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INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Not for submission under 37 CFR 1.99)

Application Number	
Filing Date	2012-07-11
First Named Inventor	Lane et al.
Art Unit	
Examiner Name	
Attorney Docket Number	031671-US-CNT03 (62 C 3)

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Examiner Initial*	Cite No	Patent Number	Kind Code[1]	Issue Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
	1	6333348		2001-12-25	VOGEL et al.	
	2	4885171		1989-12-05	SURENDRA et al.	
	3	5194447		1993-03-16	KAO	
	4	5985890		1999-11-16	COTTENS et al.	
	5	5066493		1991-11-19	SEHGAL et al.	
	6	5206018		1993-04-27	SEHGAL et al.	
	7	5362718		1994-08-11	SKOTNICKI et al.	
	8	5922730		1999-07-13	HUE et al.	

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	9	6569463		2003-05-27	PATEL et al.	
	10	6617333		2003-09-09	RABINDRAN et al.	
	11	6641822		2003-11-04	SUTHANTHIRAN et al.	

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	1	20020022054		2002-02-21	SAWADA et al.	
	2	20020098278		2002-07-25	BATES et al.	
	3	20030100886		2003-05-29	SEGAL et al.	
	4	20030100887		2003-05-29	SCOTT et al.	

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	1	1074263	EP		2001-02-07	NEUER et al.		☐
	2	9409010	WO		1994-04-28	COTTENS		☐
	3	9516691	WO		1995-06-22	COTTENS		☐
	4	9528406	WO		1995-10-26	SKOTNICKI et al.		☐
	5	9641807	WO		1996-12-27	COTTENS et al.		☐
	6	9747317	WO		1997-12-18	WECKBECKER		☐
	7	9809970	WO		1998-03-12	HU et al.		☐
	8	0149338	WO		2001-07-12	LI et al.		☐
	9	0151049	WO		2001-07-19	WASIK et al.		☐
	10	0205791	WO		2002-01-24	MASSIMINI et al.		☐
	11	0213802	WO		2002-02-21	ZHANG et al.		☐

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	12	02080975	WO		2002-10-17	GIBBONS et al.		☐
	13	02098416	WO		2002-12-12	DUKART et al.		☐
	14	0240000	WO		2002-05-23	DUKART		☐
	15	9811908	WO		1998-03-26	WOOD et al.		☐
	16	0187372	WO		2001-11-22	KOPIA et al.		☐

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Examiner Initials*	Cite No	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc), date, pages(s), volume-issue number(s), publisher, city and/or country where published.	T[5]
	1	LIEN et al. "Therapeutic anti-VEGF antibodies. Therapeutic Antibodies, Handbook of Experimental Pharmacology 181. Y. Chernajovsky et al. (eds). 2008; 131-150.	☐
	2	Wikipedia (http://en.wikipedia.org/wiki/Angiogenesis.	☐
	3	GEOERGER et al. "Antitumor Activity of the Rapamycin Analog CCI-779 in Human Primitive Neuroectodermal Tumor/ Medulloblastoma Models as SingleAgent and in Combination Chemotherapy", Cancer Res 2001, 61(4): 1527-1532.	☐
	4	GUBA et al. "Rapamycin Inhibits Tumor Growth and Metastasis by Antiangiogenesis", Chirurgisches Forum Fuer Experimentelle und Klinische Forschung, 2001, 37-39.	☐

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	5	LAW et al. "Farnesyltransferase Inhibitor Induces Rapid Growth Arrest and Blocks p70s6k Activation by Multiple Stimuli", J Biol Chem 2000, 275(15): 10796-10801.	☐
	6	PENG et al. "Novel Pyrrolo-quinoline Derivatives as Potent Inhibitors for P13-Kinase Related Kinases", Bioorg Med Chem 2002, 10(1): 167-174.	☐
	7	SHI et al. "Rapamycin Enhances Apoptosis and Increases Sensivity to Cisplatin in Vitro", Cancer Res. 1995, 55(9): 1982-1988.	☐
	8	ZHONG et al. "Modulation of Hypoxia-inducible Factor 1-alpha Expression by the Epidermal Growth Factor/ Phosphatidylinositol 3-Kinase/PTEN/AKT/ FRAP Pathway...", Cancer Res. 2000, 60(6): 1541-1545.	☐
	9	SHI et al. "Rapamycin enhances apoptosis and increases sensitivity to cisplatin in vitro" Cancer Research 1995, 55: 1982-1988.	☐
	10	FOSSA et al. "Survival of patients with advanced urothelial cancer treated with cisplatin-based chemotherapy" British Journal of Cancer 1996, 74: 1655-1659.	☐
	11	Renal Pelvis (medical dictionary definition 12/12/1998, accessed via http://www.mondofacto.com/facts/dictionary?renal +pelvis on May 19, 2011).	☐
	12	ARECCI et al. "Immunosuppresants FK506 and Rapamycin Function as Reversal Agents of the Multidrug Resistance Phenotype", Blood 1992, 80(6): 1528-1536.	☐
	13	DAYANIR et al. "Identification of Tyrosine Residues in Vascular Endothelial Growth", J Biol Chem 2001, 276(21): 17686-17692.	☐
	14	ENG et al. "Activity of Rapamycin (AY-22,989) Against Transplanted Tumors", J Antiobotics 1984, XXXVII(10): 1231-1237.	☐
	15	ZHU et al. "Inhibition of tumor growth and metastasis by targeting tumor-associated angiogenesis with antagonists to the receptors of vascular endothelial growth factor", Investigational New Drugs 1999, 17: 195-212.	☐

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CERTIFICATION STATEMENT

Please see 37 CFR 1.97 and 1.98 to make the appropriate selection(s):

☐ That each item of information contained in the information disclosure statement was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(1).

OR

☐ That no item of information contained in the information disclosure statement was cited in a communication from a foreign patent office in a counterpart foreign application, and, to the knowledge of the person signing the certification after making reasonable inquiry, no item of information contained in the information disclosure statement was known to any individual designated in 37 CFR 1.56(c) more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(2).

☐ See attached certification statement.

☐ The fee set forth in 37 CFR 1.17 (p) has been submitted herewith.

☐ A certification statement is not submitted herewith.

SIGNATURE

A signature of the applicant or representative is required in accordance with CFR 1.33, 10.18. Please see CFR 1.4(d) for the form of the signature.

Signature	/Ann R. Pokalsky/	Date (YYYY-MM-DD)	2012-07-11
Name/Print	Ann R. Pokalsky	Registration Number	34,697

This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 1 hour to complete, including gathering, preparing and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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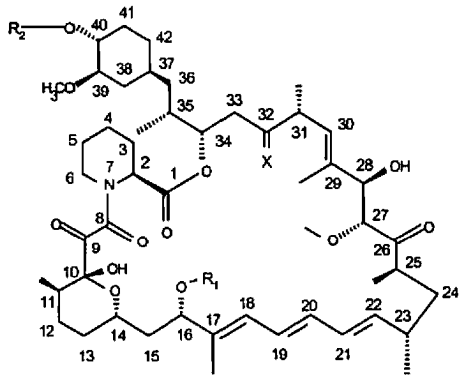
Treatment of Solid Tumours with Rapamycin Derivatives

This application is a continuation of U.S. Application No. 10/468,520, filed January 27, 2004, which is a 371 application of PCT/EP2002/01714, filed February 18, 2002, which in its entirety is herein incorporated by reference.

The present invention relates to a new use, in particular a new use for a compound group comprising rapamycin and derivatives thereof.

Rapamycin is a known macrolide antibiotic produced by Streptomyces hygroscopicus. Suitable derivatives of rapamycin include e.g. compounds of formula I



I

wherein

R_1 is CH_3 or C_{3-6}alkynyl,

R_2 is H or $-CH_2-CH_2-OH$, and

X is =O, (H,H) or (H,OH)

provided that R_2 is other than H when X is =O and R_1 is CH_3.

Compounds of formula I are disclosed e.g. in U.S. Patent Nos: 5,665,772; 6,440,990; 5,985,890; and 6,200,985, which are incorporated herein by reference. They may be prepared as diclosed or by analogy to the procedures described in these references

Preferred compounds are 32-deoxorapamycin, 16-pent-2-ynyloxy-32-deoxorapamycin, 16-pent-2-ynyloxy-32(S)-dihydro-rapamycin, 16-pent-2-ynyloxy-32(S)-dihydro-40-O-(2-hydroxyethyl)-rapamycin and, more preferably, 40-O-(2-hydroxyethyl)-rapamycin (referred thereafter as Compound A), disclosed as Example 8 in U.S. Patent Nos: 5,665,772 and 6,440,990.

Compounds of formula I have, on the basis of observed activity, e.g. binding to macrophilin-12 (also known as FK-506 binding protein or FKBP-12), e.g. as described in WO 94/09010, WO 95/16691 or WO 96/41807, been found to be useful e.g. as immunosuppressant, e.g. in the treatment of acute allograft rejection. It has now been found that Compounds of formula I have potent antiproliferative properties which make them useful for cancer chemotherapy, particularly of solid tumors, especially of advanced solid tumors. There is still the need to expand the armamentarium of cancer treatment of solid tumors, especially in cases where treatment with anticancer compounds is not associated with disease regression or stabilization.

In accordance with the particular findings of the present invention, there is provided:

1.1 A method for treating solid tumors in a subject in need thereof, comprising administering to said subject a therapeutically effective amount of a compound of formula I.

1.2 A method for inhibiting growth of solid tumors in a subject in need thereof, comprising administering to said subject a therapeutically effective amount of a compound of formula I.

1.3 A method for inducing tumor regression, e.g. tumor mass reduction, in a subject in need thereof, comprising administering to said subject a therapeutically effective amount of a compound of formula I.

1.4 A method for treating solid tumor invasiveness or symptoms associated with such tumor growth in a subject in need thereof, comprising administering to said subject a therapeutically effective amount of a compound of formula I.

1.5 A method for preventing metastatic spread of tumours or for preventing or inhibiting growth of micrometastasis in a subject in need thereof, comprising administering to said subject a therapeutically effective amount of a compound of formula I.

By "solid tumors" are meant tumors and/or metastasis (whereever located) other than lymphatic cancer, e.g. brain and other central nervous system tumors (eg. tumors of the meninges, brain, spinal cord, cranial nerves and other parts of central nervous system, e.g. glioblastomas or medulla blastomas); head and/or neck cancer; breast tumors; circulatory system tumors (e.g. heart, mediastinum and pleura, and other intrathoracic organs, vascular tumors and tumor-associated vascular tissue); excretory system tumors (e.g. kidney, renal pelvis, ureter, bladder, other and unspecified urinary organs); gastrointestinal tract tumors (e.g. oesophagus, stomach, small intestine, colon, colorectal, rectosigmoid junction, rectum, anus and anal canal), tumors involving the liver and intrahepatic bile ducts, gall bladder,

other and unspecified parts of biliary tract, pancreas, other and digestive organs); head and neck; oral cavity (lip, tongue, gum, floor of mouth, palate, and other parts of mouth, parotid gland, and other parts of the salivary glands, tonsil, oropharynx, nasopharynx, pyriform sinus, hypopharynx, and other sites in the lip, oral cavity and pharynx); reproductive system tumors (e.g. vulva, vagina, Cervix uteri, Corpus uteri, uterus, ovary, and other sites associated with female genital organs, placenta, penis, prostate, testis, and other sites associated with male genital organs); respiratory tract tumors (e.g. nasal cavity and middle ear, accessory sinuses, larynx, trachea, bronchus and lung, e.g. small cell lung cancer or non-small cell lung cancer); skeletal system tumors (e.g. bone and articular cartilage of limbs, bone articular cartilage and other sites); skin tumors (e.g. malignant melanoma of the skin, non-melanoma skin cancer, basal cell carcinoma of skin, squamous cell carcinoma of skin, mesothelioma, Kaposi's sarcoma); and tumors involving other tissues incluing peripheral nerves and autonomic nervous system, connective and soft tissue, retroperitoneum and peritoneum, eye and adnexa, thyroid, adrenal gland and other endocrine glands and related structures, secondary and unspecified malignant neoplasm of lymph nodes, secondary malignant neoplasm of respiratory and digestive systems and secondary malignant neoplasm of other sites.

Where hereinbefore and subsequently a tumor, a tumor disease, a carcinoma or a cancer is mentioned, also metastasis in the original organ or tissue and/or in any other location are implied alternatively or in addition, whatever the location of the tumor and/or metastasis is.

In a series of further specific or alternative embodiments, the present invention also provides

1.6 A method for the treatment of a disease associated with deregulated angiogenesis in a subject in need thereof, comprising administering to said subject a therapeutically effective amount of rapamycin or a derivative thereof, e.g. CCI779, ABT578 or a compound of formula I.

1.7 A method for inhibiting or controlling deregulated angiogenesis in a subject in need thereof, comprising administering to said subject a therapeutically effective amount of rapamycin or a derivative thereof, e.g. CCI779, ABT578 or a compound of formula I.

1.8 A method for enhancing the activity of a chemotherapeutic agent or for overcoming resistance to a chemotherapeutic agent in a subject in need thereof, comprising administering to said subject a therapeutically effective amount of rapamycin or a derivative thereof, e.g. CCI779, ABT578 or a compound of formula I, either concomitantly or sequentially with said chemotherapeutic agent.

1.9 A method according to 1.8 wherein the chemotherapeutic agent is an inhibitor of signal transduction pathways directed either against host cells or processes involved in tumor formation and/or metastases formation or utilised by tumour cells for proliferation, survival, differentiation or development of drug resistance.

1.10 A method as indicated above, wherein rapamycin or a derivative thereof, e.g. CCI779, ABT578 or a compound of formula I is administered intermittently.

CCI779 is a rapamycin derivative, i.e. 40- [3-hydroxy-2-(hydroxymethyl)-2-methylpropanoate]-rapamycin or a pharmaceutically acceptable salt thereof, and is disclosed e.g. in USP 5,362,718. ABT578 is a 40-substituted rapamycin derivative further comprising a diene reduction.

Examples of diseases associated with deregulated angiogenesis include without limitation e.g. neoplastic diseases, e.g. solid tumors. Angiogenesis is regarded as a prerequisite for those tumors which grow beyond a certain diameter, e.g. about 1-2 mm.

In a series of further specific or alternative embodiments, the present invention also provides:

2.1 A compound of formula I for use in any method as defined under 1.1 to 1.5 above.

2.2 Rapamycin or a derivative thereof, e.g. CCI779, ABT578 or a compound of formula I for use in any method as defined under 1.6 to 1.10 above or 7 below.

