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Initial Testing (Stage 1) the Pediatric Preclinical Testing Program of RO4929097, a γ -Secretase Inhibitor Targeting Notch Signaling

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Abstract

RO4929097 is a potent and selective inhibitor of γ -secretase and as a result is able to inhibit Notch pathway signaling. The activity of RO4929097 was evaluated against the *in vivo* panels of the Pediatric Preclinical Testing Program (PTTP). RO4929097 induced significant differences in event-free survival (EFS) distribution compared to control in 6 of 26 (23%) of the evaluable solid tumor xenografts and in 0 of 8 (0%) of the evaluable ALL xenografts. The most consistent tumor growth delay effects were noted in the osteosarcoma panel. RO4929097 at the dose and schedule evaluated demonstrated little antitumor activity against childhood cancer xenografts.

Keywords

Preclinical Testing; Developmental Therapeutics; Notch

INTRODUCTION

The Notch pathway is a developmental signaling pathway that is active in a wide range of adult and pediatric cancers. The Notch pathway is involved in regulating the differentiation of progenitor cells during development and the self-renewal of stem cells in adults. Its effects are cell-type and context-dependent and can involve either promotion or inhibition of self-renewal [1]. There are multiple cell membrane Notch pathway receptors (Notch 1-4) as

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well as a set of transmembrane Notch pathway ligands (Jagged1, Jagged2, Delta-like1, Delta-like3, and Delta-like4).

Pharmacologic inhibition of Notch pathway signaling has been accomplished by exploiting the pathway's requirement for proteolytic cleavage of Notch to release an intracellular domain of Notch (NICD) that transmits cell surface signals to the nucleus leading to altered transcription. The primary protease involved in Notch cleavage is γ -secretase. This protease is not specific for Notch and has been reported to cleave 60 distinct transmembrane proteins. Among these substrates is amyloid precursor, a membrane protein that is cleaved by γ -secretase to produce beta-amyloid peptide (Ab), which has a tendency for aggregation and which forms amyloid plaques in the central nervous system. Hence γ -secretase has been extensively evaluated as a therapeutic target for Alzheimer's disease, although the clinical experience to date for this class of agents has not yielded positive results [2].

RO4929097 is a potent and selective inhibitor of γ -secretase that inhibits Notch processing in tumor cells leading to reduced expression of the Notch transcriptional targets such as HES1 [3]. RO4929097 did not block proliferation or induce apoptosis in the adult cancer cell lines studied, but instead produced a less transformed, flattened, slower-growing phenotype. RO4929097 was active following oral dosing against adult cancer xenografts.

The Notch pathway has been implicated in a number of pediatric cancers including T-cell ALL [4], osteosarcoma [5-8], Ewing sarcoma [9], ependymoma [10], and medulloblastoma [11]. Given evidence for a role for the Notch pathway in several childhood cancers, RO4929097 was selected for evaluation against the PPTP's in vivo solid tumor models. In vitro testing was not performed because of the lack of in vitro effects of RO4929097 on proliferation and apoptosis observed for adult cancer cell lines [3].

MATERIALS AND METHODS

In vivo tumor growth inhibition studies

CB17SC-M *scid*^{-/-} female mice (Taconic Farms, Germantown NY), were used to propagate subcutaneously implanted kidney/rhabdoid tumors, sarcomas (Ewing, osteosarcoma, rhabdomyosarcoma), neuroblastoma, and non-glioblastoma brain tumors, while BALB/c nu/nu mice were used for glioma models, as previously described [12]. Human leukemia cells were propagated by intravenous inoculation in female non-obese diabetic (NOD)/*scid*^{-/-} mice as described previously [13]. 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. Ten mice (solid tumors) or 8 mice (leukemia models) were used in each control or treatment group. Tumor volumes (cm³) [solid tumor xenografts] or percentages of human CD45-positive [hCD45] cells [ALL xenografts] were determined as previously described [14] and responses were determined using three activity measures as previously described [14]. 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 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. The Mann-Whitney test was used to test the difference between VEGFA expression level between groups of xenografts with greater versus lesser tumor growth inhibition (EFS T/C ≥ 2 versus < 2).

Drugs and Formulation

RO4929097 was provided to the PPTP by Roche Pharmaceuticals, Inc. through the Cancer Treatment and Evaluation Program, NCI. RO4929097 was formulated as a suspension in 1.0% Klucel in water with 0.2% Tween 80. It was administered at a dose of 10 mg/kg/day by oral gavage for a planned treatment duration of 21 days.

