US 20020183239A1

(19) United States

(12) Patent Application Publication (10) Pub. No.: US 2002/0183239 A1

Gibbons, JR. et al. (43) Pub. Date: Dec. 5, 2002

(54) ANTINEOPLASTIC COMBINATIONS

(75) Inventors: **James J. Gibbons JR.**, Westwood, NJ (US); **Gary Dukart**, Ambler, PA (US); **Jurgen Frisch**, Marburg (DE)

Correspondence Address:
Arnold S. Milowsky
5 Giralda Farms
Madison, NJ 07940 (US)

(73) Assignee: **Wyeth**, Madison, NJ

(21) Appl. No.: **10/116,902**

(22) Filed: **Apr. 5, 2002**

Related U.S. Application Data

(60) Provisional application No. 60/282,388, filed on Apr. 6, 2001.

Publication Classification

(51) **Int. Cl.**[7] **A61K 31/00**

(52) **U.S. Cl.** **514/1**

(57) **ABSTRACT**

This invention provides the use of a combination of an mTOR inhibitor and an antimetabolite antineoplastic agent in the treatment of neoplasms.

ANTINEOPLASTIC COMBINATIONS

[0001] This application claims priority from provisional application Serial No. 60/282,388, filed Apr. 6, 2001, the entire disclosure of which is hereby incorporated by reference.

BACKGROUND OF THE INVENTION

[0002] This invention relates to the use of combinations of an mTOR inhibitor and an antimetabolite antineoplastic agent in the treatment of neoplasms.

[0003] 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. Pat. Nos. 3,929,992; and 3,993,749]. Additionally, rapamycin alone (U.S. Pat. No. 4,885,171) or in combination with picibanil (U.S. Pat. No. 4,401,653) has been shown to have antitumor activity.

[0004] 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. Pat. No. 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.

[0005] Rapamycin is also useful in preventing or treating systemic lupus erythematosus [U.S. Pat. No. 5,078,999], pulmonary inflammation [U.S. Pat. No. 5,080,899], insulin dependent diabetes mellitus [U.S. Pat. No. 5,321,009], skin disorders, such as psoriasis [U.S. Pat. No. 5,286,730], bowel disorders [U.S. Pat. No. 5,286,731], smooth muscle cell proliferation and intimal thickening following vascular injury [U.S. Pat. Nos. 5,288,711 and 5,516,781], adult T-cell leukemia/lymphoma [European Patent Application 525,960 A1], ocular inflammation [U.S. Pat. No. 5,387,589], malignant carcinomas [U.S. Pat. No. 5,206,018], cardiac inflammatory disease [U.S. Pat. No. 5,496,832], and anemia [U.S. Pat. No. 5,561,138].

[0006] 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. Pat. No. 5,362,718.

[0007] 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.

[0008] 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.

[0009] 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

[0010] This invention provides the use of combinations of an mTOR inhibitor and an antimetabolite antineoplastic 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 antimetabolite antineoplastic agent for use as antineoplastic combination chemotherapy, in which the dosage of either the mTOR inhibitor or the antimetabolite antineoplastic agent or both are used in subtherapeutically effective dosages.

[0011] 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 antimetabolite antineoplastic agent with the purpose of inhibiting growth of the neoplasm in such mammal, eradication of the neoplasm, or palliation of the mammal.

[0012] 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.

[0013] 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.

[0014] 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.

[0015] 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.

[0016] 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)

[0017] Methods:

[0018] A. Preparation of Cell Lysates

[0019] Cell lines were grown in optimal basal medium supplemented with 10% fetal bovine serum and penicillin/treptomycin. For phosphorylation studies, cells were subcultured in 6-well plates. After the cells have completely attached, they were either serum-starved. Treatment with mTOR inhibitors ranged from 2 to 16 hours. After drug treatment, the cells were rinsed once with PBS (phosphate buffered saline without Mg++ and Ca++) and then lysed in 150-200 μl NuPAGE LDS sample buffer per well. The lysates were briefly sonicated and then centrifuged for 15 minutes at 14000 rpm. Lysates were stored at minus −80° C. until use.

[0020] The test procedure can also be run by incubating the cells in growth medium overnigh, after they have completely attached. The results under both sets of conditions should be the same for an mTOR inhibitor.

