Abstract 4064

Pediatric Preclinical Testing Program (PPTP) Evaluation of the mTOR Inhibitor Rapamycin

Christopher Morton[1], Peter J. Houghton[1], John M. Maris[2], Henry S. Friedman[3], Stephen T. Keir[3], Richard B. Lock[4], Hernan Carol[4], Mayamin Tajbakhsh[4], Richard Gorlick[5], E. Anders Kolb[5], Nino Keshelava[6], C. Patrick Reynolds[6], Malcolm A. Smith[7].
[1]St. Jude Children's Research Hospital, [2]Children's Hospital of Philadelphia, [3]Duke University, [4]Children's Cancer Inst., Australia, [5]Albert Einstein College of Medicine, [6]Children's Hospital of Los Angeles, [7]CTEP/NCI.

Abstract

Background: Rapamycin is a specific inhibitor of the serine/threonine kinase, mTOR, that controls cap-dependent translation. Inhibition of mTOR may have direct effects on tumor cells or indirect effects through anti-angiogenic mechanisms.
Methods: The PPTP includes an *in vitro* panel (n=27) as well as panels of xenografts (n=61) representing most of the common types of childhood solid tumors and childhood ALL. Rapamycin was tested against the *in vitro* panel at concentrations from 10 pM to 100 nM and against the *in vivo* tumor panels at a dose of 5 mg/kg IP daily X 5 (2 days off) for 42 days. Three measures of antitumor activity were used: 1) response criteria modeled after the clinical setting [e.g., partial response (PR), complete response (CR), etc.]; 2) treated to control (T/C) tumor volume at day 21; and 3) a time to event (4X increase in tumor volume) measure based on the median EFS of treated and control lines (intermediate activity required EFS T/C > 2, and high activity additionally required a net reduction in median tumor volume at the end of the experiment).
Results: Rapamycin was active against 10 of the 23 cell lines of the PPTP *in vitro* panel. The median IC_{50} for the responsive lines was 1.26nM with RAMOS being the most sensitive with an IC_{50} of 0.39nM. Rapamycin induced significant differences in EFS distribution in 28 of 36 (78%) of solid tumor xenografts and in 5 of 8 (63%) of the ALL xenografts. Using the PPTP time to event measure of efficacy, rapamycin had a high (4) or intermediate (11) level of activity against 15 of 31 evaluable solid tumor xenografts. Among the 8 ALL xenografts, 1 demonstrated high activity and 4 showed intermediate activity. Objective responses were observed in a rhabdoid xenograft (PR), 2 rhabdomyosarcoma xenografts (both PRs), and an osteosarcoma xenograft (maintained CR). A neuroblastoma xenograft achieved stable disease. Among the 8 xenografts in the ALL panel, rapamycin induced a PR in one T-cell ALL and a maintained CR in another T-cell ALL xenograft, with stable disease being observed in 1 B-precursor ALL xenograft.
Conclusions: Rapamycin demonstrated *in vitro* activity against a range of cell lines, with the most sensitive being a Burkitt lymophoma line. Rapamycin demonstrated broad activity against both the solid tumor and ALL panels with tumor growth delay being observed in all the panels. Regressions were observed in the ALL panel and three of the solid tumor panels. Further work is needed to evaluate the activity of rapamycin in combination with standard chemotherapy agents and to evaluate associations between the molecular characteristics of the PPTP's preclinical models and their responsiveness to rapamycin.

PPTP *In Vitro* Testing Methods

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 0.01 nM to 0.1 μM with replicates of 6 per data point. Data were analyzed using Kaleidagraph (Synergy), fitting a non-linear regression, sigmoidal dose-response model to the response, relative fluorescence values *vs.* the concentration. The PPTP in vitro panel contains cell lines for neuroblastoma (4), Ewing sarcoma (4), rhabdomyosarcoma (4), ALL (5), NHL (2), and others.

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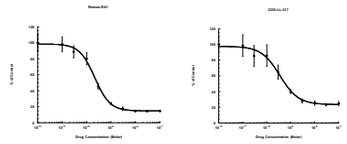
Rapamycin *In Vitro* Activity

- Ten of 23 cell lines achieved at least 50% growth inhibition, including 5 of 7 lymphoid cell lines (i.e., NHL and ALL lines).
- Maximal inhibition values ranging from 19% to 85% (median 49%). NHL cell line Ramos-RA1 and the ALL line COG-LL-317 demonstrated the greatest rapamycin effect on proliferation (85% and 77% inhibition, see below).