3.1 A compound of formula I for use in the preparation of a pharmaceutical composition for use in any method as defined under 1.1 to 1.5 above.

3.2 Rapamycin or a derivative thereof, e.g. CCI779, ABT578 or a compound of formula I for use in the preparation of a pharmaceutical composition for use in any method as defined under 1.6 to 1.10 above or 7 below.

4.1 A pharmaceutical composition for use in any method as defined under 1.1 to 1.5 above comprising a compound of formula I together with one or more pharmaceutically acceptable diluents or carriers therefor.

4.2 A pharmaceutical composition for use in any method as defined under 1.6 to 1.10 above or 7 below comprising rapamycin or a derivative thereof, e.g. CCI779, ABT578 or a compound of formula I, e.g. Compound A, together with one or more pharmaceutically acceptable diluents or carriers therefor.

5.1 A pharmaceutical combination comprising a) a first agent which is rapamycin or a derivative thereof, e.g. CCI779, ABT578 or a compound of formula I, e.g. Compound A, and b) a co-agent which is a chemotherapeutic agent, e.g. as defined hereinafter.

5.2 A pharmaceutical combination comprising an amount of a) a first agent which is rapamycin or a derivative thereof, e.g. CCI779, ABT578 or a compound of formula I, e.g. Compound A, and b) a co-agent which is a chemotherapeutic agent selected from the compounds defined under paragraph (iv) or (v) below, to produce a synergistic therapeutic effect.

6. A method as defined above comprising co-administration, e.g. concomitantly or in sequence, of a therapeutically effective amount of rapamycin or a derivative thereof, e.g. CCI779, ABT578 or a compound of formula I, e.g. Compound A, and a second drug substance, said second drug substance being a chemotherapeutic agent, e.g. as indicated hereinafter.

7. A method for treating post-transplant lymphoproliferative disorders or a lymphatic cancer, e.g. for treating tumor invasiveness or symptoms associated with such tumor growth in a subject in need thereof, comprising co-administering to said subject, e.g. concomitantly or in sequence, of rapamycin or a derivative thereof, e.g. CCI779, ABT578 or a compound of formula I, e.g. Compound A, and a second drug substance, said second drug substance being a chemotherapeutic agent, e.g. as indicated hereinafter.

By "lymphatic cancer" are meant e.g. tumors of blood and lymphatic system (e.g. Hodgkin's disease, Non-Hodgkin's lymphoma, Burkitt's lymphoma, AIDS-related lymphomas, malignant immunoproliferative diseases, multiple myeloma and malignant plasma cell neoplasms, lymphoid leukemia, myeloid leukemia, acute or chronic lymphocytic leukemia, monocytic leukemia, other leukemias of specified cell type, leukemia of unspecified cell type, other and unspecified malignant neoplasms of lymphoid, haematopoietic and related tissues, for example diffuse large cell lymphoma, T-cell lymphoma or cutaneous T-cell lymphoma).

By the term "chemotherapeutic agent" is meant especially any chemotherapeutic agent other than rapamycin or a derivative thereof. It includes but is not limited to,

i. an aromatase inhibitor,

ii. an antiestrogen, an anti-androgen (especially in the case of prostate cancer) or a gonadorelin agonist,

iii. a topoisomerase I inhibitor or a topoisomerase II inhibitor,

iv. a microtubule active agent, an alkylating agent, an antineoplastic antimetabolite or a platin compound,

v. a compound targeting/decreasing a protein or lipid kinase activity or a protein or lipid phosphatase activity, a further anti-angiogenic compound or a compound which induces cell differentiation processes,

vi. a bradykinin 1 receptor or an angiotensin II antagonist,

vii. a cyclooxygenase inhibitor, a bisphosphonate, a histone deacetylase inhibitor, a heparanase inhibitor (prevents heparan sulphate degradation), e.g. PI-88, a biological response modifier, preferably a lymphokine or interferons, e.g. interferon γ, an ubiquitination inhibitor, or an inhibitor which blocks anti-apoptotic pathways,

viii. an inhibitor of Ras oncogenic isoforms, e.g. H-Ras, K-Ras or N-Ras, or a farnesyl transferase inhibitor, e.g. L-744,832 or DK8G557,

ix. a telomerase inhibitor, e.g. telomestatin,

x. a protease inhibitor, a matrix metalloproteinase inhibitor, a methionine aminopeptidase inhibitor, e.g. bengamide or a derivative thereof, or a proteosome inhibitor, e.g. PS-341.

The term "aromatase inhibitor" as used herein relates to a compound which inhibits the estrogen production, i.e. the conversion of the substrates androstenedione and testosterone to estrone and estradiol, respectively. The term includes, but is not limited to steroids, especially atamestane, exemestane and formestane and, in particular, non-steroids, especially aminoglutethimide, roglethimide, pyridoglutethimide, trilostane, testolactone, ketokonazole, vorozole, fadrozole, anastrozole and letrozole. Exemestane can be administered, e.g., in the form as it is marketed, e.g. under the trademark AROMASIN™. Formestane can be administered, e.g., in the form as it is marketed, e.g. under the trademark LENTARON™. Fadrozole can be administered, e.g., in the form as it is marketed, e.g. under the trademark AFEMA™. Anastrozole can be administered, e.g., in the form as it is marketed, e.g. under the trademark ARIMIDEX™. Letrozole can be administered, e.g., in the form as it is marketed, e.g. under the trademark FEMARA™ or FEMAR™ Aminoglutethimide can be administered, e.g., in the form as it is marketed, e.g. under the trademark ORIMETEN™. A combination of the invention comprising a chemotherapeutic agent which is an aromatase inhibitor is particularly useful for the treatment of hormone receptor positive tumors, e.g. breast tumors.

The term "antiestrogen" as used herein relates to a compound which antagonizes the effect of estrogens at the estrogen receptor level. The term includes, but is not limited to tamoxifen, fulvestrant, raloxifene and raloxifene hydrochloride. Tamoxifen can be

administered, e.g., in the form as it is marketed, e.g. under the trademark NOLVADEX™. Raloxifene hydrochloride can be administered, e.g., in the form as it is marketed, e.g. under the trademark EVISTA™. Fulvestrant can be formulated as disclosed in US 4,659,516 or it can be administered, e.g., in the form as it is marketed, e.g. under the trademark FASLODEX™. A combination of the invention comprising a chemotherapeutic agent which is an antiestrogen is particularly useful for the treatment of estrogen receptor positive tumors, e.g. breast tumors.

The term "anti-androgen" as used herein relates to any substance which is capable of inhibiting the biological effects of androgenic hormones and includes, but is not limited to, bicalutamide (CASODEX™), which can be formulated, e.g. as disclosed in US 4,636,505.

The term "gonadorelin agonist" as used herein includes, but is not limited to abarelix, goserelin and goserelin acetate. Goserelin is disclosed in US 4,100,274 and can be administered, e.g., in the form as it is marketed, e.g. under the trademark ZOLADEX™. Abarelix can be formulated, eg. as disclosed in US 5,843,901.

The term "topoisomerase I inhibitor" as used herein includes, but is not limited to topotecan, irinotecan, 9-nitrocamptothecin and the macromolecular camptothecin conjugate PNU-166148 (compound A1 in WO99/17804). Irinotecan can be administered, e.g. in the form as it is marketed, e.g. under the trademark CAMPTOSAR™. Topotecan can be administered, e.g., in the form as it is marketed, e.g. under the trademark HYCAMTIN™.

The term "topoisomerase II inhibitor" as used herein includes, but is not limited to the anthracyclines such as doxorubicin (including liposomal formulation, e.g. CAELYX™), daunorubicin, epirubicin, idarubicin and nemorubicin, the anthraquinones mitoxantrone and losoxantrone, and the podophillotoxines etoposide and teniposide. Etoposide can be administered, e.g. in the form as it is marketed, e.g. under the trademark ETOPOPHOS™. Teniposide can be administered, e.g. in the form as it is marketed, e.g. under the trademark VM 26-BRISTOL™ Doxorubicin can be administered, e.g. in the form as it is marketed, e.g. under the trademark ADRIBLASTIN™. Epirubicin can be administered, e.g. in the form as it is marketed, e.g. under the trademark FARMORUBICIN™. Idarubicin can be administered, e.g. in the form as it is marketed, e.g. under the trademark ZAVEDOS™. Mitoxantrone can be administered, e.g. in the form as it is marketed, e.g. under the trademark NOVANTRON™.

The term "microtubule active agent" relates to microtubule stabilizing and microtubule destabilizing agents including, but not limited to taxanes, e.g. paclitaxel and docetaxel, vinca alkaloids, e.g., vinblastine, especially vinblastine sulfate, vincristine especially vincristine sulfate, and vinorelbine, discodermolides and epothilones and derivatives thereof, e.g. epothilone B or a derivative thereof. Paclitaxel may be administered e.g. in the form as it is marketed, e.g. TAXOL™. Docetaxel can be administered, e.g., in the form as it is marketed, e.g. under the trademark TAXOTERE™. Vinblastine sulfate can be administered, e.g., in the form as it is marketed, e.g. under the trademark VINBLASTIN R.P.™. Vincristine sulfate can be administered, e.g., in the form as it is marketed, e.g. under the trademark FARMISTIN™. Discodermolide can be obtained, e.g., as disclosed in US 5,010,099.

The term "alkylating agent" as used herein includes, but is not limited to cyclophosphamide, ifosfamide, melphalan or nitrosourea (BCNU or Gliadel™). Cyclophosphamide can be administered, e.g., in the form as it is marketed, e.g. under the trademark CYCLOSTIN™. Ifosfamide can be administered, e.g., in the form as it is marketed, e.g. under the trademark HOLOXAN™.

The term "antineoplastic antimetabolite" includes, but is not limited to 5-fluorouracil, capecitabine, gemcitabine, methotrexate and edatrexate. Capecitabine can be administered, e.g., in the form as it is marketed, e.g. under the trademark XELODA™. Gemcitabine can be administered, e.g., in the form as it is marketed, e.g. under the trademark GEMZAR™.

The term "platin compound" as used herein includes, but is not limited to carboplatin, cis-platin and oxaliplatin. Carboplatin can be administered, e.g., in the form as it is marketed, e.g. under the trademark CARBOPLAT™. Oxaliplatin can be administered, e.g., in the form as it is marketed, e.g. under the trademark ELOXATIN™.

The term "compounds targeting/decreasing a protein or lipid kinase activity or further anti-angiogenic compounds" as used herein includes, but is not limited to protein tyrosine kinase and/or serine and/or threonine kinase inhibitors or lipid kinase inhibitors, e.g. compounds targeting, decreasing or inhibiting the activity of the epidermal growth factor family of receptor tyrosine kinases (EGFR, ErbB2, ErbB3, ErbB4 as homo- or heterodimers), the vascular endothelial growth factor family of receptor tyrosine kinases (VEGFR), the platelet-derived growth factor-receptors (PDGFR), the fibroblast growth factor-receptors (FGFR), the insulin-like growth factor receptor 1 (IGF-1R), the Trk receptor tyrosine kinase family, the Axl receptor tyrosine kinase family, the Ret receptor tyrosine kinase, the Kit/SCFR receptor tyrosine kinase, members of the c-Abl family and their gene-fusion products (e.g. BCR-Abl),

members of the protein kinase C (PKC) and Raf family of serine/threonine kinases, members of the MEK, SRC, JAK, FAK, PDK or PI(3) kinase family, or of the PI(3)-kinase-related kinase family, and/or members of the cyclin-dependent kinase family (CDK) and anti-angiogenic compounds having another mechanism for their activity, e.g. unrelated to protein or lipid kinase inhibition.

Compounds which target, decrease or inhibit the activity of VEGFR are especially compounds, proteins or antibodies which inhibit the VEGF receptor tyrosine kinase, inhibit a VEGF receptor or bind to VEGF, and are in particular those compounds, proteins or monoclonal antibodies generically and specifically disclosed in WO 98/35958, e.g. 1-(4-chloroanilino)-4-(4-pyridylmethyl)phthalazine or a pharmaceutically acceptable salt thereof, e.g. the succinate, or in WO 00/09495, WO 00/27820, WO 00/59509, WO 98/11223, WO 00/27819 and EP 0 769 947; those as described by M. Prewett et al in Cancer Research 59 (1999) 5209-5218, by F. Yuan et al in Proc. Natl. Acad. Sci. USA, vol. 93, pp. 14765-14770, Dec. 1996, by Z. Zhu et al in Cancer Res. 58, 1998, 3209-3214, and by J. Mordenti et al in Toxicologic Pathology, Vol. 27, no. 1, pp 14-21, 1999; in WO 00/37502 and WO 94/10202; Angiostatin™, described by M. S. O'Reilly et al, Cell 79, 1994, 315-328; Endostatin™, described by M. S. O'Reilly et al, Cell 88, 1997, 277-285; anthranilic acid amides; ZD4190; ZD6474; SU5416; SU6668; or anti-VEGF antibodies or anti-VEGF receptor antibodies,e.g. RhuMab.

By antibody is meant intact monoclonal antibodies, polyclonal antibodies, multispecific antibodies formed from at least 2 intact antibodies, and antibodies fragments so long as they exhibit the desired biological activity.

Compounds which target, decrease or inhibit the activity of the epidermal growth factor receptor family are especially compounds, proteins or antibodies which inhibit members of the EGF receptor tyrosine kinase family, e.g. EGF receptor, ErbB2, ErbB3 and ErbB4 or bind to EGF or EGF related ligands, and are in particular those compounds, proteins or monoclonal antibodies generically and specifically disclosed in WO 97/02266, e.g. the compound of ex. 39, or in EP 0 564 409, WO 99/03854, EP 0520722, EP 0 566 226, EP 0 787 722, EP 0 837 063, US 5,747,498, WO 98/10767, WO 97/30034, WO 97/49688, WO 97/38983 and, especially, WO 96/30347 (e.g. compound known as CP 358774), WO 96/33980 (e.g. compound ZD 1839) and WO 95/03283 (e.g. compound ZM105180); e.g. trastuzumab (Herpetin®), cetuximab, Iressa, OSI-774, CI-1033, EKB-569, GW-2016, E1.1, E2.4, E2.5, E6.2, E6.4, E2.11, E6.3 or E7.6.3.

Compounds which target, decrease or inhibit the activity of PDGFR are especially compounds which inhibit the PDGF receptor, e.g. a N-phenyl-2-pyrimidine-amine derivative, e.g. imatinib.