[0021] B. Western Blot Analysis

[0022] 1) Prepare total protein samples by placing 22.5 μl of lysate per tube and then add 2.5 μl NuPAGE sample reducing agent. Heat samples at 70° C. for 10 minutes. Electrophoresed using NuPAGE gels and NuPAGE SDS buffers.

[0023] 2) Transfer the gel to a nitrocellulose membrane with NuPAGE transfer buffer. The membrane are blocked for 1 hour with blocking buffer (Tris buffered saline with 0.1%-Tween and 5% nonfat-milk). Rinse membranes 2× with washing buffer (Tris buffered saline with 0.1%-Tween).

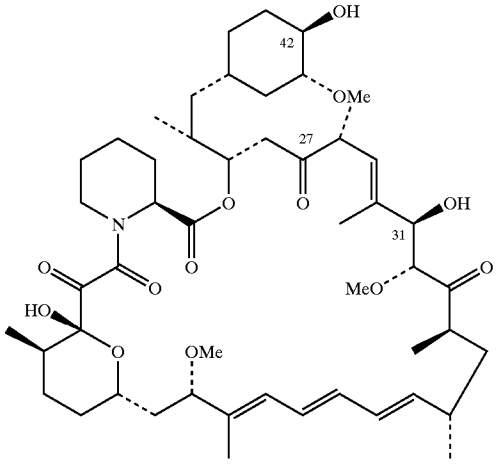
[0024] 3) Blots/membrane are incubated with the P-p70 S6K (T389) primary antibody (1:1000) in blocking buffer overnight at 4° C. in a rotating platform.

[0025] 4) Blots are rinsed 3× for 10 minutes each with washing buffer, and incubated with secondary antibody (1:2000) in blocking buffer for 1 hour at room temperature.

[0026] 5) After the secondary antibody binding, blots are washed 3× for 10 minutes each with washing buffer, and 2× for 1 minute each with Tris-buffered saline, followed by chemiluminescent (ECL) detection and then exposed to chemiluminescence films.

[0027] As used in accordance with this invention, the term "a rapamycin" defines a class of immunosuppressive compounds which contain the basic rapamycin nucleus (shown below). The rapamycins of this invention include compounds which may be chemically or biologically modified as derivatives of the rapamycin nucleus, while still retaining immunosuppressive properties. Accordingly, the term "a rapamycin" includes esters, ethers, oximes, hydrazones, and hydroxylamines of rapamycin, as well as rapamycins in which functional groups on the rapamycin nucleus have been modified, for example through reduction or oxidation. The term "a rapamycin" also includes pharmaceutically acceptable salts of rapamycins, which are capable of forming such salts, either by virtue of containing an acidic or basic moiety.

RAPAMYCIN



[0028] It is preferred that the esters and ethers of rapamycin are of the hydroxyl groups at the 42- and/or 31-positions of the rapamycin nucleus, esters and ethers of a hydroxyl group at the 27-position (following chemical reduction of the 27-ketone), and that the oximes, hydrazones, and hydroxylamines are of a ketone at the 42-position (following oxidation of the 42-hydroxyl group) and of 27-ketone of the rapamycin nucleus.

