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Initial Testing (Stage 1) of the Investigational mTOR Kinase Inhibitor MLN0128 by the Pediatric Preclinical Testing Program

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Abstract

MLN0128 is an investigational small molecule ATP-competitive inhibitor of the serine/threonine kinase mTOR. MLN0128 was tested against the *in vitro* panel at concentrations ranging from 0.1 nM to 1 μ M and against the PPTP *in vivo* panels at a dose of 1 mg/kg administered orally daily x 28. *In vitro* the median relative IC₅₀ concentration was 19 nM. *In vivo* MLN0128 induced significant differences in EFS in 24/31 (77%) solid tumor models, but 0/7 ALL xenografts. The modest activity observed for MLN0128 against the PPTP preclinical models is similar to that previously reported for another TOR kinase inhibitor.

Keywords

Preclinical Testing; Developmental Therapeutics; mTOR inhibitor

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INTRODUCTION

Direct TOR kinase inhibitors should target mTOR in both the Raptor (TORC1) and Rictor (TORC2) complexes, preventing mTORC1 signaling as well as preventing activation of Akt, assuming that mTORC2 is the only kinase to phosphorylate Akt at Ser473 [1]. Small molecule ATP-competitive inhibitors of mTOR have been identified, and several are in clinical evaluation. MLN0128 is a potent and selective inhibitor of the mTOR kinase that is in clinical phase 1 trials [2]. mTOR kinase inhibitors have been shown to suppress phosphorylation of 4E-BP1 and global translation more effectively than rapalogs [3,4]. Importantly, cellular proliferation appears to be regulated by the 4E-BP-eIF4E branch of the mTORC1 signaling pathway [5,6]. Here we have evaluated MLN0128 against the *in vitro* and *in vivo* panels of childhood cancer models of the PPTP.

MATERIALS AND METHODS

In vitro testing: *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 [7]. Cells were incubated in the presence of MLN0128 (formerly known as INK128) for 96 hours at concentrations from 0.1 nM to 1 μ M and analyzed as previously described [8].

In vivo tumor growth inhibition studies

Mouse strains used to propagate solid tumors, glioblastomas and acute lymphoblastic leukemias, and methods for assessing tumor response have been described previously [9–11]. 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 (leukemias) or 10 (solid tumors) mice were used in each control or treatment group. 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 (EFS) 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

MLN0128 was provided to the PPTP by Intellikine, through the Cancer Therapy Evaluation Program (NCI). MLN0128 was dissolved in 5% 1-methyl-2-pyrrolidinone (NMP), and sonicated. The sample was brought to volume with 15% polyvinylpyrrolidone K30 (PVP), dissolved in water sterile water, and stored at room temperature for up to 7 days. MLN0128 was administered P.O. daily for 28 days at 1 mg/kg per day.

Pharmacodynamic studies—Rh10, Rh41 rhabdomyosarcomas and KT-16 rhabdoid tumor xenografts were harvested between 0 and 24 hours post treatment on day 1, or 2 to 24 hours following treatment on day 4 (1 mg/kg/day). Tumors (3 per time point) were processed for immunoblotting as previously described [12,13].

RESULTS

MLN0128 *in vitro* testing

MLN0128 was tested against the PPTP's *in vitro* cell line panel at concentrations ranging from 0.1 nM to 1 μ M. The median relative IC₅₀ value for the PPTP cell lines was 19 nM, with a range from 2 nM (CHLA-10) to 102 nM (CCRF-CEM2). MLN0128 demonstrated cytotoxic activity against some cell lines, as evidence by Y_{min} values approaching 0% and Relative In/Out values approaching –100% (e.g. Rh18, Rh30, Rh41). For other cell lines (e.g., the Ewing sarcoma cell lines TC-71, CHLA-9, and CHLA-10 and the rhabdoid tumor line BT-12), the Relative In/Out values were closer to 0% (the value anticipated for a cytostatic agent), Table I.

MLN0128 *in vivo* testing

MLN0128 was tested against the PPTP solid tumor xenografts using a dose of 1 mg/kg administered by the P.O. route daily for 28 days. The total planned treatment and observation period was 6 weeks. MLN0128 was generally well tolerated, with only a 1.4% (5/357) toxicity rate in the treated groups, and 0.3% (1/351) toxicity in control animals. All of the 38 tested xenograft models were considered evaluable for efficacy. Complete details of testing are provided in Supplemental Table I.

MLN0128 induced significant differences in EFS distribution compared to control in 24 of 31 (77%) of the evaluable solid tumor xenografts and in 0 of 7 (0%) of the evaluable ALL xenografts 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 additionally requires a reduction in final tumor volume compared to the starting tumor volume. MLN0128 induced tumor growth inhibition meeting criteria for intermediate EFS T/C activity in 6 of 30 (20%) evaluable solid tumor xenografts. Intermediate activity for the EFS T/C metric occurred in the rhabdoid tumor panel (2 of 3) and in single xenografts in four other panels. For the ALL panel, no models met criteria for intermediate activity. There were no objective tumor regressions.