Compounds which target, decrease or inhibit the activity of c-Abl family members and their gene fusion products, e.g. a N-phenyl-2-pyrimidine-amine derivative, e.g. imatinib; PD180970; AG957; or NSC 680410.

Compounds which target, decrease or inhibit the activity of protein kinase C, Raf, MEK, SRC, JAK, FAK and PDK family members, or PI(3) kinase or PI(3) kinase-related family members, and/or members of the cyclin-dependent kinase family (CDK) are especially those staurosporine derivatives disclosed in EP 0 296 110, e.g. midostaurin; examples of further compounds include e.g. UCN-01, safingol, BAY 43-9006, Bryostatin 1, Perifosine; Ilmofosine; RO 318220 and RO 320432; GO 6976; Isis 3521; or LY333531/LY379196.

Further anti-angiogenic compounds are e.g. thalidomide (THALOMID) and TNP-470.

Compounds which target, decrease or inhibit the activity of a protein or lipid phosphatase are e.g. inhibitors of phosphatase 1, phosphatase 2A, PTEN or CDC25, e.g. okadaic acid or a derivative thereof.

Compounds which induce cell differentiation processes are e.g. retinoic acid, α–, γ– or δ-tocopherol or α–, γ– or δ-tocotrienol.

The term cyclooxygenase inhibitor as used herein includes, but is not limited to, e.g. celecoxib (Celebrex^{R}), rofecoxib (Vioxx^{R}), etoricoxib, valdecoxib or a 5-alkyl-2-arylaminophenylacetic acid, e.g. 5-methyl-2-(2'-chloro-6'-fluoroanilino)phenyl acetic acid.

The term "histone deacetylase inhibitor" as used herein includes, but is not limited to MS-27-275, SAHA, pyroxamide, FR-901228 or valproic acid.

The term "bisphosphonates" as used herein includes, but is not limited to, etridonic, clodronic, tiludronic, pamidronic, alendronic, ibandronic, risedronic and zoledronic acid. "Etridonic acid" can be administered, e.g., in the form as it is marketed, e.g. under the trademark DIDRONEL™. "Clodronic acid" can be administered, e.g., in the form as it is marketed, e.g. under the trademark BONEFOS™. "Tiludronic acid" can be administered, e.g., in the form as it is marketed, e.g. under the trademark SKELID™. "Pamidronic acid" can be administered, e.g. in the form as it is marketed, e.g. under the trademark AREDIA™. "Alendronic acid" can be administered, e.g., in the form as it is marketed, e.g. under the

trademark FOSAMAX™. "Ibandronic acid" can be administered, e.g., in the form as it is marketed, e.g. under the trademark BONDRANAT™. "Risedronic acid" can be administered, e.g., in the form as it is marketed, e.g. under the trademark ACTONEL™. "Zoledronic acid" can be administered, e.g. in the form as it is marketed, e.g. under the trademark ZOMETA™

The term "matrix metalloproteinase inhibitor" as used herein includes, but is not limited to collagen peptidomimetic and nonpetidomimetic inhibitors, tetracycline derivatives, e.g. hydroxamate peptidomimetic inhibitor batimastat and its orally bioavailable analogue marimastat, prinomastat, BMS-279251, BAY 12-9566, TAA211 or AAJ996.

In each case where citations of patent applications or scientific publications are given, the subject-matter relating to the compounds is hereby incorporated into the present application by reference. Comprised are likewise the pharmaceutically acceptable salts thereof, the corresponding racemates, diastereoisomers, enantiomers, tautomers as well as the corresponding crystal modifications of above disclosed compounds where present, e.g. solvates, hydrates and polymorphs, which are disclosed therein. The compounds used as active ingredients in the combinations of the invention can be prepared and administered as described in the cited documents, respectively. Also within the scope of this invention is the combination of more than two separate active ingredients as set forth above, i.e. a pharmaceutical combination within the scope of this invention could include three active ingredients or more. Further both the first agent and the co-agent are not the identical ingredient.

Utility of the compounds of formula I in treating solid tumors as hereinabove specified, may be demonstrated in animal test methods as well as in clinic, for example in accordance with the methods hereinafter described.

A. In Vitro

A.1 Antiproliferative activity in combination with other agents

A cell line, e.g. the compound A resistant A549 line (IC_{50} in low nM range) versus the comparative Compound A resistant KB-31 and HCT116 lines (IC_{50} in the μM range), is added to 96-well plates (1,500 cells/well in 100 μl medium) and incubated for 24 hr. Subsequently, a two-fold dilution series of each compound (Compound of formula I or a known chemotherapeutic agent) is made in separate tubes (starting at 8 x the IC_{50} of each compound) either alone or in paired combinations, and the dilutions are added to the wells. The cells are then re-incubated for 3 days. Methylene blue staining is performed on day 4 and the amount of bound dye (proportional to the number of surviving cells that bind the dye)

determined. IC_{50}s are subsequently determined using the Calcusyn program, which provides a measure of the interaction, namely the so-called non-exclusive combination index (CI), where: CI ~ 1 = the interaction is nearly additive; 0.85 – 0.9 = slight synergism; < 0.85 = synergy. In this assay, the compounds of formula I show interesting antiproliferative activity in combination with another chemotherapeutic agent. For example the following CI values are obtained with a combination of Compound A and cisplatinum, paclitaxel, gemcitabine and doxorubicin, showing synergistic effects.

	CI			
Cell line	Cisplatinum	Paclitaxel	Gemcitabine	Doxorubicin
KB-31	0.74	0.9	0.79	0.7
A549	0.47	0.74	0.76	0.64
HCT116	0.47	0.3	0.9	0.52

Furthermore, in this assay, Compound A potentiates the loss of A549 cell viability and cell death when it is used in combination with gemcitabine.

A.2 Antiangiogenic activity

In vitro assay of the antiproliferative activity of rapamycin or a derivative thereof, e.g. Compound A, against human umbilical vein endothelial cells (HUVECs) demonstrates IC_{50} values of 120 ± 22 pM and 841 ± 396, and > 10 000 pM for VEGF- and bFGF- and FBS-stimulated proliferation, respectively. Additionally, no significant effects of Compound A on bFGF-stimulated normal human dermal fibroblast (NHDF) proliferation are observed over the same concentration range. These results indicate that Compound A inhibits the proliferation of HUVECs, being particularly potent against the VEGF-induced proliferation, VEGF being a key pro-angiogenic factor.

B. In Vivo

In the following assays, antitumor activity is expressed as T/C% (mean increase in tumor volumes of treated animals divided by the mean increase of tumor volumes of control animals multiplied by 100) and % regressions (tumor volume minus initial tumor volume divided by the initial tumor volume and multiplied by 100).

B.1 Activity in A549 human lung tumor xenografts

Fragments of A549 tumors (approx. 25 mg; derived from Cell line CCL 185, ATCC, Rockville MD, USA) are transplanted subcutaneously into the left flank of BALB/c nude mice. Treatment is started on day 7 or day 12 following tumor transplantation. The compound to be tested is administered p.o. once per day from day 7/12 to day 38/55, respectively. In this assay, when administered at a daily dose ranging from 0.1 mg/kg to 2.5 mg/kg, the compounds of formula I exhibit dose-dependent inhibition of tumor growth; for example in one representative experiment Compound A when administered at a dose of 2.5 mg/kg results in persisting regressions (41 %); a dose of 0.5 mg/kg results in transient regressions (38 % on day 17), with a final T/C of 16 %, and a dose of 0.1 mg/kg slows tumor growth resulting in a final T/C of 43 % (T/C for control animals is 100%).

B.2 Activity in KB-31 human epidermoid tumor xenografts

Fragments of KB-31 tumors (approx. 25 mg; derived from the cell lines obtained from Roswell Park Memorial Institute Buffalo, NY, USA) are transplanted subcutaneously into the left flank of BALB/c nude mice. Treatment is started on day 7 or on day 10 following tumor transplantation. The compound to be tested is administered p.o. once per day from day 7/10 to day 25/35, respectively. Antitumor activity is expressed as T/C% as indicated above. In this assay, when administered at a daily dose ranging from 0.5 mg/kg to 2.5 mg/kg, the compounds of formula I inhibit tumor growth; for example in one representative experiment Compound A when administered at a dose of 2,5 mg/kg/day results in a final T/C cvalue of 25%(T/C for control animals is 100%).

B.3 Activity in CA20948 rat pancreatic tumors

Tumors are established in male Lewis rats by subcutaneous injection of CA20948 tumor cell suspension derived from donor rats into the left flank. Treatment is started on day 4 post inoculation. The compound to be tested is administered p.o. once per day (6 days a week) from day 4 to day 9-15 post inoculation. Antitumor activity is expressed as T/C% as indicated above. In this assay, when administered at a daily dose of 0.5 mg/kg to 2.5 mg/kg, the compounds of formula I inhibit tumor growth; for example in a representative experiment Compound A when administered p.o. at a daily dose of 2.5 mg/kg results in a final T/C value of 23 %. In the same experiment, intermittent administration of Compound A, 5mg/kg twice per week, results in a final T/C value of 32%. Compound A significantly and consistently decreases in these assays the rate of CA20948 pancreatic tumor growth when compared to vehicle controls (T/C for control animals is defined as 100%).

Compounds of formula I, e.g. Compound A, have been tested in further tumor models in accordance with the procedure as disclosed above. For example, a daily dosage of 2.5 mg/kg or 5 mg/kg Compound A produces final T/Cs of 18% and 9% when administered to the human NCI H-596 lung tumor model and the human MEXF 989 melanoma tumor model, respectively; 5 mg/kg produces final T/Cs of 20% (primary tumor) and 36% (cervical lymph node metastases) when administered to the orthotopic mouse B16/BL6 melanoma tumor model and 24% when administered to the human AR42J pancreatic tumor model; 2.5 mg/kg produces a final T/C of 28% when administered to the multi-drug resistant (MDR) human KB-8511 epidermoid tumor model. Good antitumor responses are also obtained when compounds of formula I, e.g. Compound A, are administered intermittently, e.g. 2 subsequent days per week or twice a week, to mice transplanted with human AR42J pancreatic tumors.

B.4 Combination with doxorubicin

Mice transplanted with human KB-31 epidermoid tumors are treated for 21 days with doxorubicin at a dose of 5 mg/kg i.v. once per week, a compound of formula I, e.g. Compound A, at a dose of 2.5 mg/kg p.o once per day, or a combination of both. Thereafter compound of formula I treatment alone is continued in the combination group in order to determine if the compound of formula I can suppress the outgrowth of tumors that respond to conventional agents. Antitumor activity is expressed as T/C% or % regressions as indicated above. For example, the combination of Compound A and doxorubicin produces greater antitumor effect (74 % regressions) as compared to either agent alone (Compound A, T/C 32 %; doxorubicin 44 % regressions). No exacerbation of the body weight losses caused by doxorubicin occurrs when Compound A treatment is added. Continuing Compound A treatment in the combination group, after ceasing doxorubicin, inhibits tumor outgrowth such that the tumor volumes of the doxorubicin monotherapy group are significantly larger than those of the combination group. Morever the combination appears to produce a greater cure rate (8/8 tumors) at 14 days post end of treatment than doxorubicin alone (3/8 tumors).

B.5 Combination with cisplatinum

Mice transplanted with human NCI H-596 lung tumors are treated for 21 days with cisplatinum at a dose of 2.5 mg/kg i.v. once per week, a compound of formula I, e.g. Compound A, at a dose of 2.5 mg/kg p.o. once per day, or a combination of both. Antitumor activity is expressed as T/C% or % regressions as indicated above. A combination of Compound A and cisplatinum produces a greater antitumor effect (5% regressions) as

compared to either agent alone (Compound A, T/C 26%; cisplatinum, T/C 26%). The combination did not lead to worsened tolerability.

B.6 Antiangiogenic activity

B16/BL6 cells (5 $X10^4$) are injected intradermally into the ear of C57BL/6 mice. Seven days later treatment with rapamycin or a derivative thereof e.g. Compound A, or vehicle is initiated. Primary tumor and cervical lymph nodes are collected after two weeks of daily treatment for measurement of vessel density. Endothelium of perfused vessels in the tumors is visualized using a nuclear staining dye (Hoechst 33342, 20 mg/kg) that is injected i.v. shortly before killing the mice. Tumors and metastases are snap frozen and sections examined under a light microscope equipped with an epifluorescent source. The fluorescence H33342-labelled endothelium cells is used to measure vessel number and size over the whole tumor section. Vessels are assigned to groups of 10 µm-size range. Distribution of vessel size is assessed using a histogram frequency analysis. At a dose of 5 mg/kg p.o., rapamycin or a derivative thereof reduces vessel density in both the primary tumor (e.g. T/C 50 % for Compound A) and the metastases (e.g.T/C 40 % for Compound A) as compared to controls. Rapamycin or a derivative thereof, e.g. Compound A, also changes vessel size distribution in the metastases.

B.7 Combination with an antiangiogenic agent

B16/BL6 cells (5 $X10^4$) are injected intradermally into the ear of C57BL/6 mice. Seven days later treatment with rapamycin or a derivative thereof, e.g. Compound A, a VEGF receptor tyrosine kinase inhibitor, e.g. 1-(4-chloroanilino)-4-(4-pyridylmethyl)phthalazine or a salt thereof, e.g. the succinate, or a combination of both is initiated and effects on the growth and weight of the primary tumor and cervical lymph node metastases are monitored, respectively. Daily administration of the antiangiogenic agent (100 mg/kg p.o.) or of rapamycin or a derivative thereof, e.g. Compound A, (1 mg/kg p.o.) alone, reduces the size of the primary tumor (final T/C: 65 % and 74 %, respectively), whereas the combination of these two agents is synergistic (T/C 12 %). Rapamycin or a derivative thereof, e.g. Compound A and the antiangiogenic agent treatment alone reduces cervical lymph node weights (related to regional metastases) (T/C: 75 % and 34 %, respectively), and the combination further reduces lymph node weights (T/C 13 %). The treatments significantly promote body weight gains as compared to controls. For the primary tumors, analysis of possible interaction shows synergy with Compound A and antiangiogenic agent as antiangiogenic agent /controls = 0.66; Compound A/controls = 0.77; Compound A and antiangiogenic agent /controls = 0.135. As Compound A and antiangiogenic agent /controls

< Compound A/controls x antiangiogenic agent /controls (0.51), this is defined as synergy. For the metastases, analysis also shows synergy with Compound A and the antiangiogenic agent as antiangiogenic agent /controls = 0.337; Compound A/controls = 0.75; Compound A and antiangiogenic agent /controls = 0.122. As Compound A and antiangiogenic agent /controls < Compound A/controls x antiangiogenic agent /controls (0.252), this is also defined as synergy (Clark, Breast Cancer Research Treatment 1997;46:255).