[0029] Preferred 42- and/or 31-esters and ethers of rapamycin are disclosed in the following patents, which are all hereby incorporated by reference: alkyl esters (U.S. Pat. No. 4,316,885); aminoalkyl esters (U.S. Pat. No. 4,650,803); fluorinated esters (U.S. Pat. No. 5,100,883); amide esters (U.S. Pat. No. 5,118,677); carbamate esters (U.S. Pat. No. 5,118,678); silyl ethers (U.S. Pat. No. 5,120,842); aminoesters (U.S. Pat. No. 5,130,307); acetals (U.S. Pat. No. 5,51,413); aminodiesters (U.S. Pat. No. 5,162,333); sulfonate and sulfate esters (U.S. Pat. No. 5,177,203); esters (U.S. Pat. No. 5,221,670); alkoxyesters (U.S. Pat. No. 5,233,036); O-aryl, -alkyl, -alkenyl, and -alkynyl ethers (U.S. Pat. No. 5,258,389); carbonate esters (U.S. Pat. No. 5,260,300); arylcarbonyl and alkoxycarbonyl carbamates (U.S. Pat. No. 5,262,423); carbamates (U.S. Pat. No. 5,302,584); hydroxyesters (U.S. Pat. No. 5,362,718); hindered esters (U.S. Pat. No. 5,385,908); heterocyclic esters (U.S. Pat. No. 5,385,909); gem-disubstituted esters (U.S. Pat. No. 5,385,910); amino alkanoic esters (U.S. Pat. No. 5,389,639); phosphorylcarbamate esters (U.S. Pat. No. 5,391,730); carbamate esters (U.S. Pat. No. 5,411,967); carbamate esters (U.S. Pat. No. 5,434,260); amidino carbamate esters (U.S. Pat. No. 5,463,048); carbamate esters (U.S. Pat. No. 5,480,988); carbamate esters (U.S. Pat. No. 5,480,989); carbamate esters (U.S. Pat. No. 5,489,680); hindered N-oxide esters (U.S. Pat. No. 5,491,231); biotin esters (U.S. Pat. No. 5,504,091); O-alkyl ethers (U.S. Pat. No. 5,665,772); and PEG esters of rapamycin (U.S. Pat. No. 5,780,462). The preparation of these esters and ethers are disclosed in the patents listed above.

[0030] Preferred 27-esters and ethers of rapamycin are disclosed in U.S. Pat. No. 5,256,790, which is hereby incorporated by reference. The preparation of these esters and ethers are disclosed in the patents listed above.

[0031] Preferred oximes, hydrazones, and hydroxylamines of rapamycin are disclosed in U.S. Pat. Nos. 5,373,014, 5,378,836, 5,023,264, and 5,563,145, which are hereby incorporated by reference. The preparation of these oximes, hydrazones, and hydroxylamines are disclosed in the above listed patents. The preparation of 42-oxorapamycin is disclosed in U.S. Pat. No. 5,023,263, which is hereby incorporated by reference.

[0032] Particularly preferred rapamycins include rapamycin [U.S. Pat. No. 3,929,992], CCI-779 [rapamycin 42-ester with 3-hydroxy-2-(hydroxymethyl)-2-methylpropionic acid; U.S. Pat. No. 5,362,718], and 42-O-(2-hydroxy)ethyl rapamycin [U.S. Pat. No. 5,665,772].

[0033] When applicable, pharmaceutically acceptable salts of the rapamycin can be formed from organic and inorganic acids, for example, acetic, propionic, lactic, citric, tartaric, succinic, fumaric, maleic, malonic, mandelic, malic, phthalic, hydrochloric, hydrobromic, phosphoric, nitric, sulfuric, methanesulfonic, napthalenesulfonic, benzenesulfonic, toluenesulfonic, camphorsulfonic, and similarly known acceptable aids when the rapamycin contains a suitable basic moiety. Salts may also be formed from organic and inorganic bases, such as alkali metal salts (for example, sodium, lithium, or potassium) alkaline earth metal salts, ammonium salts, alkylammonium salts containing 1-6 carbon atoms or dialkylammonium salts containing 1-6 carbon atoms in each alkyl group, and trialkylammonium salts containing 1-6 carbon atoms in each alkyl group, when the rapamycin contains a suitable acidic moiety.

[0034] It is preferred that the mTOR inhibitor used in the antineoplastic combinations of this invention is a rapamycin, and more preferred that the mTOR inhibitor is rapamycin, CCI-779, or 42-O-(2-hydroxy)ethyl rapamycin.

[0035] As described herein, CCI-779 was evaluated as a representative mTOR inhibitor in the mTOR inhibitor plus antimetabolite combinations of this invention.

[0036] The preparation of CCI-779 is described in U.S. Pat. No. 5,362,718, which is hereby incorporated by reference. When CCI-779 is used as an antineoplastic agent, it is projected that initial i.v. infusion dosages will be between about 0.1 and 100 mg/m^2 when administered on a daily dosage regimen (daily for 5 days, every 2-3 weeks), and between about 0.1 and 1000 mg/m^2 when administered on a once weekly dosage regimen. Oral or intravenous infusion are the preferred routes of administration, with intravenous being more preferred.