Pharmacodynamic studies

Inhibition of mTORC1 was assessed by decreased phospho-4E-BP1 (Thr37/46) and phospho-S6(Ser235/6) protein, and inhibition of mTORC2 by decreased phospho-Akt(Ser473) in Rh10, Rh41 and KT-16 xenografts following the first and fourth dose of MLN0128. As shown in Supplemental Figure 1A, the phosphorylation of both 4E-BP1 and S6 was completely suppressed in non-responding Rh41 xenografts at 4 and 8 hr after first administration of MLN0128, recovering at 24 hr. Similarly, phospho-Akt was completely abrogated and was detected only at 24 hr post dosing. Phosphorylation of Akt, S6 and 4E-

BP1 had recovered to control levels by 24 hr post daily dose 3. Following treatment on day 4, there was less complete inhibition of phospho-Akt although both S6 and 4E-BP1 appeared to be dephosphorylated with similar kinetics to those observed following day 1 dosing. Similar results were obtained for Rh10 (PD2 response) and KT-16 xenografts (PD2 response). However for phospho-Akt, recovery was seen at earlier time points following day 4 dosing (Rh10, Supplemental Figure 1B). For KT-16 tumors pAkt was not suppressed at any time point and suppression of 4E-BP1 phosphorylation was somewhat less robust after day 4 dosing, than after the first dose of drug, Supplemental Figure 1C.

DISCUSSION

MLN0128 demonstrated potent, mainly cytostatic activity *in vitro*, although it exerted cytotoxicity against several rhabdomyosarcoma cell lines including Rh41 (Relative I/O –98%). *In vivo* MLN0128 was well tolerated on the dose and schedule used. Pharmacodynamics studies indicated complete inhibition of 4E-BP1, S6 and Akt phosphorylation that recovered at 24 hr post dosing even in non-responsive tumors, such as Rh41. Thus, even in non-responsive tumors MLN0128 achieved significant TOR inhibition. Of interest, phospho-Akt was not suppressed in KT-16 tumors despite inhibition of S6 and 4E-BP1 phosphorylation. Despite evidence for target inhibition, MLN0128 induced intermediate antitumor activity (EFS T/C>2) in only 6 of 30 solid tumor models and in none of the 7 ALL models. No regressions were observed for any tumors. Thus, the activity of MLN0128 was similar to that of another TOR kinase inhibitor, AZD8055 that induced disease stabilization in two brain tumor models, but did not induce regressions [13]. When these data are combined with PPTP results reported for PI3K inhibitors [14] and AKT inhibitors [15,16], the available data suggest that kinase inhibitors targeting the PI3K pathway produce limited single agent activity for the tumor types represented by the PPTP models.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Activity of MLN0128 against the PPTP *in vitro* panel.

Cell Line	Histotype	rIC ₅₀ (nM)	Panel rIC ₅₀ /Line rIC ₅₀	Y _{min} (Observed)	Relative In/Out (Observed Y _{min})
RD	Rhabdomyosarcoma	24	0.82	4.7	-15%
Rb41	Rhabdomyosarcoma	8	2.34	0.4	-98%
Rh18	Rhabdomyosarcoma	2	10.55	0.5	-99%
Rh30	Rhabdomyosarcoma	7	2.74	2.7	-84%
BT-12	Rhabdoid	12	1.55	12.1	4%
CHLA-266	Rhabdoid	7	2.89	19.1	-27%
TC-71	Ewing sarcoma	33	0.59	3.3	2%
CHLA-9	Ewing sarcoma	5	4.14	8.2	5%
CHLA-10	Ewing sarcoma	2	10.29	7.9	2%
CHLA-258	Ewing sarcoma	5	3.97	4.3	-89%
SI-GBM2	Glioblastoma	20	0.95	5.6	-43%
NB-1643	Neuroblastoma	29	0.67	15.2	-28%
NB-EBc1	Neuroblastoma	35	0.55	11.2	-51%
CHLA-90	Neuroblastoma	18	1.06	2.1	-93%
CHLA-136	Neuroblastoma	88	0.22	39.2	15%
NALM-6	ALL	85	0.23	8.9	6%
COG-LL-317	ALL	13	1.51	6.7	2%
RS4;11	ALL	35	0.55	10.2	-32%
MOLT-4	ALL	95	0.20	7.8	-22%
CCRF-CEM (1)	ALL	52	0.37	8.4	2%
CCRF-CEM (2)	ALL	102	0.19	10.5	5%
Kasumi-1	AML	13	1.52	8.6	-70%
Karpas-299	ALCL	46	0.42	6.7	-13%
Ramos-RA1	NHL	10	1.92	0.1	-90%
Median		19	1.00	7.9	-24%
Minimum		2	0.19	0.1	-99%

Cell Line	Histotype	rIC ₅₀ (nM)	Panel rIC ₅₀ /Line rIC ₅₀	Y _{min} (Observed)	Relative In/Out (Observed Y _{min})
Maximum		102	10.55	39.2	15%

rIC₅₀: relative inhibitory concentration 50; Y_{min}: minimal survival fraction at the highest concentration tested; Relative In/Out: (Observed Y_{min} – Predicted Y_{min})/(100 – Predicted Y_{min}) if Observed Y_{min}>Predicted Y_{min} and (Predicted Y_{min} – Observed Y_{min})/(Predicted Y_{min}) if Observed Y_{min}<Predicted Y_{min}

Table II

Activity of MLN0128 against the PPTP *in vivo* panel.