C. Clinical Trial

C.1 Investigation of clinical benefit of a compound of formula I, e.g. Compound A as monotherapy in solid tumours

Aim of the study: To identify the optimal dose of said compound, given orally once weekly, in a dose escalating study and the efficacy of the optimal dosage in solid tumours.

The study is divided into 2 parts:

Part 1:

Primary Aim: Identify the optimal dose of a compound of formula I, e.g. Compound A, given p.o. once weekly, assuming this should be the minimum dose associated with prolonged inhibition of mTOR and blood levels of said compound at least equivalent to those achieving an anti-tumor effect in in-vivo preclinical levels.

Secondary Aim: Assess safety of said compound when given alone to cancer patients and assess changes in tumor metabolic activity.

Design: Successive groups of 4 patients with advanced malignant solid tumors, refractory or resistant to standard therapies to receive a compound of formula I, e.g. Compound A, every 7 days different doses (group 1 to receive 5 mg; group 2 to receive 10 mg, group 3 to receive 20 mg) for 4 weeks. In week 4, establish the pharmacokinetic profile and the profile of mTOR inhibition as reflected by the inhibition of p70s6 kinase in peripheral lymphocytes. Carry out comparative 18-fluorodeoxyglucose (FDG) positron-emission tomography (FDG-PET) imaging (before 1st dose, after 3rd dose) to explore the change in tumor metabolism.

Patients main selection criteria: Adults with advanced-stage (III-V) solid tumors, resistant or refractory to standard therapies. At least one tumoral lesion should be measurable (>20 mm in one dimension).

Main variables for evaluation: Safety (adverse events), standard serum biochemistry and haematology, blood levels of the compound to be tested, lymphocyte p70-s6kinase activity, changes in tumor glucose uptake by FDG-PET.

Part 2:

Primary Aim: Explore the efficacy of a compound of formula I, e.g. Compound A, in patients with advanced solid tumors when given once a week at the optimal dosage, as identified in Part 1 as shown by tumor response.

Secondary Aim: Assess the safety of said compound at this dosage.

Design: 20 patients with progressing, advanced-stage solid tumors, resistant or refractory to standard therapies, to receive said compound at the dosage recommended as a result of Part 1. The general clinical state of the patient is investigated weekly by physical and laboratory examination. Changes in tumor burden are assessed every 2 months by radiological examination. Initially patients receive treatment for 2 months. Thereafter, they remain on treatment for as long as their disease does not progress and the drug is satisfactorily tolerated.

Main variables for evaluation: Safety (adverse events), standard serum biochemistry and haematology, tumor dimensions by computerised tomographic (CT) scan or magnetic resonance imaging (MRI).

C.2 Combined Treatment

Suitable clinical studies are, for example, open label non-randomized, dose escalation studies in patients with advanced solid tumors. Such studies prove in particular the synergism of the active ingredients of the combination of the invention. The beneficial effects on proliferative diseases can be determined directly through the results of these studies or by changes in the study design which are known as such to a person skilled in the art. Such studies are, in particular, suitable to compare the effects of a monotherapy using the active ingredients and a combination of the invention. Preferably, the dose of agent (a) is escalated until the Maximum Tolerated Dosage is reached, and the co-agent (b) is administered with a fixed dose. Alternatively, the agent (a) is administered in a fixed dose and the dose of co-agent (b) is escalated. Each patient receives doses of the agent (a) either daily or intermittent. The efficacy of the treatment can be determined in such studies, e.g., after 12, 18 or 24 weeks by radiologic evaluation of the tumors every 6 weeks.

Alternatively, a placebo-controlled, double blind study can be used in order to prove the benefits of the combination of the invention mentioned herein.

Daily dosages required in practicing the method of the present invention when a compound of formula I alone is used will vary depending upon, for example, the compound used, the host, the mode of administration and the severity of the condition to be treated. A preferred daily dosage range is about from 0.1 to 25 mg as a single dose or in divided doses. Suitable

daily dosages for patients are on the order of from e.g. 0.1 to 25 mg p.o. Compound A may be administered by any conventional route, in particular enterally, e.g. orally, e.g. in the form of tablets, capsules, drink solutions, nasally, pulmonary (by inhalation) or parenterally, e.g. in the form of injectable solutions or suspensions. Suitable unit dosage forms for oral administration comprise from ca. 0.05 to 12.5 mg, usually 0.25 to 10 mg Compound A, together with one or more pharmaceutically acceptable diluents or carriers therefor.

The combination of the invention can also be applied in combination with surgical intervention, mild prolonged whole body hyperthermia and/or irradiation therapy.

The administration of a pharmaceutical combination of the invention results not only in a beneficial effect, e.g. a synergistic therapeutic effect, e.g. with regard to slowing down, arresting or reversing the neoplasm formation or a longer duration of tumor response, but also in further surprising beneficial effects, e.g. less side-effects, an improved quality of life or a decreased mortality and morbidity, compared to a monotherapy applying only one of the pharmaceutically active ingredients used in the combination of the invention, in particular in the treatment of a tumor that is refractory to other chemotherapeutics known as anti-cancer agents. In particular, an increased up-take of the co-agent (b) in tumor tissue and tumor cells is observed, when applied in combination with the first agent (a).
A further benefit is that lower doses of the active ingredients of the combination of the invention can be used, for example, that the dosages need not only often be smaller but are also applied less frequently, or can be used in order to diminish the incidence of side-effects, while controlling the growth of neoplasm formation. This is in accordance with the desires and requirements of the patients to be treated.

According to one embodiment of the invention, a preferred pharmaceutical combination comprises
a) a compound of formula I, e.g. Compound A, and
b) as co-agent, one or more compounds as indicated in paragraphs (ii), (iii) or (iv) above, e.g. carboplatin, cisplatinum, paclitaxel, docetaxel, gemcitabine or doxorubicin.
A synergistic combination of a compound of formula I, e.g. Compound A, with carboplatin, cisplatinum, paclitaxel, docetaxel, gemcitabine or doxorubicin is particularly preferred.

A further preferred pharmaceutical combination is e.g. a combination comprising
a) rapamycin or a derivative thereof, e.g. CCI-779, ABT578 or Compound A, and
b) as co-agent, one or more compounds as indicated under paragraphs (i) and (v) to (x) above, preferably one or more compounds as specified in paragraph (v) above.

Preferred is e.g. a synergistic combination of rapamycin or a derivative thereof, e.g. CCI-779, ABT578 or Compound A, with a compound which target, decrease or inhibit the activity of VEGFR, EGFR family, PDGFR, c-ABl family members or protein kinase C, e.g. as disclosed above.

One specific embodiment of the invention relates to the use of a combination of the invention for the prevention, delay of progression or treatment of or for the preparation of a medicament for the prevention, delay of progression or treatment of breast cancer. Preferably, in such embodiment the combination comprises as co-agent b) an aromatase inhibitor, e.g. the aromatase inhibitor letrozole, an anti-estrogen, e.g. tamoxifen, a topoisomerase II inhibitor, e.g. doxorubicin, or a microtubule active agent, e.g. paclitaxel.

Another embodiment of the invention relates to the use of a combination of the invention for the prevention, delay of progression or treatment of or for the preparation of a medicament for the prevention, delay of progression or treatment of lung cancer. Preferably, in such embodiment the combination of the invention comprises as co-agent b) a platin compound, e.g. carboplatin, or a microtubule active agent, e.g. paclitaxel.

Another embodiment of the invention relates to the use of a combination of the invention for the prevention, delay of progression or treatment of or for the preparation of a medicament for the prevention, delay of progression or treatment of pancreatic cancer. Preferably, in such embodiment the combination of the invention comprises as co-agent b) an antineoplastic antimetabolite, e.g. gemcitabine.

Another embodiment of the invention relates to the use of a combination of the invention for the prevention, delay of progression or treatment of or for the preparation of a medicament for the prevention, delay of progression or treatment of glioblastomas. Preferably, in such embodiment the combination of the invention comprises as co-agent b) an alkylating agent, e.g. BCNU.

A further embodiment of the invention relates to the use of rapamycin or a derivative thereof in combination with a chemotherapeutic agent in the treatment of a lymphatic cancer, e.g. as disclosed above. The combination may additionally comprise as co-agent b) busulfan, cytarabine, 6-thioguanine, fludarabine, hydroxyurea, procarbazine, bleomycin or methotrexate. Topoisomerase II inhibitors e,g. daunorubicin or, particularly, compounds which target, decrease or inhibit the activity of PDGFR or of c-Abl family members and their gene fusion products, e.g. imatinib are preferred as co-agent (b).

The terms "co-administration" or "combined administration" or the like as utilized herein are meant to encompass administration of the selected therapeutic agents to a single patient, and are intended to include treatment regimens in which the agents are not necessarily administered by the same route of administration or at the same time.

It is one objective of this invention to provide a pharmaceutical composition comprising a quantity, which is jointly therapeutically effective against a proliferative malignant disease comprising a combination of the invention. In this composition, the first agent a) and co-agent (b) can be administered together, one after the other or separately in one combined unit dosage form or in two separate unit dosage forms. The unit dosage form may also be a fixed combination.

The pharmaceutical compositions for separate administration of the first agent a) and co-agent b) and for the administration in a fixed combination, i.e. a single galenical composition comprising at least two combination partners a) and b), according to the invention can be prepared in a manner known per se and are those suitable for enteral, such as oral or rectal, and parenteral administration to mammals (warm-blooded animals), including humans, comprising a therapeutically effective amount of at least one pharmacologically active combination partner alone, e.g. as indicated above, or in combination with one or more pharmaceutically acceptable carriers or diluents, especially suitable for enteral or parenteral application.

Suitable pharmaceutical compositions contain, for example, from about 0.1 % to about 99.9%, preferably from about 1 % to about 60 %, of the active ingredient(s). Pharmaceutical preparations for the combination therapy for enteral or parenteral administration are, for example, those in unit dosage forms, such as sugar-coated tablets, tablets, capsules or suppositories, or ampoules. If not indicated otherwise, these are prepared in a manner known per se, for example by means of conventional mixing, granulating, sugar-coating, dissolving or lyophilizing processes. It will be appreciated that the unit content of a combination partner contained in an individual dose of each dosage form need not in itself constitute an effective amount since the necessary effective amount can be reached by administration of a plurality of dosage units.

In particular, a therapeutically effective amount of each of the combination partner of the combination of the invention may be administered simultaneously or sequentially and in any order, and the components may be administered separately or as a fixed combination. For example, the method of delay of progression or treatment of a proliferative malignant

disease according to the invention may comprise (i) administration of the first agent a) in free or pharmaceutically acceptable salt form and (ii) administration of a co-agent b) in free or pharmaceutically acceptable salt form, simultaneously or sequentially in any order, in jointly therapeutically effective amounts, preferably in synergistically effective amounts, e.g. in daily or intermittently dosages corresponding to the amounts described herein. The individual combination partners of the combination of the invention may be administered separately at different times during the course of therapy or concurrently in divided or single combination forms. Furthermore, the term administering also encompasses the use of a pro-drug of a combination partner that convert *in vivo* to the combination partner as such. The instant invention is therefore to be understood as embracing all such regimens of simultaneous or alternating treatment and the term "administering" is to be interpreted accordingly.

The effective dosage of each of the combination partners employed in the combination of the invention may vary depending on the particular compound or pharmaceutical composition employed, the mode of administration, the condition being treated, the severity of the condition being treated. Thus, the dosage regimen of the combination of the invention is selected in accordance with a variety of factors including the route of administration and the renal and hepatic function of the patient. A physician, clinician or veterinarian of ordinary skill can readily determine and prescribe the effective amount of the single active ingredients required to prevent, counter or arrest the progress of the condition. Optimal precision in achieving concentration of the active ingredients within the range that yields efficacy without toxicity requires a regimen based on the kinetics of the active ingredients' availability to target sites.

Daily dosages for the first agent a) will, of course, vary depending on a variety of factors, for example the compound chosen, the particular condition to be treated and the desired effect. In general, however, satisfactory results are achieved on administration of rapamycin or a derivative thereof at daily dosage rates of the order of ca. 0.1 to 25 mg as a single dose or in divided doses. Rapamycin or a derivative thereof, e.g. a compound of formula I, may be administered by any conventional route, in particular enterally, e.g. orally, e.g. in the form of tablets, capsules, drink solutions or parenterally, e.g. in the form of injectable solutions or suspensions. Suitable unit dosage forms for oral administration comprise from ca. 0.05 to 10 mg active ingredient, e.g. Compound A, together with one or more pharmaceutically acceptable diluents or carriers therefor.

Fadrozole may be administered orally to a human in a dosage range varying from about 0.5 to about 10 mg/day, preferably from about 1 to about 2.5 mg/day. Exemestane may be administered orally to a human in a dosage range varying from about 5 to about 200 mg/day, preferably from about 10 to about 25 mg/day, or parenterally from about 50 to 500 mg/day, preferably from about 100 to about 250 mg/day. If the drug shall be administered in a separate pharmaceutical composition, it can be administered in the form disclosed in GB 2,177,700. Formestane may be administered parenterally to a human in a dosage range varying from about 100 to 500 mg/day, preferably from about 250 to about 300 mg/day. Anastrozole may be administered orallly to a human in a dosage range varying from about 0.25 to 20 mg/day, preferably from about 0.5 to about 2.5 mg/day. Aminogluthemide may be administered to a human in a dosage range varying from about 200 to 500 mg/day.

Tamoxifen citrate may be administered to a human in a dosage range varying from about 10 to 40 mg/day.

Vinblastine may be administered to a human in a dosage range varying from about 1.5 to 10 mg/m^{2}day. Vincristine sulfate may be administered parenterally to a human in a dosage range varying from about 0.025 to 0.05 mg/kg body weight • week. Vinorelbine may be administered to a human in a dosage range varying from about 10 to 50 mg/m^{2}day.

Etoposide phosphate may be administered to a human in a dosage range varying from about 25 to 115 mg/m^{2}day, e.g. 56.8 or 113.6 mg/m^{2}day.

