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(71) Applicant: **WYETH** [US/US]; Five Giralda Farms, Madison, NJ 07940-0874 (US).

(72) Inventors: **DUKART, Gary**; 1714 Benjamin Drive, Ambler, PA 19002 (US). **GIBBONS, James, Joseph, Jr.**; 33 Terrace Drive, Westwood, NJ 07675 (US). **SPEICHER, Lisa, Anne**; 509 Covington Road, Havertown, PA 19083 (US). **FROST, Philip**; 4 Emerson Court, Morris Township, NJ 07960 (US). **DISCAFANI-MARRO, Carolyn, Mary**; 8 Brookside Avenue, Cortlandt Manor, NY 10567 (US).

(74) Agents: **MILOWSKY, Arnold, S.**; Wyeth, Patent Law Department, Five Giralda Farms, Madison, NJ 07940-0874 et al. (US).

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(54) Title: USE OF CCI-779 AS AN ANTINEOPLASTIC AGENT

(57) Abstract: This invention provides the use of CCI-779 in the treatment of neoplasms.

USE OF CCI-779 AS AN ANTINEOPLASTIC AGENT

This invention relates to the use of rapamycin 42-ester with 3-hydroxy-2-(hydroxymethyl)-2-methylpropionic acid (CCI-779) as an antineoplastic agent.

Rapamycin is a macrocyclic triene antibiotic produced by Streptomyces hygroscopicus, which was found to have antifungal activity, particularly against Candida albicans, both in vitro and in vivo [C. Vezina et al., J. Antibiot. 28, 721 (1975); S.N. Sehgal et al., J. Antibiot. 28, 727 (1975); H. A. Baker et al., J. Antibiot. 31, 539 (1978); U.S. Patent 3,929,992; and U.S. Patent 3,993,749]. Additionally, rapamycin alone (U.S. Patent 4,885,171) or in combination with picibanil (U.S. Patent 4,401,653) has been shown to have antitumor activity.

The immunosuppressive effects of rapamycin have been disclosed in FASEB 3, 3411 (1989). Cyclosporin A and FK-506, other macrocyclic molecules, also have been shown to be effective as immunosuppressive agents, therefore useful in preventing transplant rejection [FASEB 3, 3411 (1989); FASEB 3, 5256 (1989); R. Y. Calne et al., Lancet 1183 (1978); and U.S. Patent 5,100,899]. R. Martel et al. [Can. J. Physiol. Pharmacol. 55, 48 (1977)] disclosed that rapamycin is effective in the experimental allergic encephalomyelitis model, a model for multiple sclerosis; in the adjuvant arthritis model, a model for rheumatoid arthritis; and effectively inhibited the formation of IgE-like antibodies.

Rapamycin is also useful in preventing or treating systemic lupus erythematosus [U.S. Patent 5,078,999], pulmonary inflammation [U.S. Patent 5,080,899], insulin dependent diabetes mellitus [U.S. Patent 5,321,009], skin disorders, such as psoriasis [U.S. Patent 5,286,730], bowel disorders [U.S. Patent 5,286,731], smooth muscle cell proliferation and intimal thickening following vascular injury [U.S. Patents 5,288,711 and 5,516,781], adult T-cell leukemia/-lymphoma [European Patent Application 525,960 A1], ocular inflammation [U.S. Patent 5,387,589], malignant carcinomas [U.S. Patent 5,206,018], cardiac inflammatory disease [U.S. Patent 5,496,832], and anemia [U.S. Patent 5,561,138].

The preparation and use of hydroxyesters of rapamycin, including CCI-779, are disclosed in U.S. Patent 5,362,718.

DESCRIPTION OF THE INVENTION

This invention provides the use of CCI-779 as an antineoplastic agent, and particularly for neoplasms which are refractory to standard therapy, or for whom standard therapy is not appropriate. In particular CCI-779 is useful in the treatment of renal cancer, soft tissue cancer, breast cancer, neuroendocrine tumor of the lung, cervical cancer, uterine cancer, head and neck cancer, glioblastoma, non-small lung cell cancer, prostate cancer, pancreatic cancer, lymphoma, melanoma, small cell lung cancer, ovarian cancer, colon cancer.

As used in accordance with this invention, the term "treatment" means treating a mammal having a neoplastic disease by providing said mammal an effective amount of CCI-779 with the purpose of inhibiting growth of the neoplasm in such mammal, eradication of the neoplasm, or palliation of the neoplasm.

