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Pediatric Preclinical Testing Program (PPTP) evaluation of rapamycin combined with cytotoxic drugs used frequently in treatment of childhood cancer

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Abstract

Background: Rapamycin (Rap) is a specific inhibitor of mTOR that has demonstrated broad-spectrum antitumor activity as a single agent against the PPTP in vivo panels of childhood tumors. Here we have extended the studies with Rap to combinations with agents used frequently in the treatment of childhood malignancies.
Methods: Rap was tested against the PPTP in vitro panel of 23 cell lines at a concentration of 10nM alone or in combination with increasing concentrations of melphalan, cisplatin, vincristine or dexamethasone [acute lymphoblastic leukemia (ALL) models only]. Rap was tested in vivo at a dose of 5 mg/kg i.p. 5 days per week for 6 weeks for solid tumors or 4 weeks for leukemia models. Cytotoxic agents were administered at their maximum tolerated dose (MTD, approximately LD10), and 0.5 x MTD. Three measures of antitumor activity were used: 1) response criteria modeled after the clinical setting; 2) treated to control (T/C) tumor volume at day 21; and 3) a time to event (4-fold increase in tumor volume) measure based on the median EFS of treated and control lines.
Results: Combining Rap with cytotoxic agents in vitro gave predominantly <-additive or additive effects, except with dexamethasone in ALL models for which the effect was >-additive. In vivo Rap significantly increased the toxicity of cisplatin but not vincristine or cyclophosphamide. Rap combined with vincristine (MTD) was additive or >-additive in 10 of 12 models and with cyclophosphamide (MTD) was additive or >-additive activity in 8 of 9 models and antagonistic in 1 model. Cisplatin (0.63 x MTD) – Rap combination gave additive or >-additive activity in 9 of 9 models. Against the ALL panel the combination with vincristine was predominantly <-additive, while with cyclophosphamide the effect was additive or <-additive. Rap combined with dexamethasone was >-additive, additive, or antagonistic, respectively, in 3 ALL models.
Conclusions: Rap combined with cyclophosphamide or vincristine appeared superior to either single agent against several tumor models. There was little evidence that rapamycin potentiated the toxicity of these agents. Rap significantly potentiated the toxicity of cisplatin. However, the antitumor activity of Rap combined with either cisplatin administered at 0.63 x MTD or with vincristine or cyclophosphamide (both at 0.5 x MTD) was greater than that for each cytotoxic agent alone administered at its MTD in most solid tumor models. (Supported by NCI NO1CM42216)

Methods for PPTP *In Vivo* Testing

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 $(\pi/6)\times d3$, 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 administered intraperitoneally daily x 5 for 6 consecutive weeks at a dose of 5 mg/kg in the solid tumor models and for 4 weeks in ALL models. Cyclophosphamide was administered weekly for 6 weeks by I.P. injection (150 mg/kg q 7d x 6; MTD), as was vincristine (1 mg/kg q7d x 6; MTD). Cisplatin was administered at 5.5 mg/kg on day 1 and 21 (MTD). For leukemia models in NOD/scid mice, the dose of cyclophosphamide was 112.5mg/kg alone and 84.4 mg/kg in combination with rapamycin. Dexamethasone, given alone, was administered at 30 mg/kg daily x5 for four consecutive weeks, but the dose was reduced to 7.5 mg/kg when combined with rapamycin.

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 and were not adjusted for multiple comparisons given the exploratory nature of this study. P-values < 0.05 were considered to be significant.

Calculation of Log Cell Kill: Log_{10} cell kill (LCK), a frequently used measure of antitumor activity, corresponds to the difference in the median times to event between the treated and control mice (or mice treated with the combination *versus* a single-agent). The formula below was used to calculate LCK for *solid tumor xenografts*:

$$LCK_{ST} = (T_d(T) - T_d(C)) / (3.32 * T_2(C)),$$

where $T_d(T)$ is the median time to event in the treatment group [or combination group], $T_d(C)$ is the median time to event in the control group [or single-agent group], and $T_2(C)$ is the median time to tumor doubling in the control group. The constant 3.32 is the inverse of the tumor doubling required for a population on $\log_{10}$ unit, that is the $\log_2(10)$. A high LCK value would indicate treatment efficacy.

Times to event and doubling were calculated using interpolation and were estimated for each group of mice from the Kaplan-Meier survival distribution. If no group median existed (e.g., there were not enough events), then a raw median was calculated (i.e., by taking the median of the imputed time to event for mice with events or the last day of observation for mice without events) for use in the calculation of LCK and is denoted with a ">" sign in the result.