- The median EC_{50} for the *in vitro* panel was 0.7 nM (range 0.2 – 3.9 nM).
- The median IC_{50} was >100 nM, as the highest concentration of rapamycin tested failed to reduce proliferation by 50% in a majority of the lines.
- Rapamycin response data for the entire *in vitro* panel are shown in the table below:

Cell Line	Histology	Maximal Inhibition (%)	EC_{50} (nM)	IC_{50} (nM)
RD	Rhabdomyosarcoma	37	3.9	>100
Rh41	Rhabdomyosarcoma	71	0.6	1.3
Rh18	Rhabdomyosarcoma	19	0.9	>100
Rh30	Rhabdomyosarcoma	57	0.7	3.9
BT-12	Rhabdoid	40	1.4	>100
CHLA-266	Rhabdoid	40	0.7	>100
TC-71[1]	Ewing	20	0	>100
CHLA-9	Ewing	50	0.8	100
CHLA-10	Ewing	74	0.6	1
CHLA-258	Ewing	49	0.8	>100
SJ-GBM2	Glioblastoma	49	1.4	>100
NB-1643	Neuroblastoma	39	0.5	>100
NB-EBc1	Neuroblastoma	34	1.2	>100
CHLA-90	Neuroblastoma	57	0.4	2.2
CHLA-136	Neuroblastoma	24	1	>100
COG-LL-317	ALL T-cell	77	0.4	0.6
NALM-6	ALL B-precursor	28	1.7	>100
RS4;11[1]	ALL B-precursor	23	3.5	>100
MOLT-4	ALL T-cell	72	0.7	1.2
CCRF-CEM	ALL T-cell	71	0.5	0.9
Kasumi-1	AML	49	1.2	>100
Karpas-299	ALCL	76	0.7	1.4
Ramos-RA1	NHL	85	0.2	0.3
Median		**49**	**0.7**	**>100**
Minimum		**19**	**0**	**0.3**
Maximum		**85**	**3.9**	**>100**

[1] R^2 values for these lines < 0.90

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 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 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: Rapamycin was purchased from LC Laboratories (Woburn, MA). Rapamycin was dissolved in DMSO (5% final concentration) and diluted in 5% Tween 80 in water, and administered intraperitoneally daily for 5 for 6 consecutive weeks at a dose of 5 mg/kg. Rapamycin 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% ↑ 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 cm3 tumor volume	8
MCR (Maintained CR)	<0.1 cm3 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.

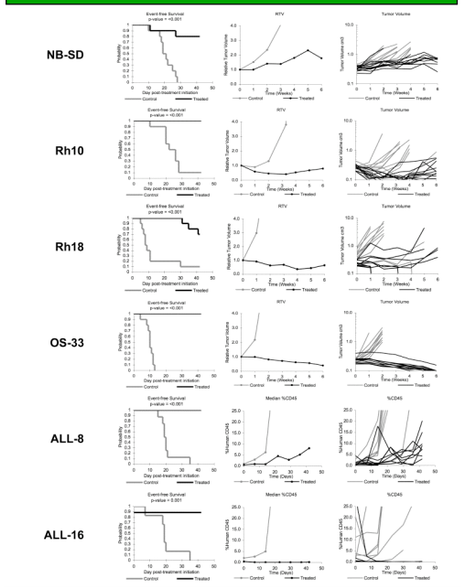
Rapamycin *In Vivo* Activity

- Rapamycin induced significant differences in EFS distributions compared to controls in 27/36 (75%) solid tumor models and 5/8 (63%) ALL models .
- Nineteen xenografts met criteria for intermediate (15) or high (4) activity for the EFS T/C time to event activity measure. Activity was observed within most of the PPTP's *in vivo* tumor panels.
- Objective responses were seen in 4 of 36 solid tumor models with a maintained CR in the osteosarcoma line OS-33. One rhabdoid line (KT-16) and 2 rhabdomyosarcoma lines (Rh10, and Rh18) achieved partial responses.
- Objective responses were seen in two T-cell ALL models, with one partial response (ALL-8) and one maintained complete response (ALL-16). A B-precursor ALL line demonstrated stable disease.