RESULTS

RO4929097 was evaluated against 34 xenograft models. Three of 641 mice died during the study (0.5%), with 1 of 318 in the control arm (0.3%) and 2 of 323 in the RO4929097 treatment arm (0.6%). No tumor lines were excluded from analysis due to toxicity greater than 25 percent, but one rhabdomyosarcoma xenograft (Rh10) was not evaluable because of poor growth of tumor in control animals. A complete summary of results is provided in Supplemental Table I, including total numbers of mice, number of mice that died (or were otherwise excluded), numbers of mice with events and average times to event, tumor growth delay, as well as numbers of responses and T/C values.

RO4929097 induced significant differences in EFS distribution compared to control in 6 of 26 (23%) of the evaluable solid tumor xenografts and in 0 of 8 (0%) of the evaluable ALL xenografts (Table I). 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 more substantial agent effect in slowing tumor growth. High activity further requires a reduction in final tumor volume compared to the starting tumor volume. RO4929097 did not induce tumor growth inhibition meeting criteria for intermediate EFS T/C activity in the evaluable solid tumor or ALL xenografts. The most consistent tumor growth delay effects were noted in the osteosarcoma panel, with 4 of 6 evaluable xenografts showing significant differences in EFS distribution between the treated and control groups. Objective responses (i.e., regressions meeting criteria for PR, CR, or MCR) were not observed for either the solid tumor or ALL xenografts.

DISCUSSION

The oncogenic role of Notch signaling is illustrated by T-cell ALL, for which NOTCH1 activating mutations are present in over 50% of cases [4]. Two types of NOTCH1 mutations are observed in T-cell ALL: mutations in the extracellular heterodimerization (HD) domains that lead to increased susceptibility to ligand-independent proteolytic cleavage, and mutations in the intracellular domain that result in increased NICD stability. Mutations in FBXW7, a ubiquitin ligase implicated in NICD turnover, are also observed in T-cell ALL, and like PEST domain mutations they lead to increased NICD stability [4]. The genomic characteristics of T-cell ALL indicate that it should be an ideal setting in which to evaluate inhibitors of Notch signaling. However, the initial clinical experience evaluating the γ -secretase inhibitor MK-0752 against T-cell ALL was disappointing, as significant gastrointestinal toxicity was observed and none of the patients showed a significant clinical response [15]. Both of the PPTP T-cell ALL xenografts tested (ALL-8 and ALL-16) have heterozygous NOTCH1 mutations, ALL-8 in the PEST Domain and ALL-16 in the heterodimerization domain. However, the PPTP results for RO4929097 with these two T-cell ALL xenografts are consistent with the MK-0752 clinical results in showing little activity.

The gene expression patterns of the PPTP xenograft panels suggest that Notch signaling is activated in some of the solid tumor panels, as evidenced by expression patterns of NOTCH1-4, and the Notch transcriptional targets HES1 and HEY1/HEY2 (Figure 1). Notch

pathway activation has been described for several childhood cancers, including osteosarcoma [5-8] and medulloblastoma [11], and NOTCH1 mutations have also been reported for a small subset of patients with ependymoma [10].

Previous studies evaluating the effect of Notch pathway inhibition for osteosarcoma have produced contradictory results. One report described an effect of Notch inhibition on metastasis and cell migration, but no effect on cell proliferation [8]. Two other reports described effects for Notch inhibition on cell proliferation, and both showed *in vivo* tumor growth delay as a result of Notch inhibition [6,7]. The PPTP results show a clear treatment effect for RO4929097 against most of the osteosarcoma xenografts, but the magnitude of this effect is small.

The RO4929097 dose and schedule selected for testing was based on prior published work describing its tumor growth inhibition activity against 6 of 7 established adult cancer xenografts and the absence of a clear dose-response effect for higher doses [3]. Furthermore, pharmacodynamic testing showed that Notch signaling was inhibited at day 21 following 14 days treatment with RO4929097 at 3 mg/kg [3]. Thus, at the dose and schedule used in the PPTP study (10 mg/kg daily x 21) it is anticipated that the target, γ -secretase, is fully inhibited.

Phase 1 evaluation of single agent RO4929097 has been completed [16], and phase 1 and phase 2 studies with RO4929097 alone and in combination are ongoing for various adult cancer indications. More intensive Notch pathway inhibition may be required for activity as regressions were observed with the use of higher doses (60 mg/kg) administered for 7 to 14 days [3]. Other γ -secretase inhibitors are under clinical evaluation for cancer indications and other approaches to Notch pathway inhibition are in development [17], and it is possible that one of these approaches may show a more favorable activity profile against pediatric preclinical models including T-ALL.

