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(54) Title: ANTINEOPLASTIC COMBINATIONS

(57) Abstract: This invention provides the use of a combination of an mTOR inhibitor and an antinoeplastic alkylating agent in the treatment of neoplasms.

ANTINEOPLASTIC COMBINATIONS

This invention relates to the use of combinations of an mTOR inhibitor (e.g rapamycin 42-ester with 3-hydroxy-2-(hydroxymethyl)-2-methylpropionic acid (CCI-779)) and an antineoplastic alkylating agent in the treatment of neoplasms, to the use of an mTOR inhibitor and an antineoplastic alkylating agent in the preparation of a medicament for the treatment of a neoplasm, to a product comprising an mTOR inhibitor and an antineoplastic alkylating agent as a combined preparation for simultaneous, separate or sequential use in the treatment of a neoplasm, and to pharmaceutical compositions comprising an mTOR inhibitor, an antineoplastic alkylating agent and a pharmaceutically acceptable carrier.

BACKGROUND OF THE INVENTION

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].

Rapamycin 42-ester with 3-hydroxy-2-(hydroxymethyl)-2-methylpropionic acid (CCI-779) is ester of rapamycin which has demonstrated significant inhibitory effects on tumor growth in both in vitro and in vivo models. The preparation and use of hydroxyesters of rapamycin, including CCI-779, are disclosed in U.S. Patent 5,362,718.

CCI-779 exhibits cytostatic, as opposed to cytotoxic properties, and may delay the time to progression of tumors or time to tumor recurrence. CCI-779 is considered to have a mechanism of action that is similar to that of sirolimus. CCI-779 binds to and forms a complex with the cytoplasmic protein FKBP, which inhibits an enzyme, mTOR (mammalian target of rapamycin, also known as FKBP12-rapamycin associated protein [FRAP]). Inhibition of mTOR's kinase activity inhibits a variety of signal transduction pathways, including cytokine-stimulated cell proliferation, translation of mRNAs for several key proteins that regulate the G1 phase of the cell cycle, and IL-2-induced transcription, leading to inhibition of progression of the cell cycle from G1 to S. The mechanism of action of CCI-779 that results in the G1 S phase block is novel for an anticancer drug.

In vitro, CCI-779 has been shown to inhibit the growth of a number of histologically diverse tumor cells. Central nervous system (CNS) cancer, leukemia (T-cell), breast cancer, prostate cancer, and melanoma lines were among the most sensitive to CCI-779. The compound arrested cells in the G1 phase of the cell cycle.

In vivo studies in nude mice have demonstrated that CCI-779 has activity against human tumor xenografts of diverse histological types. Gliomas were particularly sensitive to CCI-779 and the compound was active in an orthotopic glioma model in nude mice. Growth factor (platelet-derived)-induced stimulation of a human glioblastoma cell line in vitro was markedly suppressed by CCI-779. The growth of several human pancreatic tumors in nude mice as well as one of two breast cancer lines studied in vivo also was inhibited by CCI-779.

DESCRIPTION OF THE INVENTION

This invention provides the use of combinations of an mTOR inhibitor and an antineoplastic alkylating agent as antineoplastic combination chemotherapy. In particular, these combinations are 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, glioma, non-small lung cell cancer, prostate cancer, pancreatic cancer, lymphoma, melanoma, small cell lung cancer, ovarian cancer, colon cancer, esophageal cancer, gastric cancer, leukemia, colorectal cancer, and unknown primary cancer. This invention also provides combinations of an mTOR inhibitor and an antineoplastic alkylating agent for use as antineoplastic combination chemotherapy, in which the dosage of either the mTOR inhibitor or the antineoplastic alkylating agent or both are used in subtherapeutically effective dosages.

In another aspect, the invention provides the use of combinations of an mTOR inhibitor and an antineoplastic alkylating agent in the preparation of a medicament for the treatment of a neoplasm. In a further aspect, the invention provides a product comprising an mTOR inhibitor and an antineoplastic alkylating agent as a combined *preparation for simultaneous, separate or sequential use in the treatment of a* neoplasm in a mammal. In a still further aspect, the invention provides a pharmaceutical composition comprising an mTOR inhibitor, an antineoplastic alkylating agent and a pharmaceutically acceptable carrier.

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 a combination of an mTOR inhibitor and an antineoplastic alkylating agent with the purpose of inhibiting growth of the neoplasm in such mammal, eradication of the neoplasm, or palliation of the mammal.

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

mTOR is the mammalian target of rapamycin, also known as FKBP12-rapamycin associated protein [FRAP]. Inhibition of mTOR's kinase activity inhibits a variety of signal transduction pathways, including cytokine-stimulated cell proliferation,

translation of mRNAs for several key proteins that regulate the G1 phase of the cell cycle, and IL-2-induced transcription, leading to inhibition of progression of the cell cycle from G1 to S.

mTOR regulates the activity of at least two proteins involved in the translation of specific cell cycle regulatory proteins (Burnett, P.E., PNAS 95: 1432 (1998) and Isotani, S., J. Biol. Chem. 274: 33493 (1999)). One of these proteins p70s6 kinase is phosphorylated by mTOR on serine 389 as well as threonine 412. This phosphorylation can be observed in growth factor treated cells by Western blotting of whole cell extracts of these cells with antibody specific for the phosphoserine 389 residue.

As used in accordance with this invention, an "mTOR inhibitor" means a compound or ligand which inhibits cell replication by blocking progression of the cell cycle from G1 to S by inhibiting the phosphorylation of serine 389 of p70s6 kinase by mTOR.

The following standard pharmacological test procedure can be used to determine whether a compound is an mTOR inhibitor, as defined herein. Treatment of growth factor stimulated cells with an mTOR inhibitor like rapamycin completely blocks phosphorylation of serine 389 as evidenced by Western blot and as such constitutes a good assay for mTOR inhibition. Thus whole cell lysates from cells stimulated by a growth factor (eg. IGF1) in culture in the presence of an mTOR inhibitor should fail to show a band on an acrylamide gel capable of being labeled with an antibody specific for serine 389 of p70s6K.

Materials:

NuPAGE LDS Sample Buffer	(Novex Cat # NP0007)
NuPAGE Sample Reducing Agent	(Novex Cat # NP0004)
NuPAGE 4-12% Bis-Tris Gel	(Novex Cat # NP0321)
NuPAGE MOPS SDS Running Buffer	(Novex Cat # NP0001)
Nitrocellulose	(Novex Cat # LC2001)
NuPAGE Transfer Buffer	(Novex Cat # NP0006)
Hyperfilm ECL	(Amersham Cat # RPN3114H)
ECL Western Blotting Detection Reagent	(Amersham Cat # RPN2134)

Primary antibody:	Phospho-p70 S6 Kinase (Thr389)	(Cell Signaling Cat #9205)
Secondary antibody:	Goat anti-rabbit IgG-HRP conjugate	(Santa Cruz Cat #sc-2004)

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