Xenograft Line	Histology	P-value	EFS T/C	Median Final RTV	Tumor Volume T/C	Heat Map
BT-29	Rhabdoid	<0.001	> 3.1	1.5	0.24	PD2
KT-16	Rhabdoid	<0.001	> 3.5	0.5	0.21	PR
KT-14	Rhabdoid	<0.001	> 1.6	1.5	0.69	PD2
KT-10	Wilms	0.979	1	>4	0.96	PD1
KT-11	Wilms	0.052	1.6	>4	0.81	PD2
KT-13	Wilms	0.307	1.2	>4	0.79	PD1
SK-NEP-1	Ewings	0.325	1.7	>4	0.76	PD2
EW5	Ewings	<0.001	4.9	>4	0.26	PD2
EW8	Ewings	0.082	1.5	>4	0.68	PD2
TC-71	Ewings	0.113	1.2	>4	0.79	PD1
CHLA258	Ewings	<0.001	2.4	>4	0.39	PD2
Rh10	ALV RMS	<0.001	> 1.8	0.8	0.19	PR
Rh28	ALV RMS	0.282	1.2	>4	0.66	PD1
Rh30	ALV RMS	<0.001	2.4	>4	0.43	PD2
Rh30R	ALV RMS	<0.001	2.1	>4	0.7	PD2
Rh41	ALV RMS	<0.001	1.7	>4	0.56	PD2
Rh18	EMB RMS	<0.001	> 4.9	0.6	0.41	PR
BT-28	Medulloblastoma	<0.001	1.9	>4	0.38	PD2
BT-46	Medulloblastoma	0.01	1.4	>4	0.94	PD1
BT-44	Ependymoma	0.005	1.2	>4	0.73	PD1
GBM2	Glioblastoma	<0.001	2.6	>4	0.42	PD2
BT-39	Glioblastoma	0.009	1.4	>4	0.6	PD1
D645	Glioblastoma	<0.001	> 3.7	>4	0.32	PD2
D456	Glioblastoma	<0.001	3.7	>4	0.54	PD2
NB-SD	Neuroblastoma	<0.001	> 2.1	1.8	0.71	PD2
NB-1771	Neuroblastoma	0.029	1.2	>4	0.68	PD1
NB-1691	Neuroblastoma	0.077	1.2	>4	0.91	PD1
NB-EBc1	Neuroblastoma	0.019	2	>4	0.71	PD2
CHLA-79	Neuroblastoma	0.099	1.4	>4	0.72	PD1
NB-1643	Neuroblastoma	0.028	1.3	>4	0.56	PD1
OS-1	Osteosarcoma	<0.001	> 1.4	3.1	0.48	PD2
OS-2	Osteosarcoma	<0.001	> 2.7	3.1	0.37	PD2
OS-17	Osteosarcoma	<0.001	> 2.5	1.5	0.3	PD2
OS-9	Osteosarcoma	0.002	> 1.3	1.3	0.53	PD2
OS-33	Osteosarcoma	<0.001	> 4.1	0.4	0.34	MCR
OS-31	Osteosarcoma	<0.001	> 1.9	3.6	0.41	PD2
ALL-2	ALL B-precursor	0.001	2.1	>25	.	PD2
ALL-3	ALL B-precursor	0.008	> 6.0	>25	.	PD2
ALL-4	ALL B-precursor	0.704	0.8	>25	.	PD1
ALL-7	ALL B-precursor	<0.001	> 6.6	10.8	.	SD
ALL-8	ALL T-cell	<0.001	> 2.2	8.1	.	PR
ALL-16	ALL T-cell	<0.001	> 2.2	0.2	.	MCR
ALL-17	ALL B-precursor	0.521	1.7	>25	.	PD2
ALL-19	ALL B-precursor	0.171	3.3	>25	.	PD2

Red shading in the p-value column indicates a significant difference in EFS distribution 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.

Rapamycin *In Vivo* Activity



CONCLUSIONS

- Rapamycin variably inhibited growth of the PPTP's *in vitro* cell lines, with maximal inhibition values generally less than 70%. These *in vitro* results are consistent with rapamycin's well described effects on cell cycle progression.
- Rapamycin demonstrated broad growth inhibitory activity against the PPTP's *in vivo* solid tumor panels, with particularly noteworthy activity for selected sarcoma xenografts.
- Slowly developing objective responses were noted in the rhabdomyosarcoma, osteosarcoma, and rhabdoid *in vivo* tumor panels.
- Several ALL xenografts responded to rapamycin, with both T-cell ALL xenografts demonstrating objective responses.
- Ongoing projects are evaluating PK-PD activity relationships for rapamycin and evaluating combinations of rapamycin with standard chemotherapy agents and with other molecularly targeted agents.

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