Teniposide may be administered to a human in a dosage range varying from about 75 to 150 mg about every two weeks. Doxorubicin may be administered to a human in a dosage range varying from about 10 to 100 mg/m^{2}day, e.g. 25 or 50 mg/m^{2}day. Epirubicin may be administered to a human in a dosage range varying from about 10 to 200 mg/m^{2}day. Idarubicin may be administered to a human in a dosage range varying from about 0.5 to 50 mg/m^{2}day. Mitoxantrone may be administered to a human in a dosage range varying from about 2.5 to 25 mg/m^{2}day.

Paclitaxel may be administered to a human in a dosage range varying from about 50 to 300 mg/m^{2}day. Docetaxel may be administered to a human in a dosage range varying from about 25 to 100 mg/m^{2}day.

Cyclophosphamide may be administered to a human in a dosage range varying from about 50 to 1500 mg/m^{2}day. Melphalan may be administered to a human in a dosage range varying from about 0.5 to 10 mg/m^{2}day.

5-Fluorouracil may be administered to a human in a dosage range varying from about 50 to 1000 mg/m^{2}day, e.g. 500 mg/m^{2}day. Capecitabine may be administered to a human in a dosage range varying from about 10 to 1000 mg/m^{2}day. Gemcitabine hydrochloride may be administered to a human in a dosage range varying from about 1000 mg/m^2/week. Methotrexate may be administered to a human in a dosage range varying from about 5 to 500 mg/m^{2}day.

Topotecan may be administered to a human in a dosage range varying from about 1 to 5 mg/m^{2}day. Irinotecan may be administered to a human in a dosage range varying from about 50 to 350 mg/m^{2}day.

Carboplatin may be administered to a human in a dosage range varying from about 200 to 400 mg/m^2 about every four weeks. Cisplatin may be administered to a human in a dosage range varying from about 25 to 75 mg/m^2 about every three weeks. Oxaliplatin may be administered to a human in a dosage range varying from about 50 to 85 mg/m^2 every two weeks.

Imatinib may be administered to a human in a dosage in the range of about 2.5 to 850 mg/day, more preferably 5 to 600 mg/day and most preferably 20 to 300 mg/day.

Alendronic acid may be administered to a human in a dosage range varying from about 5 to 10 mg/day. Clodronic acid may be administered to a human e.g. in a dosage range varying from about 750 to 1500 mg/day. Etridonic acid may be administered to a human in a dosage range varying from about 200 to 400 mg/day. Ibandronic acid may be administered to a human in a dosage range varying from about 1 to 4 mg every three to four weeks. Risedronic acid may be administered to a human in a dosage range varying from about 20 to 30 mg/day. Pamidronic acid may be administered to a human in a dosage range varying from about 15 to 90 mg every three to four weeks. Tiludronic acid may be administered to a human in a dosage range varying from about 200 to 400 mg/day.

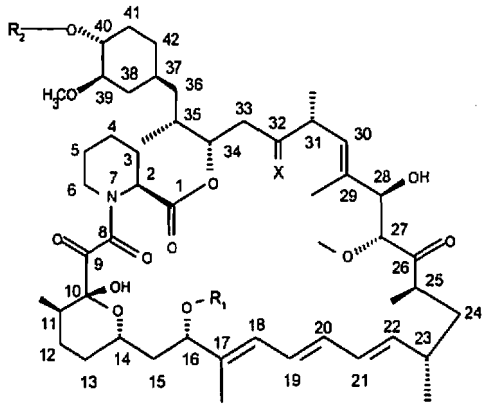
Trastuzumab may be administered to a human in a dosage range varying from about 1 to 4 mg/m^2/week.

Bicalutamide may be administered to a human in a dosage range varying from about 25 to 50 mg/m^{2}day.

1-(4-chloroanilino)-4-(4-pyridylmethyl)phthalazine or salt thereof, e.g. succinate, may be administered to a human in a dosage range of about 50 to 1500, more preferably about 100 to 750, and most preferably 250 to 500, mg/day.

IN THE CLAIMS:

Claim 1. A method for inhibiting growth of solid tumors of the brain in a subject, said method comprising administering to said subject a therapeutically effective amount of a compound of formula I



I

wherein

R_1 is CH_3,

R_2 is $-CH_2-CH_2-OH$, and

X is =O.

Claim 2. The method of claim 1 wherein the solid tumor of the brain is a carcinoma.

Claim 2. The method of claim 1 wherein the compound of formula I is administered orally.

Claim 3. The method of claim 1 wherein the compound of formula I is administered at a daily dose range of from about 0.1 to 25 mg, as a single dose or in divided doses.

Claim 4. The method of claim 1 wherein the compound of formula I is administered in a unit dosage form of from about 0.05 to 12.5 mg.

Claim 5. The method of claim 1 wherein the compound of formula I is administered in a unit dosage form of from about 0.25 to 10 mg.

Claim 6. The method of claim 1 wherein the compound of formula I is administered in a unit dosage form of 10 mg.

Abstract

Rapamycin derivatives have interesting effects in the treatment of solid tumours, optionally in combination with a chemotherapeutic agent.

Electronic Patent Application Fee Transmittal

Application Number:	
Filing Date:	
Title of Invention:	TREATMENT OF SOLID TUMORS WITH RAPAMYCIN DERIVATIVES
First Named Inventor/Applicant Name:	Heidi Lane
Filer:	Ann R. Pokalsky/Maggi Leone
Attorney Docket Number:	PAT031671-US-CNT03 (62C3)

Filed as Large Entity

Utility under 35 USC 111(a) Filing Fees

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Utility application filing	1011	1	380	380
Utility Search Fee	1111	1	620	620
Utility Examination Fee	1311	1	250	250
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				
Miscellaneous:				
		Total in USD ($)		**1250**

Electronic Acknowledgement Receipt

EFS ID:	13228116
Application Number:	13546686
International Application Number:	
Confirmation Number:	8586
Title of Invention:	TREATMENT OF SOLID TUMORS WITH RAPAMYCIN DERIVATIVES
First Named Inventor/Applicant Name:	Heidi Lane
Customer Number:	28249
Filer:	Ann R. Pokalsky/Maggi Leone
Filer Authorized By:	Ann R. Pokalsky
Attorney Docket Number:	PAT031671-US-CNT03 (62C3)
Receipt Date:	11-JUL-2012
Filing Date:	
Time Stamp:	16:49:31
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	Credit Card
Payment was successfully received in RAM	$1250
RAM confirmation Number	4048
Deposit Account	041121
Authorized User	HARRISON,HELENE

The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:

Charge any Additional Fees required under 37 C.F.R. Section 1.16 (National application filing, search, and examination fees)

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File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Application Data Sheet	Appln_Data_Form.pdf	1680302 b14dd727f439c487167de27bd63ef69736252b02	no	5
Warnings:					
Information:					
2	Oath or Declaration filed	Declaration.pdf	164519 6bb873be06a0a7e1f2af90079d3816c7642685b1	no	4
Warnings:					
Information:					
3	Transmittal Letter	Information_Disclosure_Statement.pdf	79239 4ef064726ee2875c158665c8bbda9172a11ac7a7	no	2
Warnings:					
Information:					
4	Information Disclosure Statement (IDS) Form (SB08)	US_IDS_Form_SB_08a.pdf	1281037 d572f8b2b95aceb63e312f729eb601bcbeba3a60	no	8
Warnings:					
Information:					
5		Specification.pdf	1688703 b25d706b4f54da63e8aed711ad8cc892fe058e58	yes	26

Multipart Description/PDF files in .zip description

Document Description	Start	End
Specification	1	23
Claims	24	25
Abstract	26	26

Warnings:					
Information:					
6	Fee Worksheet (SB06)	fee-info.pdf	33008 7ee225fdc9e991a5ced5e775f67d8b304e76731e	no	2
Warnings:					
Information:					
			Total Files Size (in bytes):	4926808	

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New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	031671-US-CNT03 167-62 C3
	Application Number	
Title of Invention	TREATMENT OF SOLID TUMORS WITH RAPAMYCIN DERIVATIVES	

The application data sheet is part of the provisional or nonprovisional application for which it is being submitted. The following form contains the bibliographic data arranged in a format specified by the United States Patent and Trademark Office as outlined in 37 CFR 1.76.
This document may be completed electronically and submitted to the Office in electronic format using the Electronic Filing System (EFS) or the document may be printed and included in a paper filed application.

Secrecy Order 37 CFR 5.2

☐ Portions or all of the application associated with this Application Data Sheet may fall under a Secrecy Order pursuant to 37 CFR 5.2 (Paper filers only. Applications that fall under Secrecy Order may not be filed electronically.)

Applicant Information:

Applicant 1 Remove

Applicant Authority ◉ Inventor ○ Legal Representative under 35 U.S.C. 117 ○ Party of Interest under 35 U.S.C. 118

Prefix	Given Name	Middle Name	Family Name	Suffix
	Heidi		Lane	

Residence Information (Select One) ○ US Residency ◉ Non US Residency ○ Active US Military Service

City	Basel	Country Of Residence[i]	CH
Citizenship under 37 CFR 1.41(b)[i]	CH		

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Address 2			
City	Basel	State/Province	
Postal Code	4052	Country[i]	CH

Applicant 2 Remove

Applicant Authority ◉ Inventor ○ Legal Representative under 35 U.S.C. 117 ○ Party of Interest under 35 U.S.C. 118

Prefix	Given Name	Middle Name	Family Name	Suffix
	Terence		O'Reilly	

Residence Information (Select One) ○ US Residency ◉ Non US Residency ○ Active US Military Service

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Citizenship under 37 CFR 1.41(b)[i]	CH		

Mailing Address of Applicant:

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Address 2			
City	Basel	State/Province	CH
Postal Code	4057	Country[i]	CH

Applicant 3 Remove

Applicant Authority ◉ Inventor ○ Legal Representative under 35 U.S.C. 117 ○ Party of Interest under 35 U.S.C. 118

Prefix	Given Name	Middle Name	Family Name	Suffix
	Jeanette	Marjorie	Wood	

Residence Information (Select One) ○ US Residency ◉ Non US Residency ○ Active US Military Service

City	Biel-Benken	Country Of Residence[i]	CH

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	031671-US-CNT03 167-62 C3
	Application Number	
Title of Invention	TREATMENT OF SOLID TUMORS WITH RAPAMYCIN DERIVATIVES	

Citizenship under 37 CFR 1.41(b) [i]	NZ		
Mailing Address of Applicant:			
Address 1	In den Kleematen 18		
Address 2			
City	Biel-Benken	**State/Province**	
Postal Code	4105	**Country**[i]	CH

All Inventors Must Be Listed - Additional Inventor Information blocks may be generated within this form by selecting the **Add** button. Add

Correspondence Information:

Enter either Customer Number or complete the Correspondence Information section below.
For further information see 37 CFR 1.33(a).

☐ **An Address is being provided for the correspondence Information of this application.**

Customer Number	28249		
Email Address	iplaw@dlworthbarrese.com	Add Email	Remove Email

Application Information:

Title of the Invention	TREATMENT OF SOLID TUMORS WITH RAPAMYCIN DERIVATIVES		
Attorney Docket Number	031671-US-CNT03 167-62 C3	**Small Entity Status Claimed**	☐
Application Type	Nonprovisional		
Subject Matter	Utility		
Suggested Class (if any)		**Sub Class (if any)**	
Suggested Technology Center (if any)			
Total Number of Drawing Sheets (if any)		**Suggested Figure for Publication (if any)**	

Publication Information:

☐ Request Early Publication (Fee required at time of Request 37 CFR 1.219)

☐ **Request Not to Publish.** I hereby request that the attached application not be published under 35 U.S. C. 122(b) and certify that the invention disclosed in the attached application **has not and will not** be the subject of an application filed in another country, or under a multilateral international agreement, that requires publication at eighteen months after filing.

Representative Information:

Representative information should be provided for all practitioners having a power of attorney in the application. Providing this information in the Application Data Sheet does not constitute a power of attorney in the application (see 37 CFR 1.32).
Enter either Customer Number or complete the Representative Name section below. If both sections are completed the Customer Number will be used for the Representative Information during processing.

Please Select One:	◉ Customer Number	○ US Patent Practitioner	○ Limited Recognition (37 CFR 11.9)

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	031671-US-CNT03 167-62 C3
	Application Number	
Title of Invention	TREATMENT OF SOLID TUMORS WITH RAPAMYCIN DERIVATIVES	

Customer Number	28249

Domestic Benefit/National Stage Information:

This section allows for the applicant to either claim benefit under 35 U.S.C. 119(e), 120, 121, or 365(c) or indicate National Stage entry from a PCT application. Providing this information in the application data sheet constitutes the specific reference required by 35 U.S.C. 119(e) or 120, and 37 CFR 1.78(a)(2) or CFR 1.78(a)(4), and need not otherwise be made part of the specification.

Prior Application Status			Remove
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)
	Continuation of	10468520	2004-01-27
Prior Application Status			Remove
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)
10468520	a 371 of international	PCT/EP02/01714	2002-02-18

Additional Domestic Benefit/National Stage Data may be generated within this form by selecting the **Add** button. Add

Foreign Priority Information:

This section allows for the applicant to claim benefit of foreign priority and to identify any prior foreign application for which priority is not claimed. Providing this information in the application data sheet constitutes the claim for priority as required by 35 U.S.C. 119(b) and 37 CFR 1.55(a).

Remove

Application Number	Country [i]	Parent Filing Date (YYYY-MM-DD)	Priority Claimed
0104072.4	GB	2001-02-19	(•) Yes ( ) No

Remove

Application Number	Country [i]	Parent Filing Date (YYYY-MM-DD)	Priority Claimed
0124957.2	GB	2001-10-17	(•) Yes ( ) No

Additional Foreign Priority Data may be generated within this form by selecting the **Add** button. Add

Assignee Information:

Providing this information in the application data sheet does not substitute for compliance with any requirement of part 3 of Title 37 of the CFR to have an assignment recorded in the Office.

Assignee 1 Remove

If the Assignee is an Organization check here. ☐

Prefix	**Given Name**	Middle Name	**Family Name**	Suffix

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	031671-US-CNT03 167-62 C3
	Application Number	
Title of Invention	TREATMENT OF SOLID TUMORS WITH RAPAMYCIN DERIVATIVES	

Mailing Address Information:			
Address 1			
Address 2			
City		**State/Province**	
Country [i]		Postal Code	
Phone Number		Fax Number	
Email Address			
Additional Assignee Data may be generated within this form by selecting the **Add** button.		Add	

Signature:

A signature of the applicant or representative is required in accordance with 37 CFR 1.33 and 10.18. Please see 37 CFR 1.4(d) for the form of the signature.					
Signature	/Ann R. Pokalsky/			Date (YYYY-MM-DD)	2012-07-11
First Name	Ann	Last Name	Pokalsky	Registration Number	34697

This collection of information is required by 37 CFR 1.76. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 23 minutes to complete, including gathering, preparing, and submitting the completed application data sheet form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether the Freedom of Information Act requires disclosure of these records.