[0037] As used in accordance with this invention, the term "antimetabolite" means a substance which is structurally similar to a critical natural intermediate (metabolite) in a biochemical pathway leading to DNA or RNA synthesis which is used by the host in that pathway, but acts to inhibit the completion of that pathway (i.e., synthesis of DNA or RNA). More specifically, antimetabolites typically function by (1) competing with metabolites for the catalytic or regulatory site of a key enzyme in DNA or RNA synthesis, or (2) substitute for a metabolite that is normally incorporated into DNA or RNA, and thereby producing a DNA or RNA that cannot support replication. Major categories of antimetabolites include (1) folic acid analogs, which are inhibitors of dihydrofolate reductase (DHFR); (2) purine analogs, which mimic the natural purines (adenine or guanine) but are structurally different so thy competitively or irreversibly inhibit nuclear processing of DNA or RNA; and (3) pyrimidine analogs. which mimic the natural pyrimidines (cytosine, thymidine, and uracil) but are structurally different so thy competitively or irreversibly inhibit nuclear processing of DNA or RNA.

[0038] The following are representative examples of antimetabolites of this invention.

[0039] 5-Fluorouracil (5-FU; 5-fluoro-2,4(1H,3H)-pyrimidinedione) is commercially available in a topical cream (FLUOROPLEX or EFUDEX) a topical solution (FLUOROPLEX or EFUDEX), and as an injectable containing 50 mg/mL 5-fluorouracil (ADRUCIL or flurouracil).

[0040] Floxuradine (2'-deoxy-5-fluorouridine) is commercially available as an injectable containing 500 mg/vial of floxuradine (FUDR or floxuradine).

[0041] Thioguanine (2-amino-1,7-dihydro-6-H-purine-6-thione) is commercially available in 40 mg oral tablets (thioguanine).

[0042] Cytarabine (4-amino-1-(beta)-D-arabinofuranosyl-2(1H)-pyrimidinone) is commercially available as a liposomal injectable containing 10 mg/mL cytarabine (DEPOCYT) or as a liquid injectable containing between 1 mg-1 g/vial or 20 mg/mL (cytarabine or CYTOSAR-U).

[0043] Fludarabine (9-H-Purin-6-amine,2-fluoro-9-(5-O-phosphono-(beta)-D-arabinofuranosyl) is commercially available as a liquid injectable containing 50 mg/vial (FLUDARA).

[0044] 6-Mercaptopurine (1,7-dihydro-6H-purine-6-thione) is commercially available in 50 mg oral tablets (PURINETHOL).

[0045] Methotrexate (MTX; N-[4-[[(2,4-diamino-6-pteridinyl)methyl]methylamino]benzoyl]-L-glutamic acid) is commercially available as a liquid injectable containing between 2.5-25 mg/mL and 20 mg-1 g/vial (methotrexate sodium or FOLEX) and in 2.5 mg oral tablets (methotrexate sodium).

[0046] Gemcitabine (2'-deoxy-2',2'-difluorocytidine monohydrochloride ((beta)-isomer)), is commercially available as a liquid injectable containing between 200 mg-1 g/vial (GEMZAR).

[0047] Capecitabine (5'-deoxy-5-fluoro-N-[(pentyloxy-)carbonyl]-cytidine) is commercially available as a 150 or 500 mg oral tablet (XELODA).

[0048] Pentostatin ((R)-3-(2-deoxy-(beta)-D-erythropentofuranosyl)-3,6,7,8-tetrahydroimidazo[4,5-d][1,3]diazepin-8-ol) is commercially available as a liquid injectable containing 10 mg/vial (NIPENT).

[0049] Trimetrexate (2,4-diamino-5-methyl-6-[(3,4,5-trimethoxyanilino)methyl]quinazoline mono-D-glucuronate) is commercially available as a liquid injectable containing between 25-200 mg/vial (NEUTREXIN).

[0050] Cladribine (2-chloro-6-amino-9-(2-deoxy-(beta)-D-erythropento-furanosyl) purine) is commercially available as a liquid injectable containing 1 mg/mL (LEUSTATIN).

