.5	0.75	0.07526	PD2
ALL-2	ALL B-precursor	20 mg/kg	0.0016	> 3.8	0.3	-	-	MCR
		10 mg/kg	0.0016	> 3.8	3.1	-	-	CR
		5 mg/kg	0.0033	> 3.8	>25	-	-	CR
		2.5 mg/kg	0.0016	2.3	>25	-	-	PD2
ALL-8	ALL T-cell	20 mg/kg	0.00240	> 3.3	0.2	-	-	MCR
		10 mg/kg	0.00020	> 3.3	6.2	-	-	CR
		5 mg/kg	0.00131	2.5	>25	-	-	PR
		2.5 mg/kg	0.05235	1.8	>25	-	-	PD2
ALL-19	ALL B-precursor	20 mg/kg	0.02597	16.8	>25	-	-	CR
		10 mg/kg	0.01232	14.9	>25	-	-	CR
		5 mg/kg	0.04096	9.2	>25	-	-	PD2
		2.5 mg/kg	0.74242	1.0	>25	-	-	PD1

* Red shading in the p-value column indicates a significant difference in EFS distribution or Tumor Volume T/C values between treated and control groups.
 * Shading in the EFS column indicates xenografts that have either high (dark blue), intermediate (light blue), or indeterminate (gray) activity.

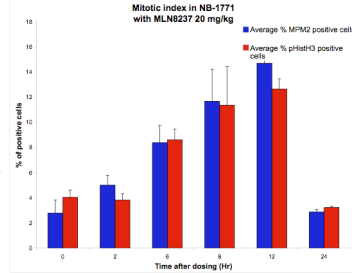


MLN8237 *In Vivo* Activity



MLN8237 Pharmacodynamic (PD) Studies

- MLN8237 PD effect was evaluated by determining the % mitotic cells (measured by MPM2 staining) and the % phospho-histone H3 positive cells following a single oral dose of MLN8237 (20 mg/kg). Ser10 of histone H3 is a specific Aurora B substrate.
- MLN8237 induced an ~ 5-fold increase in % mitotic cells that peaked at ~ 12 hrs and returned to baseline by 24 hrs. A similar magnitude of effect and time course was observed for % phospho-histone H3 positive cells.
- MLN8237 plasma levels at 12 hours post-dosing were ~1 μM and ~2 μM for the 10 mg/kg and 20 mg/kg dose, respectively (data not shown). 1 μM is the *in vivo* EC90 concentration for MLN8237 based on adult cancer preclinical modeling.
- The MLN8237-induced increase in % mitotic cells was matched by an equal increase in % phospho-histone H3 positive cells, consistent with an Aurora A specific effect with minimal Aurora B kinase inhibition.



Conclusions

- MLN8237 demonstrated promising anti-tumor activity at its MTD in Stage 1 testing by the PPTP, particularly for the neuroblastoma and ALL xenograft panels.
- Dose response testing confirmed the high level of activity for MLN8237 against ALL xenografts and selected neuroblastoma xenografts.
 - Against neuroblastoma xenografts, MLN8237 induced maintained complete responses at 50% of its MTD in 2 of 3 responsive models.
 - Against 3 ALL xenografts studied, MLN8237 at 50% of its MTD induced CRs that were maintained during 3 weeks of treatment, and 2 of 3 models showed objective responses at 25% of the MLN8237 MTD.
- MLN8237 induced pharmacodynamic effects consistent with specific inhibition of Aurora A kinase with little Aurora B kinase inhibition.
- MLN8237 has entered pediatric phase 1 evaluation with plans to quickly investigate its clinical activity against neuroblastoma and ALL.

MLN8237 was provided by Millennium Pharmaceuticals and testing was supported by NCI NO1CM42216