Xenograft Line	Histology	Median Time to Event	P-value	EFS T/C	Median Final RTV	T/C	T/C Activity	EFS Activity	Overall Group Response
BT-29	Rhabdoid	.	0.001	> 1.6	3.1	0.57	Low	NE	PD2
KT-16	Rhabdoid	24.5	<0.001	4.1	>4	0.24	Int	Int	PD2
KT-14	Rhabdoid	40.5	<0.001	2.1	>4	0.19	Int	Int	PD2
KT-10	Wilms	16.6	<0.001	1.7	>4	0.46	Low	Low	PD2
KT-13	Wilms	15.2	0.007	1.4	>4	0.61	Low	Low	PD1
SK-NEP-1	Ewing	16.0	0.011	1.3	>4	0.52	Low	Low	PD1
EW5	Ewing	17.7	<0.001	2.7	>4	0.28	Int	Int	PD2
EW8	Ewing	15.8	<0.001	1.8	>4	0.55	Low	Low	PD2
TC-71	Ewing	12.8	0.007	2.0	>4	0.49	Low	Low	PD2
CHLA258	Ewing	24.3	<0.001	1.5	>4	0.56	Low	Low	PD1
Rh10	Alveolar RMS	23.6	<0.001	1.9	>4	0.37	Int	Low	PD2
Rh30	Alveolar RMS	20.1	<0.001	1.8	>4	0.40	Int	Low	PD2
Rh30R	Alveolar RMS	22.5	<0.001	2.1	>4	0.33	Int	Int	PD2
Rh41	Alveolar RMS	13.5	0.002	1.2	>4	0.69	Low	Low	PD1
BT-28	Medulloblastoma	19.4	0.005	1.5	>4	0.53	Low	Low	PD2
GBM2	Glioblastoma	18.5	0.752	1.2	>4	0.76	Low	Low	PD1
BT-39	Glioblastoma	11.0	0.285	1.3	>4	0.86	Low	Low	PD1
D645	Glioblastoma	10.8	0.003	1.4	>4	0.53	Low	Low	PD1
D456	Glioblastoma	16.5	0.006	2.5	>4	0.25	Int	Int	PD2
NB-SD	Neuroblastoma	21.3	0.001	2.1	>4	0.71	Low	Int	PD2
NB-1771	Neuroblastoma	8.5	0.059	1.3	>4	0.76	Low	Low	PD1
NB-1691	Neuroblastoma	7.7	0.149	1.3	>4	0.72	Low	Low	PD1
NB-EBc1	Neuroblastoma	8.9	0.070	1.3	>4	0.70	Low	Low	PD1
CHLA-79	Neuroblastoma	13.1	<0.001	1.7	>4	0.49	Low	Low	PD2
NB-1643	Neuroblastoma	16.8	0.025	1.5	>4	0.72	Low	Low	PD2
OS-1	Osteosarcoma	36.1	<0.001	1.3	>4	0.79	Low	Low	PD1
OS-2	Osteosarcoma	19.3	0.323	1.1	>4	0.88	Low	Low	PD1

Xenograft Line	Histology	Median Time to Event	P-value	EFS T/C	Median Final RTV	T/C	T/C Activity	EFS Activity	Overall Group Response
OS-17	Osteosarcoma	20.1	0.096	1.1	>4	0.86	Low	Low	PDI
OS-9	Osteosarcoma	21.5	0.002	1.2	>4	0.83	Low	Low	PDI
OS-33	Osteosarcoma	25.1	<0.001	1.6	>4	0.59	Low	Low	PD2
OS-31	Osteosarcoma	21.1	<0.001	1.3	>4	0.69	Low	Low	PDI
ALL-4	ALL B-precursor	6.5	0.984	1.0	>25	.		Low	PDI
ALL-7	ALL B-precursor	7.4	0.997	1.0	>25	.		Low	PDI
ALL-8	ALL T-cell	5.8	0.961	1.1	>25	.		Low	PDI
ALL-17	ALL B-precursor	6.9	0.706	0.9	>25	.		Low	PDI
ALL-19	ALL B-precursor	5.3	0.928	1.1	>25	.		Low	PDI
ALL-31	ALL T-cell	13.1	0.183	1.2	>25	.		Low	PDI
MLL-7	ALL, MLL rearranged	9.0	0.566	1.0	>25	.		Low	PDI

RMS = rhabdomyosarcoma. **Event**: 4-fold increase in tumor volume. **EFS T/C**: Ratio of median time to event for treated versus control animals. **RTV**: Relative tumor volume (ratio to day 1 tumor volume). **T/C**: Ratio of average tumor volume of treated versus control tumors on last measurement date before any animal in treated or control groups reached “event” status. **PDI** (Progressive Disease 1): >25% ↑ in tumor volume, EFS T/C value 1.5. **PD2** (Progressive Disease 2): >25% ent dateor volume, EFS T/C value > 1.5