An LCK value was computed similarly for ALL xenografts. Since the event of interest in ALL lines is the percent of cells expressing human CD45 reaching or exceeding 25%, we substituted time to CD45% reaching or exceeding half of the "event," that is CD45% ≥ 12.5%, for the tumor doubling time in the solid tumor lines. The formula below was used to calculate LCK for *ALL xenografts*:

$$LCK_{ALL} = (T_{25}(T) - T_{25}(C)) / (3.32 * T_{12.5}(C)),$$

where $T_{25}(T)$ is the median time to event in the treatment group [or combination group], $T_{25}(C)$ is the median time to event in the control group [or single-agent group], and $T_{12.5}(C)$ is the median time to CD45% ≥ 12.5% in the control group. Times to event or half of the event were calculated using interpolation.

Therapeutic Synergy: Therapeutic synergy was denoted when the LCK value for a combination treatment exceeded the maximum LCK value of either single-agent treatment, and when the EFS distribution for the combination group was significantly higher than ($p<0.05$ using unadjusted p-values) the EFS distributions of both single agent treatment groups.

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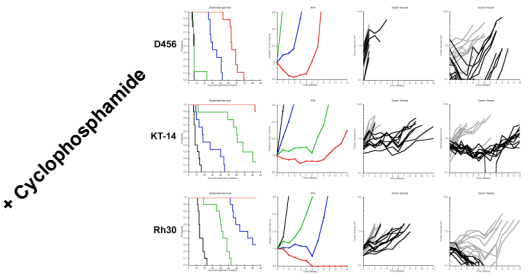
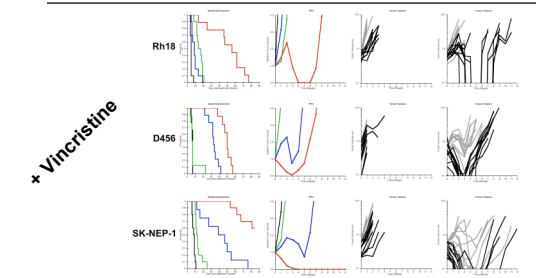
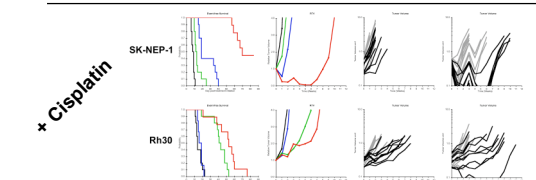
Rapamycin *In Vivo* Growth Curves



Panel 1 shows the Kaplan-Meier curves for EFS, control (black), rapamycin (green), cyclophosphamide (blue), or rapamycin + cyclophosphamide (red). Panel 2 shows median relative tumor volumes, control (black), rapamycin (green), cyclophosphamide (blue), or rapamycin + cyclophosphamide (red). Individual tumor growth curves are shown in panel 3, control (light gray), rapamycin (dark lines), and panel 4 cyclophosphamide (light gray), cyclophosphamide + rapamycin (dark lines).



Panel 1 shows the Kaplan-Meier curves for EFS, control (black), rapamycin (green), vincristine (blue), or rapamycin + vincristine (red). Panel 2 shows median relative tumor volumes, control (black), rapamycin (green), vincristine (blue), or rapamycin + vincristine (red). Individual tumor growth curves are shown in panel 3, control (light gray), rapamycin (dark lines), and panel 4 vincristine (light gray), vincristine + rapamycin (dark lines).



Panel 1 shows the Kaplan-Meier curves for EFS, control (black), rapamycin (green), cisplatin (blue), or rapamycin + cisplatin (red). Panel 2 shows median relative tumor volumes, control (black), rapamycin (green), cisplatin (blue), or rapamycin + cisplatin (red). Individual tumor growth curves are shown in panel 3, control (light gray), rapamycin (dark lines), and panel 4 cisplatin (light gray), cisplatin + rapamycin (dark lines).