[0051] The following table briefly summarizes some of the recommended dosages for the antimetabolites listed above.

Drug	Dosage	Regimen
5-Fluorouracil	12 mg/kg oral	daily for 4 days
	6 mg/kg oral	days 6, 8, 10, 12
		no drug on days 5, 7, 9, and 11; doses cut in half if toxicity observed
	370–600 mg/m^2 i.v.	daily for 5 days, every 3–4 weeks
Floxuradine (FUDR)	0.1–0.6 mg/kg	daily by arterial infusion
Cytarabine (DEPOCYT)	50 mg	every 14 days for 5 doses during induction period; followed by every 28 days for maintenance
Cytarabine	100 mg/m^2	daily for 7 days
(injectable)	2–3 g/m^2	twice daily for 2–6 days
Fludarabine (FLUDARA)	25 mg/m^2	30 min infusion for 5 consecutive days; every 28 days
6-Mercaptopurine	2.5–5 mg/kg	daily for induction
(PURINETHOL)	1.5–2.5 mg/kg	daily for maintenance
Methotrexate	15–30 mg oral	daily for 5 day course; repeated 3–5 times
Gemcitabine (GEMZAR)	1000 mg/m^2/30 min	single agent: once weekly for 7 weeks, followed by 1 week rest, then once weekly for 3 out of every 4 weeks
	1000–1250 mg/m^2/30 min	combination therapy: days 1, 8, 15 per 28 day cycle, or days 1 and 8 per 21 day cycle
Capecitabine (XELODA)	2500 mg/m^2	daily for 2 weeks followed by 1 week rest period
Pentostatin (NIPENT)	4 mg/m^2	as bolus injection or diluted as i.v. infusion; every other week
Trimetrexate (NEUTREXIN)	45 mg/m^2	i.v. infusion once daily for 21 days
Cladribine (LEUSTATIN)	0.09 mg/kg/day	continuous infusion for 7 consecutive days

[0052] This invention also covers the use of an mTOR inhibitor plus an antimetabolite in which a biochemical modifying agent is part of the chemotherapeutic regimen. The term "biochemical modifying agent" is well known and understood to those skilled in the art as an agent given as an adjunct to antimetabolite therapy, which serves to potentate its antineoplastic activity, as well as counteract the side effects of the antimetabolite. Leucovorin and levofolinate are typically used as biochemical modifying agents for methotrexate and 5-FU therapy.

[0053] Leucovorin (5-formyl-5,6,7,8-tetrahydrofolic acid) is commercially available as an injectable liquid containing between 5-10 mg/mL or 50-350 mg/vial (leucovorin calcium or WELLCOVORIN) and as 5-25 mg oral tablets (leucovorin calcium).

[0054] Levofolinate (pharmacologically active isomer of 5-formyltetrahydrofolic acid) is commercially available as an injectable containing 25-75 mg levofolinate (ISOVORIN) or as 2.5-7.5 mg oral tablets (ISOVORIN).

[0055] Preferred mTOR inhibitor plus antimetabolite combinations of this invention include CCI-779 plus gemcitabine; CCI-779 plus 5-fluorouracil; and CCI-779 plus 5-fluorouracil plus leucovorin. It is preferred that the CCI-779 plus gemcitabine combination be used in treating pancreatic cancer and that the CCI-779 plus 5-fluorouracil combination (with or without leucovorin) be used in treating colorectal cancer.

[0056] The antineoplastic activity of the CCI-779 plus antimetabolite combination was confirmed in in vitro and in vivo standard pharmacological test procedures using combinations of CCI-779 plus gemcitabine; and CCI-779 plus 5-fluorouracil as representative combinations of this invention. The following briefly describes the procedures used and the results obtained.

[0057] Human rhabdomyosarcoma lines Rh30 and Rh1 and the human glioblastoma line SJ-GBM2 were used for in vitro combination studies with CCI-779 and antimetabolite agents. In vivo studies used a human neuroblastoma (NB1643) and human colon line GC3.

[0058] Dose response curves were determined for each of the drugs of interest. The cell lines Rh30, Rh1 and SJ-G2

DOCKET ALARM

Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts

Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research

With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips

Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS
Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS
Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS
Sync your system to PACER to automate legal marketing.