2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.

3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.

4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).

5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.

6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).

7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.

8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspections or an issued patent.

9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

DECLARATION AND POWER OF ATTORNEY FOR UNITED STATES PATENT APPLICATION

☒ Original ☐ Supplemental ☐ Substitute

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name, and

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if more than one name is listed below) of the subject matter which is claimed and for which a United States patent is sought on the invention entitled

TREATMENT OF SOLID TUMOURS WITH RAPAMYCIN DERIVATIVES

the specification of which:

☐ is attached hereto.

☐ was filed on _____ (day/month/year) as Application No. _____

and, if this box (☐) contains an *

☐ was amended on _____ (day/month/year)

☒ was filed as Patent Cooperation Treaty international Application No.

PCT/EP 02/01714 on 18.02.2002 (day/month/year)

and, if this box (☐) contains an *

☐ entered the national stage in the United States and was accorded Application No.

_____

and, if this box (☐) contains an *

☐ was amended, subsequent to entry into the national stage, on _____ (day/month/year)

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment(s) specifically referred to above and, if this application was filed as a Patent Cooperation Treaty international application, by any amendments made during the international stage (including any made under Patent Cooperation Treaty Rule 91, Article 19 and Article 34).

I acknowledge my duty to disclose information which is material to patentability as defined in 37 C.F.R. 1.56, including, for continuation-in-part applications, material information which became available between the filing date of the prior application and the national or Patent Cooperation Treaty international filing date of the continuation-in-part application.

I hereby claim the benefit under 35 U.S.C. 119(a)-(d) or (f) or 365(b) of any foreign application(s) for patent, inventor's certificate or plant breeder's right certificate listed below and under 35 U.S.C. 365(a) of any Patent Cooperation Treaty international application(s) designating at least one country other than the United States listed below and have also listed below any foreign application(s) for patent, inventor's certificate or plant breeder's right certificate and Patent Cooperation Treaty international application(s) designating at least one country other than the United States for the same subject matter and having a filing date before that of the application the priority of which is claimed for that subject matter:

COUNTRY/REGION (OR P.C.T.)	APPLICATION No.	FILING DATE (day/month/year)	PRIORITY CLAIMED	
Great Britain	**0104072.4**	**19/02/ 2001**	☒ Yes	☐ No
Great Britain	**0124957.2**	**17/10/ 2001**	☒ Yes	☐ No
			☐ Yes	☐ No
			☐ Yes	☐ No
			☐ Yes	☐ No

I hereby claim the benefit under 35 U.S.C. 119(e) of any United States provisional application(s) listed below:

APPLICATION NO.	FILING DATE (day/month/year)

I hereby claim the benefit under 35 U.S.C. 120 of any United States application(s) listed below and under 35 U.S.C. 365(c) of any Patent Cooperation Treaty international application(s) designating the United States listed below:

United States Application No.	United States Filing Date (day/month/year)	Status (Pending, Abandoned or U.S. Patent No.)	International Application No. and Filing Date (day/month/year)

I hereby appoint the registered practitioners associated with Customer No. 001095, respectively and individually, as my attorneys and agents, with full power of substitution and revocation, to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith.

If this box (☐) contains an x ☒, I hereby authorize the registered practitioners associated with Customer No. 001095 and any others acting on my behalf to take any action relating to this application based on communications from Corporate Intellectual Property of Novartis International AG, Basle, Switzerland, or an affiliate thereof or a successor thereto, without direct communication from me.

Please address all communications to the address associated with Customer No. 001095, which is currently Thomas Hoxie, Novartis, Corporate Intellectual Property, One Health Plaza, Bldg. 430, East Hanover, NJ 07936-1080.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001 and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Full name of sole or first joint inventor: **Heidi LANE**

Inventor's signature: H. A. Lane Date: 06.10.2003 (day/month/year)

Residence: **Basel, Switzerland**

Citizenship: **Swiss**

Post Office Address: **Lehenmattstr. 189**
4052 Basel
Switzerland

Full name of second joint inventor, if any: **Terence O'REILLY**

Inventor's signature: Date: 14-oct-2003 (day/month/year)

Residence: **Basel, Switzerland**

Citizenship: **Canadian**

Post Office Address: **Drahtzugstrasse 51**
4057 Basel
Switzerland

IMPORTANT: Before this declaration is signed, the patent application (the specification, the claims and this declaration) must be read and understood by each person signing it, and no changes may be made in the application after this declaration has been signed.

Full name of third joint inventor, if any	**Jeanette Marjorie WOOD**
Inventor's signature	Jeanette M Wood — Date 14.10.03 (day/month/year)
Residence	**Biel-Benken, Switzerland**
Citizenship	**New Zealand**
Post Office Address	**In den Kleematten 18** **4105 Biel-Benken** **Switzerland**

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s): Lane et al.

Docket: PAT031671-US-CNT03
(167-62 CON III)

Serial No.: Unknown

Dated: July 11, 2012

Filed: Herewith

For: TREATMENT OF SOLID TUMORS WITH RAPAMYCIN DERIVATIVES

INFORMATION DISCLOSURE STATEMENT

Sir:

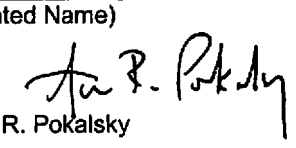
Pursuant to Applicants duty of disclosure, the information listed in the attached Form SB 08a is brought to the attention of the Examiner. Each of the items listed on the attached Form SB 08a were either cited by, or submitted to, the PTO in prior application Serial No. 10/468,520 filed January 27, 2004. Accordingly, pursuant to 37 C.F.R. §1.98(d), copies of the listed items are not being provided.

The citation of the items listed in the attached Form PTO/SB/08a is not a representation that they constitute a complete or exhaustive listing of the relevant art or that the items are prior art.

Certificate of EFS-Web Transmission

I hereby certify that this correspondence is being transmitted to the U.S. Patent and Trademark Office via the Office's electronic filing system on July 11, 2012.

Ann R. Pokalsky
(Printed Name)



Ann R. Pokalsky

The items listed are submitted in good faith, but are not intended to substitute for the Examiner's search. It is hoped, however, that in addition to apprising the Examiner of these particular items, they will assist in identifying fields of search and in making as full and complete a search as possible.

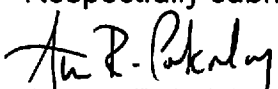
The filing of this information disclosure statement is not an admission that the information cited herein is, or is considered to be, material to patentability as defined in 37 C.F.R. § 1.56(b).

This information disclosure statement is being filed concurrently with this application.

Please charge any deficiency as well as any other fee(s) which may become due under 37 C.F.R. § 1.16 and/or 1.17 at any time during the pendency of this application, or credit any overpayment of such fee(s) to Deposit Account **04-1121**. Also, in the event any extensions of time for responding are required for the pending application(s), please treat this paper as a petition to extend the time as required and charge Deposit Account No. **04-1121** therefor.

Early and favorable consideration of the case is respectfully requested.

Respectfully submitted,



Ann R. Pokalsky
Reg. No. 34,697
Attorney for Applicants

DILWORTH & BARRESE
1000 Woodbury Road, Suite 405
Woodbury, NY 11797
fax (516) 228-8516
phone (516) 228-8484

PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875	Application or Docket Number 13/546,686

APPLICATION AS FILED - PART I

	(Column 1)	(Column 2)	SMALL ENTITY		OR	OTHER THAN SMALL ENTITY	
FOR	NUMBER FILED	NUMBER EXTRA	RATE($)	FEE($)		RATE($)	FEE($)
BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A			N/A	380
SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A			N/A	620
EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A			N/A	250
TOTAL CLAIMS (37 CFR 1.16(i))	7 minus 20 =	*			OR	x 60 =	0.00
INDEPENDENT CLAIMS (37 CFR 1.16(h))	1 minus 3 =	*				x 250 =	0.00
APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $310 ($155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).						0.00
MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))							0.00
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL			TOTAL	1250

APPLICATION AS AMENDED - PART II

		(Column 1)		(Column 2)	(Column 3)	SMALL ENTITY		OR	OTHER THAN SMALL ENTITY	
		CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA	RATE($)	ADDITIONAL FEE($)		RATE($)	ADDITIONAL FEE($)
AMENDMENT A	Total (37 CFR 1.16(i))	*	Minus	**	=	x =		OR	x =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	x =		OR	x =	
	Application Size Fee (37 CFR 1.16(s))									
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
						TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

		(Column 1)		(Column 2)	(Column 3)					
		CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA	RATE($)	ADDITIONAL FEE($)		RATE($)	ADDITIONAL FEE($)
AMENDMENT B	Total (37 CFR 1.16(i))	*	Minus	**	=	x =		OR	x =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	x =		OR	x =	
	Application Size Fee (37 CFR 1.16(s))									
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
						TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".
The "Highest Number Previously Paid For" (Total or Independent) is the highest found in the appropriate box in column 1.

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NUMBER	FILING or 371(c) DATE	GRP ART UNIT	FIL FEE REC'D	ATTY.DOCKET.NO	TOT CLAIMS	IND CLAIMS
13/546,686	07/11/2012	1629	1250	031671-US-CNT03 167-62 C3	7	1

CONFIRMATION NO. 8586

28249
DILWORTH & BARRESE, LLP
1000 WOODBURY ROAD
SUITE 405
WOODBURY, NY 11797

FILING RECEIPT

OC000000055562376

Date Mailed: 07/30/2012

Receipt is acknowledged of this non-provisional patent application. The application will be taken up for examination in due course. Applicant will be notified as to the results of the examination. Any correspondence concerning the application must include the following identification information: the U.S. APPLICATION NUMBER, FILING DATE, NAME OF APPLICANT, and TITLE OF INVENTION. Fees transmitted by check or draft are subject to collection. Please verify the accuracy of the data presented on this receipt. **If an error is noted on this Filing Receipt, please submit a written request for a Filing Receipt Correction. Please provide a copy of this Filing Receipt with the changes noted thereon. If you received a "Notice to File Missing Parts" for this application, please submit any corrections to this Filing Receipt with your reply to the Notice. When the USPTO processes the reply to the Notice, the USPTO will generate another Filing Receipt incorporating the requested corrections**

Applicant(s)

Heidi Lane, Basel, SWITZERLAND;
Terence O'Reilly, Basel, SWITZERLAND;
Jeanette Marjorie Wood, Biel-Benken, SWITZERLAND;

Power of Attorney: The patent practitioners associated with Customer Number 001095

Domestic Priority data as claimed by applicant

This application is a CON of 10/468,520 01/27/2004
which is a 371 of PCT/EP02/01714 02/18/2002

Foreign Applications (You may be eligible to benefit from the **Patent Prosecution Highway** program at the USPTO. Please see http://www.uspto.gov for more information.)
UNITED KINGDOM 0104072.4 02/19/2001
UNITED KINGDOM 0124957.2 10/17/2001

Request to Retrieve - This application either claims priority to one or more applications filed in an intellectual property Office that participates in the Priority Document Exchange (PDX) program or contains a proper **Request to Retrieve Electronic Priority Application(s)** (PTO/SB/38 or its equivalent). Consequently, the USPTO will attempt to electronically retrieve these priority documents.

If Required, Foreign Filing License Granted: 07/24/2012

The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 13/546,686**

Projected Publication Date: 11/08/2012

Non-Publication Request: No

Early Publication Request: No
Title

TREATMENT OF SOLID TUMORS WITH RAPAMYCIN DERIVATIVES

Preliminary Class

514

PROTECTING YOUR INVENTION OUTSIDE THE UNITED STATES

Since the rights granted by a U.S. patent extend only throughout the territory of the United States and have no effect in a foreign country, an inventor who wishes patent protection in another country must apply for a patent in a specific country or in regional patent offices. Applicants may wish to consider the filing of an international application under the Patent Cooperation Treaty (PCT). An international (PCT) application generally has the same effect as a regular national patent application in each PCT-member country. The PCT process **simplifies** the filing of patent applications on the same invention in member countries, but **does not result** in a grant of "an international patent" and does not eliminate the need of applicants to file additional documents and fees in countries where patent protection is desired.

Almost every country has its own patent law, and a person desiring a patent in a particular country must make an application for patent in that country in accordance with its particular laws. Since the laws of many countries differ in various respects from the patent law of the United States, applicants are advised to seek guidance from specific foreign countries to ensure that patent rights are not lost prematurely.

Applicants also are advised that in the case of inventions made in the United States, the Director of the USPTO must issue a license before applicants can apply for a patent in a foreign country. The filing of a U.S. patent application serves as a request for a foreign filing license. The application's filing receipt contains further information and guidance as to the status of applicant's license for foreign filing.

Applicants may wish to consult the USPTO booklet, "General Information Concerning Patents" (specifically, the section entitled "Treaties and Foreign Patents") for more information on timeframes and deadlines for filing foreign patent applications. The guide is available either by contacting the USPTO Contact Center at 800-786-9199, or it can be viewed on the USPTO website at http://www.uspto.gov/web/offices/pac/doc/general/index.html.

For information on preventing theft of your intellectual property (patents, trademarks and copyrights), you may wish to consult the U.S. Government website, http://www.stopfakes.gov. Part of a Department of Commerce initiative, this website includes self-help "toolkits" giving innovators guidance on how to protect intellectual property in specific countries such as China, Korea and Mexico. For questions regarding patent enforcement issues, applicants may call the U.S. Government hotline at 1-866-999-HALT (1-866-999-4158).

LICENSE FOR FOREIGN FILING UNDER

Title 35, United States Code, Section 184

Title 37, Code of Federal Regulations, 5.11 & 5.15

GRANTED

The applicant has been granted a license under 35 U.S.C. 184, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" followed by a date appears on this form. Such licenses are issued in all applications where

the conditions for issuance of a license have been met, regardless of whether or not a license may be required as set forth in 37 CFR 5.15. The scope and limitations of this license are set forth in 37 CFR 5.15(a) unless an earlier license has been issued under 37 CFR 5.15(b). The license is subject to revocation upon written notification. The date indicated is the effective date of the license, unless an earlier license of similar scope has been granted under 37 CFR 5.13 or 5.14.

This license is to be retained by the licensee and may be used at any time on or after the effective date thereof unless it is revoked. This license is automatically transferred to any related applications(s) filed under 37 CFR 1.53(d). This license is not retroactive.