Rapamycin *In Vivo* Activity

Xenograft Line	Drug	EFS T/C	LCK value	Overall Group Response
BT-29	Rapamycin	5.4	2.943	PD2
	VCR MTD	1.5	0.363	PD1
	Rap + VCR MTD	5.2	2.812	PD2
	CTX MTD	4.3	2.224	PD2
	Rap + CTX MTD	6.8	3.887	PD2
	CDDP 0.63 MTD	1.6	0.438	PD2
	Rap + CDDP 0.63 MTD	5	2.734	PD2
KT-14	Rapamycin	7.7	4.245	PD2
	VCR MTD	5.3	2.759	PD2
	Rap + VCR MTD	8.5	4.761	PD2
	CTX MTD	2.3	0.843	PD2
	Rap + CTX MTD	>10.6	> 6.102	PR
	CDDP 0.63 MTD	4.2	2.008	PD2
	Rap + CDDP 0.63 MTD	7.3	4.021	PD2
SK-NEP-1	Rapamycin	1.5	0.307	PD1
	VCR MTD	5.2	2.616	PD2
	Rap + VCR MTD	>10.3	> 5.725	MCR
	CTX MTD	>10.3	> 5.725	MCR
	Rap + CTX MTD	>10.3	> 5.725	MCR
	CDDP 0.63 MTD	1.9	0.569	PD2
	Rap + CDDP 0.63 MTD	5	2.492	PD2
EW5	Rapamycin	1.8	0.474	PD2
	VCR MTD	1.5	0.287	PD2
	Rap + VCR MTD	1.7	0.38	PD2
	CTX MTD	2.3	0.701	PD2
	Rap + CTX MTD	5.1	2.294	PD2
	CDDP 0.63 MTD	1.7	0.411	PD2
	Rap + CDDP 0.63 MTD	2.1	0.601	PD2
Rh30	Rapamycin	3.1	1.007	PD2
	VCR MTD	1.2	0.11	PD1
	Rap + VCR MTD	4.7	1.768	SD
	CTX MTD	5.1	1.966	SD
	Rap + CTX MTD	> 6.2	> 2.495	MCR
	CDDP 0.63 MTD	1.3	0.122	PD1
	Rap + CDDP 0.63 MTD	3.9	1.396	PD2
Rh18	Rapamycin	3.3	1.384	PD2
	CTX MTD	>17.9	> 8.826	MCR
	Rap + CTX MTD	>17.9	> 7.928	MCR
	CDDP 0.63 MTD	1.7	0.45	PD2
	Rap + CDDP 0.63 MTD	3	1.188	PD2
D645	Rapamycin	1.9	0.461	PD2
	VCR MTD	2.2	0.579	PD2
	Rap + VCR MTD	4.5	1.76	SD
	CTX MTD	> 6.6	> 2.766	MCR
	Rap + CTX MTD	> 6.6	> 2.766	CR
D456	Rapamycin	1	-0.011	PD1
	VCR MTD	4.9	2.374	PD2
	Rap + VCR MTD	7.5	3.888	CR
	CTX MTD	4.4	2.076	PD2
	Rap + CTX MTD	8.1	4.263	CR
	CDDP 0.63 MTD	1.6	0.386	PD2
	Rap + CDDP 0.63 MTD	3.1	1.259	PD2
OS-2	Rapamycin	3.1	1.298	PD2
	VCR MTD	3.7	1.624	CR
	Rap + VCR MTD	3.9	1.784	CR
OS-31	Rapamycin	1.4	0.197	PD1
	VCR MTD	2.5	0.769	PD2
	Rap + VCR MTD	2.8	0.915	PD2
	CTX 0.5 MTD	> 6.0	> 2.509	MCR
	Rap + CTX 0.5 MTD	> 6.0	> 2.509	PR
	CDDP 0.63 MTD	1.3	0.158	PD2
	Rap + CDDP 0.63 MTD	2	0.517	PD2

* Blue shading denotes combinations resulting in Therapeutic Synergy

Xenograft Line	Drug	EFS T/C	LCK value	Overall Group Response
ALL-4	Rapamycin	2.1	0.832	PD2
	VCR	9.6	6.472	CR
	CTX	>13.3	> 8.541	CR
	DEX	1.4	0.31	PD1
	Rap + VCR	8.7	5.803	CR
	Rap + CTX	12.4	8.537	CR
	Rap + DEX	0.7	-0.228	PD1
ALL-8	Rapamycin	2.8	0.814	PD2
	VCR	3.3	1.07	CR
	CTX	> 6.6	> 2.037	CR
	DEX	2.6	0.707	PD2
	Rap + VCR	3.6	1.172	CR
	Rap + CTX	> 6.6	> 2.565	CR
	Rap + DEX	3.1	0.982	CR
ALL-19	Rapamycin	2.2	1.248	PD1
	VCR	23.5	23.377	CR
	CTX	9	8.27	PR
	DEX	9.4	8.758	PD2
	Rap + VCR	23.2	23.05	CR
	Rap + CTX	15.8	15.359	CR
	Rap + DEX	12.8	12.212	CR

CONCLUSIONS

- In vitro, rapamycin combined with cytotoxic agents had predominantly sub-additive to additive activity, but was supra-additive with dexamethasone in leukemia models (data not shown).
- In vivo, rapamycin potentiated the toxicity of cisplatin, requiring dose reduction to 0.63 x MTD, but did not significantly potentiate the toxicity of cyclophosphamide (CPM) or vincristine (VCR).
- The rapamycin and VCR (MTD) combination demonstrated:
  - Significant extension of median EFS compared to single agent VCR in 7 of 9 solid tumor models.
  - Therapeutic synergy in 4 of 9 evaluable models.
- The rapamycin CPM (MTD) combination demonstrated:
  - Significant extension of median EFS compared to single agent CPM in 6 of 6 informative models
  - Therapeutic synergy in 5 of 8 evaluable models.
- The rapamycin and cisplatin (0.63 x MTD) combination demonstrated:
  - Significant extension of median EFS compared to single agent cisplatin (MTD) in 4 of 4 informative models.
  - Therapeutic synergy in 2 of 7 evaluable xenografts.

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