In summary, the γ -secretase inhibitor RO4929097 showed limited *in vivo* activity against the PPTP's solid tumor and ALL xenografts at a dose and schedule that showed tumor growth inhibition and on-target pharmacodynamic effects in adult cancer preclinical models.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1. Expression of Notch pathway genes (Affymetrix U133 Plus 2.0) in PPTP cell lines and xenografts as visualized using GeneSifter software (VizX Labs, Seattle, WA). Gray indicates an absent call from Affymetrix quality control. Gene expression analysis methods are as previously described [18].

Table 1
Activity of RO4929097 against the PPTP *in vivo* panel

Line Description	Tum or Type	Median Time to Event ¹	P-value	EFS T/C ²	Median RTV/CD45 at End of Study ³	Tumor Volume T/C ⁴	Median Group Response ⁵	EFS Activity
KT-12	Rhabdoid	13.3	0.060	1.2	>4	0.76	PD1	Low
KT-10	Wilms	11.9	0.327	1.1	>4	0.71	PD1	Low
KT-13	Wilms	10.9	0.897	0.9	>4	1.06	PD1	Low
SK-NEP-1	Ewing	13.2	0.268	1.0	>4	1.52	PD1	Low
EW5	Ewing	9.8	0.105	1.4	>4	0.85	PD1	Low
EW8	Ewing	6.0	0.224	0.7	>4	1.70	PD1	Low
TC-71	Ewing	4.7	0.498	0.9	>4	1.21	PD1	Low
Rh10	ALV RMS	31.8	0.023	.	>4	0.93	PD1	NE
Rh28	ALV RMS	27.0	0.755	1.0	>4	0.87	PD1	Low
Rh30	ALV RMS	11.0	0.059	1.3	>4	0.69	PD1	Low
Rh30R	ALV RMS	16.5	0.003	1.5	>4	0.71	PD1	Low
Rh41	ALV RMS	13.8	0.367	1.0	>4	0.83	PD1	Low
Rh18	EMB RMS	10.5	0.804	1.1	>4	1.08	PD1	Low
Rh36	EMB RMS	9.9	0.529	1.0	>4	1.01	PD1	Low
BT-28	Medulloblastoma	4.3	0.871	1.0	>4	1.06	PD1	Low
BT-45	Medulloblastoma	6.3	0.099	1.1	>4	0.89	PD1	Low
GBM2	Glioblastoma	15.8	<0.001	1.5	>4	0.64	PD2	Low
BT-39	Glioblastoma	10.5	0.732	1.2	>4	0.79	PD1	Low
D645	Glioblastoma	9.9	0.693	1.0	>4	0.92	PD1	Low
D456	Glioblastoma	5.9	0.550	1.0	>4	1.10	PD1	Low
OS-1	Osteosarcoma	25.0	0.221	1.0	>4	0.84	PD1	Low
OS-2	Osteosarcoma	20.2	0.033	1.1	>4	0.87	PD1	Low
OS-17	Osteosarcoma	18.9	<0.001	1.4	>4	0.69	PD1	Low
OS-9	Osteosarcoma	19.5	0.002	1.4	>4	0.79	PD1	Low
OS-33	Osteosarcoma	21.1	0.018	1.2	>4	0.70	PD1	Low

Line Description	Tum or Type	Median Time to Event ¹	P-value	EFS T/C ²	Median RTV/CD45 at End of Study ³	Tumor Volume T/C ⁴	Median Group Response ⁵	EFS Activity
OS-31	Osteosarcoma	18.3	0.401	1.3	>4	0.85	PD1	Low
ALL-2	ALL B-precursor	6.5	0.608	0.6	>25	.	PD1	Low
ALL-3	ALL B-precursor	5.0	1.000	1.0	>25	.	PD1	Low
ALL-4	ALL B-precursor	5.0	0.948	1.0	>25	.	PD1	Low
ALL-7	ALL B-precursor	3.1	0.117	1.3	>25	.	PD1	Low
ALL-8	ALL T-cell	5.2	0.142	1.2	>25	.	PD1	Low
ALL-16	ALL T-cell	15.1	0.264	1.6	>25	.	PD2	Low
ALL-17	ALL B-precursor	6.6	0.249	1.3	>25	.	PD1	Low
ALL-19	ALL B-precursor	7.1	0.438	0.8	>25	.	PD1	Low

¹ Kaplan-Meier estimate of the median time to event for the treatment group

² EFS T/C value = ratio of the median time to event of the treatment group and of the respective control group

³ RTV (Relative tumor volume) = ratio of tumor volume at specified time post-treatment to pretreatment tumor volume

⁴ Tumor Volume T/C = ratio of RTV for treatment and control groups calculated at day 21 or when all mice in the control and treated groups still had measurable tumor volumes (if less than 21 days).

⁵ See Supplemental Response Definitions.