The grant of a license does not in any way lessen the responsibility of a licensee for the security of the subject matter as imposed by any Government contract or the provisions of existing laws relating to espionage and the national security or the export of technical data. Licensees should apprise themselves of current regulations especially with respect to certain countries, of other agencies, particularly the Office of Defense Trade Controls, Department of State (with respect to Arms, Munitions and Implements of War (22 CFR 121-128)); the Bureau of Industry and Security, Department of Commerce (15 CFR parts 730-774); the Office of Foreign AssetsControl, Department of Treasury (31 CFR Parts 500+) and the Department of Energy.

NOT GRANTED

No license under 35 U.S.C. 184 has been granted at this time, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" DOES NOT appear on this form. Applicant may still petition for a license under 37 CFR 5.12, if a license is desired before the expiration of 6 months from the filing date of the application. If 6 months has lapsed from the filing date of this application and the licensee has not received any indication of a secrecy order under 35 U.S.C. 181, the licensee may foreign file the application pursuant to 37 CFR 5.15(b).

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
13/546,686	07/11/2012	Heidi Lane	031671-US-CNT03 167-62 C3	8586

28249 7590 10/09/2012

DILWORTH & BARRESE, LLP
1000 WOODBURY ROAD
SUITE 405
WOODBURY, NY 11797

EXAMINER	
KLINKEL, KORTNEY L	
ART UNIT	**PAPER NUMBER**
1611	

MAIL DATE	DELIVERY MODE
10/09/2012	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	13/546,686	LANE ET AL.	
	Examiner	Art Unit	
	Kortney L. Klinkel	1611	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *11 July 2012*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

4)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

5)☒ Claim(s) *1-7* is/are pending in the application.

5a) Of the above claim(s) _____ is/are withdrawn from consideration.

6)☐ Claim(s) _____ is/are allowed.

7)☒ Claim(s) *1-7* is/are rejected.

8)☐ Claim(s) _____ is/are objected to.

9)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

10)☐ The specification is objected to by the Examiner.

11)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

12)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

13)☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☒ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☒ Certified copies of the priority documents have been received in Application No. 10/468520.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *7/11/2012*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Claims

Please note, that the claims submitted 7/11/2012 contain two claim 2's. **The second claim 2 drawn to oral administration has been renumbered as claim 7.** Claims 1-7 are pending in the instant Office action.

Information Disclosure Statement

Acknowledgement is made of applicant's submitting an information disclosure statement on 7/11/2012. The submission is in compliance with the provisions of 37 CFR 1.97. Accordingly, the information disclosure statement has been considered by the examiner.

Priority

Acknowledgement is made that the instant application is a CON of 10/468520 filed 1/27/2004 which is a 371 of PCT/EP02/01714 filed 2/18/2002. Acknowledgement is also made of applicant's foreign priority claim to UK patent applications 0104072.4 filed 2/19/2001 and 0124957.2 filed 10/17/2001. Receipt is acknowledged of papers submitted under 35 U.S.C. 119(a)-(d), which papers have been placed of record in the file.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

The factual inquiries set forth in *Graham* v. *John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966), that are applied for establishing a background for determining obviousness under 35 U.S.C. 103(a) are summarized as follows:

1. Determining the scope and contents of the prior art.
2. Ascertaining the differences between the prior art and the claims at issue.
3. Resolving the level of ordinary skill in the pertinent art.
4. Considering objective evidence present in the application indicating obviousness or nonobviousness.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

Claims 1-7 are rejected under 35 U.S.C. 103(a) as being unpatentable over Geoerger et al. (“Antitumor Activity of the Rapamycin Analog CCI-779 in Human Primitive Neuroectodermal Tumor/Medulloblastoma Models as Single Agent and in

Combination Chemotherapy", Cancer Research, 61, 2/15/2001, 1527-1532, as per Applicant's IDS) in view of Cottens et al. (WO 94/09010, as per Applicant's IDS). The Examiner notes that WO 94/09010 was initially cited on the International Search Report, however, the Examiner was unable to find a copy of this reference in the parent or instant file. Accordingly, a copy of this reference is provided herewith for good measure.

Geoerger et al. teach that administration of rapamycin has antitumor activity (p. 1527, 1st column). Co-administration of rapamycin with cisplatin, or 5-fluouracil and cyclophosphamide exhibited enhanced apoptosis in human cell lines and cytotoxicity in colon tumor models respectively (p. 1527, 1st column). Rapamycin and its 40-O substituted analog CCI-779 are effective brain tumor therapeutics both alone and in combination with chemotherapeutics such as cisplatin and camptothecin (p. 1527, abstract and 2nd column). Geoerger et al. teach that brain tumor cell lines are exquisitely sensitive to rapamycin (p. 1527, 2nd column, first full paragraph). Geoerger et al. teach that rapamycin in combination with cisplatin or camptothecin has an additive effect in cell lines resistant to rapamycin (p. 1528, 1st paragraph of Results section). The antitumor activity of rapamycin has been demonstrated in tumors. The antitumor activity of rapamycin has been demonstrated in human rhabdomyosarcoma and neuroblastoma tumor cell lines *in vitro* and in B16 melanocarcinoma, Colon 38 tumors, CD8F1 mammary tumors, EM ependymoblastoma, and U251 glioblastoma brain tumors *in vivo* (p. 1530, Discussion). Geoerger et al. also teach that tumor toxicity can be increased by using combination chemotherapy with a rapamycin without the risk of

increased systemic cyctotoxicity (p. 1530, Discussion). Geoerger et al. teach that cisplatin, camptothecin, CPT 11 and topotecan are effective agents in the chemotherapeutic treatment of brain tumors but that dosages of these agents are limited due to their toxicity. Because rapamycin and the 40-O-substituted derivative CCI-779 show at least an additive effect when combined with chemotherapeutics and they have low toxicity, they are good adjuvants for these toxic chemotherapeutics (p. 1532, first column). Additionally CCI-779 exhibits an enhanced antitumor effect when combined with cisplatin in vivo (p. 1532, first column).

Geoerger et al. also teach that either 20 mg/kg/d in a single dose or 100 mg/kg/d in a divided dose of the rapamycin derivative CCI-779 is administered via intraperitoneal injection (p. 1528 1st col., p. 1532 1st col.). Dosages of 100, 200, 400 or 800 mg/kg/d of rapamycin are also taught to be effective (p. 1531, 1st col.).

The teachings of Geoerger et al. differ from the instant claims in that rapamycin or the 40-O substituted rapamycin derivative CCI-779 are administered either alone or in combination with other chemotherapeutics for the treatment of brain tumors *inter alia*, rather than the claimed rapamycin derivative 40-O-(2-hydroxyethyl) rapamycin (AKA everolimus). Geoerger et al. also fail to teach explicit dosages in terms of mg administered, but rather teaches dosages in terms of mg/kg. The dosages described by Geoerger et al. are all administered intraperiotoneally rather than orally as required by instant claim 7.

Cottens et al. teach compounds of formula I, including the instant claimed compound i.e. 40-O-(2-hydroxyethyl) rapamycin (pages 2-4, see particularly p. 3

compound 8, last line; 21-22; Example 8 p. 21-22; claim 2, compound 8) and that these derivatives of rapamycin have an improved pharmacologic profile over rapamycin, exhibit greater stability and bioavailability and allow for greater ease in producing gelenic formulations (p. 2, first full paragraph). Cottens et al. teach that the use of rapamycin as an antitumor agent is restricted by its low and variable bioavailability (p. 2, lines 1-4).

Cottens et al. teach that compounds of formula I have demonstrated antitumor activity and the ability to enhance performance of antitumor agents by alleviating multidrug resistance e.g. by administration with anticancer agent e.g. colchicine or etoposide, to multidrug resistant cells and drug sensitive cells in vitro or to animals having multidrug resistant or drug sensitive tumors (page 12, first full para.). Cottens et al. teach that the compounds may be administered as the sole active ingredient or together with other drugs e.g. corticosteroids, azathioprine, immunosuppressive monoclonal antibodies (page 8, second full para.).

Cottens et al. teach a method of treating tumors or hyperproliferative disorders comprising administering a compound of formula I (page 6, items “d and e;” page 40, claim 8). Cottens et al. teach that generally the dose of the instant claimed compounds is from 0.05 to 10 mg/kg/d orally in individual dosages of 0.1 to 7.5 mg/kg/day for up to 4 divided doses per day. Typical dosages for intravenous injection range from 0.01 to 5 mg/kg/day (page 7, first para to page 8, first para.). In total, for an average human, dosages range from 5 to 100 mg p.o. up to 500 mg/d p.o. or on the order of 0.5 to 250

mg i.v. with individual dosages from 2.5 to 50 mg i.v. (p. 8 first para.). These absolute dosage amounts overlap with the dosage amounts required by claims 3-6.

It would have been prima facie obvious to one of ordinary skill in the art at the time of the instant invention to substitute rapamycin or CCI-779 of Geoerger et al. for the claimed rapamycin derivative 40-O-(2-hydroxyethyl)rapamycin of Cottens et al. with the reasonable expectation that solid tumors, including brain tumors or brain carcinoma would be treated when administered alone or in combination with other chemotherapeutics such as cisplatin, 5-fluoruracil, and topotecan. One would have been motivated to do so because it is well known in the art that 40-O-(2-hydroxyethyl)rapamycin is useful for treating tumors and hyperproliferative disorders and that it exhibits an improved pharmacologic profile over rapamycin, exhibits greater stability and bioavailability and allows for greater ease in formulating. One of ordinary skill in the art would be imbued with the reasonable expectation that the combination of 40-O-(2-hydroxyethyl)rapamycin with the chemotherapeutics 5-fluorouracil and topotecan would exhibit at least an additive effect as this is what is observed for the combination of rapamycin or CCI-779 with these agents. One would be imbued with the reasonable expectation that the combination of 40-O-(2-hydroxyethyl)rapamycin with cisplatin would exhibit an enhanced antitumor effect, as this is what is observed for the 40-O-substituted rapamycin derivative CCI-779. Additionally, "[i]t is *prima facie* obvious to combine two compositions each of which is taught by the prior art to be useful for the same purpose, in order to form a third composition to be used for the very same purpose.... [T]he idea of combining them flows logically from their having been

individually taught in the prior art." *In re Kerkhoven*, 626 F.2d 846, 850, 205 USPQ 1069, 1072 (CCPA 1980) (citations omitted).

Regarding the dosage amounts of about 0.1-25 mg as a single or divided dosage of claim 3, a unit dosage of about 0.05 to 12.5 mg of claim 4, a unit dosage from about 0.25 to 10 mg of claim 5 and a unit dosage form of 10 mg of claim 6, the Examiner notes that depending on the size of the subject, both the teachings of Geoerger et al. and Cottens et al. teach amounts which fall within or overlap with the claimed amounts. Generally, differences in concentration or temperature will not support the patentability of subject matter encompassed by the prior art unless there is evidence indicating such concentration or temperature is critical. "[W]here the general conditions of a claim are disclosed in the prior art, it is not inventive to discover the optimum or workable ranges by routine experimentation." *In re Aller*, 220 F.2d 454, 456, 105 USPQ 233, 235 (CCPA 1955). Here, one of ordinary skill in the art would be motivated to adjust the relative amount of drug administered to suite the subject's mass and condition and to balance beneficial effects with negative side effects. It is well within the purview of one of ordinary skill in the art to determine the optimal dosage amount.

Conclusion

Claims 1-7 are rejected. No claim is allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Kortney Klinkel whose telephone number is (571)270-5239. The examiner can normally be reached on Monday-Friday 10 am to 7 pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Daniel Sullivan can be reached at (571)272-0779. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

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/Kortney L. Klinkel/
Examiner, Art Unit 1611

Notice of References Cited	Application/Control No. 13/546,686	Applicant(s)/Patent Under Reexamination LANE ET AL.	
	Examiner Kortney L. Klinkel	Art Unit 1611	Page 1 of 1

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	N	WO 94/09010	04-1994	WO	Cottens et al.	C07D 498/18
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EAST Search History (Prior Art)

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L1	32	("20020022054" | "20020098278" | "20030100886" | "20030100887" | "4885171" | "5066493" | "5194447" | "5206018" | "5362718" | "5922730" | "5985890" | "6333348" | "6569463" | "6617333" | "6641822").PN.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/09/28 13:06
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L6	4	2 and 3 and 4	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/09/28 13:07
L7	604202	cancer or carcinoma or tumor	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/09/28 13:09

L8	83	5 and 7	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/09/28 13:10
L9	25275	rapamycin or everolimus	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/09/28 13:10
L10	19	8 and 9	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/09/28 13:10
L11	5952358	brain or cns or central near2 nervous near2 system	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/09/28 13:23
L12	109650	7 same 11	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/09/28 13:24
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	Examiner	Art Unit
	KORTNEY L KLINKEL	1611

SEARCHED			
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SEARCH NOTES		
Search Notes	Date	Examiner
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First Named Inventor	Lane et al.
Art Unit	
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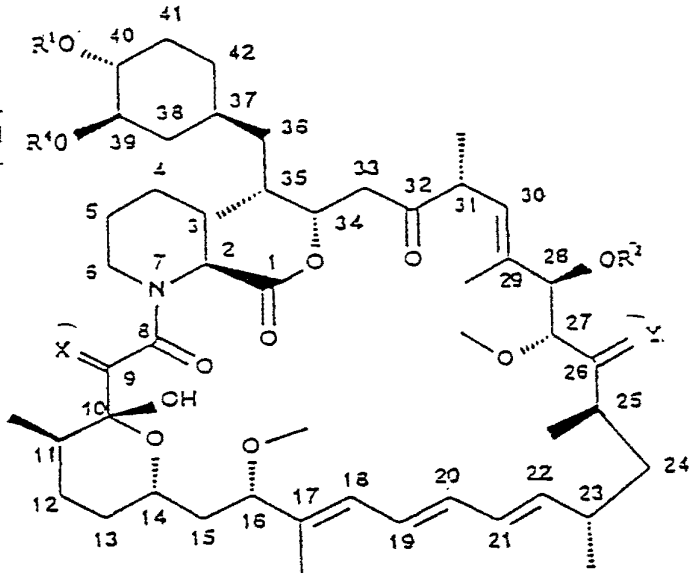
Published
With international search report.

(54) Title: O-ALKYLATED RAPAMYCIN DERIVATIVES AND THEIR USE, PARTICULARLY AS IMMUNOSUPPRESSANTS

(57) Abstract

Novel O-alkylated derivatives of rapamycin of formula (I), especially 40-O-alkylated derivatives, are found to have pharmaceutical utility, particularly as immunosuppressants.