As used in accordance with this invention, the term "providing," with respect to providing CCI-779, means either directly administering CCI-779, or administering a prodrug, derivative, or analog which will form an effective amount of CCI-779 within the body.

As used in accordance with this invention, the term "refractory neoplasm" refers to neoplasms in patients which typically had progressed following treatment with standard chemotherapy that was appropriate for that given neoplasm.

The preparation of CCI-779 is described in U.S. Patent 5,362,718, which is hereby incorporated by reference.

The antineoplastic activity of CCI-779 was confirmed in a preclinical *in vitro* and *in vivo* standard pharmacological test procedure which measured the ability of

CCI-779 to treat human renal cell cancer (a rapidly progressive disease with very limited treatment options), as well as in two Phase I human clinical trials. The procedures used and results obtained are briefly described below.

Preclinical Test Procedures

In vitro test procedure: Renal tumor lines HTB-44 and CRL-1161 were obtained from the American Tissue Culture Collection (ATCC), Bethesda, MD. SN12-C line was obtained from Dr. J. Fidler, M.D. Anderson Hospital, Houston, TX. Cells were plated in MEM (Gibco) supplemented with 2 mM glutamine, 1 mM sodium pyruvate, 5 ml penicillin-streptomycin solution, 1 mM non-essential amino acid solution, 10% fetal bovine solution. Cells (5×10^3) were plated in 96 well plates with a final volume of 200 ml and incubated for 24 hours at 37°C. Log dilutions of CCI-779 beginning at 100 µg/ml were then added to the cultures for 48 hours. Over the last 5 hours, cells were pulsed with 1 µci ^{3}H-thymidine (New England Nuclear, 6.7 ci/m Mol). Cells were then harvested and the degree of thymidine uptake determined by liquid scintillation spectrometry. The IC_{50} was determined as the concentration that produced 50% of the maximum uptake of thymidine in control untreated cells.

In vivo test procedure: Female Balb/c nu/nu mice were obtained from Charles River Labs, Wilmington, DE, at 6-8 weeks of age. Mice (n=10/group) were injected sc with 5×10^6 cells resuspended in a 50% solution of Matrigel (BD Biosciences) and tumors allowed to develop. When tumor size reached 100 mg, mice were treated orally with CCI-779 at 25 mg/kg. CCI-779 was dosed for 5 consecutive days with repeated 14 day cycles throughout the duration of the experiment. The formulation used for CCI-779 was a 50% ethanol, 49% phosal, 1% tween 80 vehicle for resuspending CCI-779, where the stock was resuspended into a 1:10 dilution of the vehicle prior to dosing. Tumor growth was evaluated using a vernier caliper and volume (l x w x h) was converted to mass using the formula: $l \times w^2 / 2$.

Results:

Human renal cell tumors were cultured *in vitro* in the presence or absence of CCI-779 for 3 days and the effect on growth determined by ^{3}H-thymidine incorporation of control versus treated cells. Table 1 shows that IC_{50} (50% growth inhibitory concentration) for all 3 lines tested was in the low nM range.

Table 1 The effect of CCI-779 on the growth of human renal tumor cells *in vitro*

Renal Tumor Line	CCI-779 IC_{50} (nM)
HTB-44	5.0
CRL-1161	2.0
SN12-C	5.5

The effect of CCI-779 in two human renal lines (HTB-44 and CRL-1161) were was evaluated *in vivo* by engrafting tumor cells on the flanks of nude mice. Once tumors were established at a size of about 100 mg, mice were treated with CCI-779 or a vehicle control. Treatment with CCI-779 at 25 mg/kg resulted in significant inhibition of tumor cell growth in the mice (Table 2).

Table 2 Effect of CCI-779 on the growth of human renal tumor cells in nude mouse xenografts

		Tumor Mass (mg)					
		Days					
Cell Line	Drug Treatment	0	7	21	35	49	55
HTB-44	Control	288±21	219±18	616±55	1095±44	2033±247	2412±342
	CCI	290±15	156±13*	252±48*	453±85*	980±155*	1050±183*
	% T/C	101	71	41	41	48	44
CRL-1161	Control	273±18	355±36	413±60	480±127	546±170	507±156
	CCI	272±14	219±16*	226±17*	200±21*	229±28*	268±30
	% T/C	100	62	60	42	42	53

* p value - < .05
% T/C - Treated/Control x 100

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