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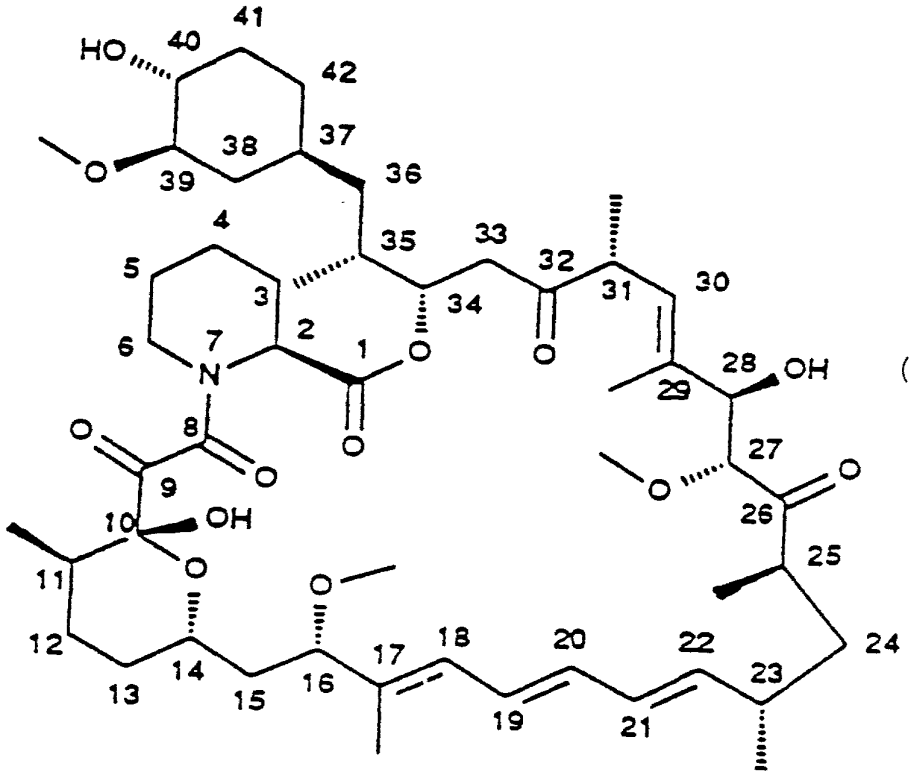
O-ALKYLATED RAPAMYCIN DERIVATIVES AND THEIR USE, PARTICULARLY AS IMMUNO-SUPPRESSANTS

This invention comprises novel alkylated derivatives of rapamycin having pharmaceutical utility, especially as immunosuppressants.

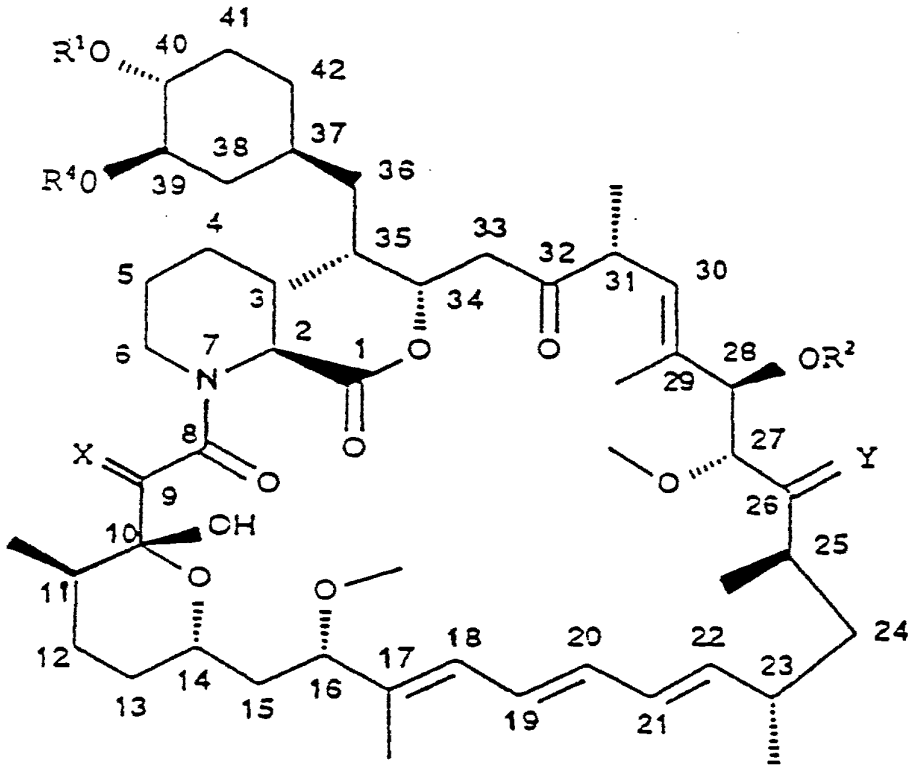
Rapamycin is a known macrolide antibiotic produced by Streptomyces hygroscopicus, having the structure depicted in Formula A:



See, e.g., McAlpine, J.B., et al., J. Antibiotics (1991) 44: 688; Schreiber, S.L., et al., J. Am. Chem. Soc. (1991) 113: 7433; US Patent No. 3 929 992. Rapamycin is an extremely

potent immunosuppressant and has also been shown to have antitumor and antifungal activity. Its utility as a pharmaceutical, however, is restricted by its very low and variable bioavailability as well as its high toxicity. Moreover, rapamycin is highly insoluble, making it difficult to formulate stable galenic compositions.

It has now surprisingly been discovered that certain novel derivatives of rapamycin (the Novel Compounds) have an improved pharmacologic profile over rapamycin, exhibit greater stability and bioavailability, and allow for greater ease in producing galenic formulations. The Novel Compounds are alkylated derivatives of rapamycin having the structure of Formula I:



(I)

wherein

X is (H,H) or O;

Y is (H,OH) or O;

R^1 and R^2 are independently selected from

H, alkyl, thioalkyl, arylalkyl, hydroxyalkyl, dihydroxyalkyl, hydroxyalkylarylalkyl, dihydroxyalkylarylalkyl, alkoxyalkyl, acyloxyalkyl, aminoalkyl, alkylaminoalkyl, alkoxycarbonylaminoalkyl, acylaminoalkyl, arylsulfonamidoalkyl, allyl, dihydroxyalkylallyl, dioxolanylallyl, carbalkoxyalkyl, and $(R^3)_3Si$ where each R^3 is independently selected from H, methyl, ethyl, isopropyl, t-butyl, and phenyl; wherein "alk-" or "alkyl" refers to C_{1-6} alkyl, branched or linear preferably C_{1-3} alkyl, in which the carbon chain may be optionally interrupted by an ether (-O-) linkage; and

R^4 is methyl, or R^4 and R^1 together form C_{2-6} alkylene;

provided that R^1 and R^2 are not both H; and
provided that where R^1 is $(R^3)_3Si$ or carbalkoxyalkyl, X and Y are not both O.

Preferred Novel Compounds include the following:

1. 40-O-Benzyl-rapamycin
2. 40-O-(4'-Hydroxymethyl)benzyl-rapamycin
3. 40-O-[4'-(1,2-Dihydroxyethyl)]benzyl-rapamycin
4. 40-O-Allyl-rapamycin
5. 40-O-[3'-(2,2-Dimethyl-1,3-dioxolan-4(S)-yl)-prop-2'-en-1'-yl]-rapamycin
6. (2'E, 4'S)-40-O-(4',5'-Dihydroxypent-2'-en-1'-yl)-rapamycin
7. 40-O-(2-Hydroxy)ethoxycarbonylmethyl-rapamycin
8. 40-O-(2-Hydroxy)ethyl-rapamycin

9. 40-O-(3-Hydroxy)propyl-rapamycin
10. 40-O-(6-Hydroxy)hexyl-rapamycin
11. 40-O-[2-(2-Hydroxy)ethoxy]ethyl-rapamycin
12. 40-O-[(3S)-2,2-Dimethyldioxolan-3-yl]methyl-rapamycin
13. 40-O-[(2S)-2,3-Dihydroxyprop-1-yl]-rapamycin
14. 40-O-(2-Acetoxy)ethyl-rapamycin
15. 40-O-(2-Nicotinoyloxy)ethyl-rapamycin
16. 40-O-[2-(N-Morpholino)acetoxy]ethyl-rapamycin
17. 40-O-(2-N-Imidazolylacetoxy)ethyl-rapamycin
18. 40-O-[2-(N-Methyl-N'-piperazinyl)acetoxy]ethyl-rapamycin
19. 39-O-Desmethyl-39,40-O,O-ethylene-rapamycin
20. (26R)-26-Dihydro-40-O-(2-hydroxy)ethyl-rapamycin
21. 28-O-Methyl-rapamycin
22. 40-O-(2-Aminoethyl)-rapamycin
23. 40-O-(2-Acetaminoethyl)-rapamycin
24. 40-O-(2-Nicotinamidoethyl)-rapamycin
25. 40-O-(2-(N-Methyl-imidazo-2'-ylcarbethoxamido)ethyl)-rapamycin
26. 40-O-(2-Ethoxycarbonylaminoethyl)-rapamycin
27. 40-O-(2-Tolylsulfonamidoethyl)-rapamycin
28. 40-O-[2-(4',5'-Dicarboethoxy-1',2',3'-triazol-1'-yl)-ethyl]-rapamycin

The Novel Compounds for immunosuppressive use are preferably the 40-O-substituted rapamycins where X and Y are both O, R^2 is H, R^4 is methyl, and R^1 is other than H; most preferably where R^1 is selected from hydroxyalkyl, hydroxyalkoxyalkyl, acylaminoalkyl, and aminoalkyl; especially 40-O-(2-hydroxy)ethyl-rapamycin, 40-O-(3-hydroxy)propyl-rapamycin, 40-O-[2-(2-hydroxy)ethoxy]ethyl-rapamycin, and 40-O-(2-acetaminoethyl)-rapamycin).

Preferably, O-substitution at C40 or O,O-disubstitution at C28 and C40 is performed

according to the following general process: Rapamycin (or dihydro or deoxorapamycin) is reacted with an organic radical attached to a leaving group (e.g., RX where R is the organic radical, e.g., an alkyl, allyl, or benzyl moiety, which is desired as the O-substituent, and X is the leaving group, e.g., $CCl_3C(NH)O$ or CF_3SO_3) under suitable reaction conditions, preferably acidic or neutral conditions, e.g., in the presence of an acid like trifluoromethanesulfonic acid, camphorsulfonic acid, p-toluenesulfonic acid or their respective pyridinium or substituted pyridinium salts when X is $CCl_3C(NH)O$ or in the presence of a base like pyridine, a substituted pyridine, diisopropylethylamine or pentamethylpiperidine when X is CF_3SO_3. O-substitutions at C28 only are accomplished in the same manner, but with prior protection at C40. Further modifications are possible. For example, where the substituent is allyl, the isolated, monosubstituted double bond of the allyl moiety is highly amenable to further modification.

The 9-deoxorapamycin compounds are preferably produced by reducing a rapamycin using hydrogen sulfide, by reacting rapamycin with diphenyldiselenide and tributylphosphine or by other suitable reduction reaction.

The 26-dihydro-rapamycins are preferably produced by reducing rapamycins or 9-deoxorapamycins from keto to hydroxy at C26 by a mild reduction reaction, such as a borohydride reduction reaction.

The Novel Compounds are particularly useful for the following conditions:

a) Treatment and prevention of organ or tissue transplant rejection, e.g. for the treatment of recipients of e.g. heart, lung, combined heart-lung, liver, kidney, pancreatic, skin or corneal transplants. They are also indicated for the prevention of graft-versus-host disease, such as following bone marrow transplantation.

b) Treatment and prevention of autoimmune disease and of inflammatory conditions, in particular inflammatory conditions with an etiology including an autoimmune

component such as arthritis (for example rheumatoid arthritis, arthritis chronica progrediente and arthritis deformans) and rheumatic diseases. Specific autoimmune diseases for which the compounds of the invention may be employed include, autoimmune hematological disorders (including e.g. hemolytic anaemia, aplastic anaemia, pure red cell anaemia and idiopathic thrombocytopenia), systemic lupus erythematosus, polychondritis, sclerodoma, Wegener granulamatosis, dermatomyositis, chronic active hepatitis, myasthenia gravis, psoriasis, Steven-Johnson syndrome, idiopathic sprue, autoimmune inflammatory bowel disease (including e.g. ulcerative colitis and Crohn's disease) endocrine ophthalmopathy, Graves disease, sarcoidosis, multiple sclerosis, primary billiary cirrhosis, juvenile diabetes (diabetes mellitus type I), uveitis (anterior and posterior), keratoconjunctivitis sicca and vernal keratoconjunctivitis, interstitial lung fibrosis, psoriatic arthritis, glomerulonephritis (with and without nephrotic syndrome, e.g. including idiopathic nephrotic syndrome or minimal change nephropathy) and juvenile dermatomyositis.

c) Treatment and prevention of asthma.

d) Treatment of multi-drug resistance (MDR). The Novel Compounds suppress P-glycoproteins (Pgp), which are the membrane transport molecules associated with MDR. MDR is particularly problematic in cancer patients and AIDS patients who will not respond to conventional chemotherapy because the medication is pumped out of the cells by Pgp. The Novel Compounds are therefore useful for enhancing the efficacy of other chemotherapeutic agents in the treatment and control of multidrug resistant conditions such as multidrug resistant cancer or multidrug resistant AIDS.

e) Treatment of proliferative disorders, e.g. tumors, hyperproliferative skin disorder and the like.

f) Treatment of fungal infections.

g) Treatment and prevention of inflammation, especially in potentiating the action of steroids.

h) Treatment and prevention of infection, especially infection by pathogens having Mip or Mip-like factors.

i) Treatment of overdoses of FK-506, rapamycin, immunosuppressive Novel

Compounds, and other macrophilin binding immunosuppressants.

The invention thus provides the Novel Compounds described herein, for use as novel intermediates or as pharmaceuticals, methods of treating or preventing the above-described disorders by administering an effective amount of a Novel Compound to a patient in need thereof, use of a Novel Compound in the manufacture of a medicament for treatment or prevention of the above-described disorders, and pharmaceutical compositions comprising a Novel Compound in combination or association with a pharmaceutically acceptable diluent or carrier.

Most of the Novel Compounds described herein are highly immunosuppressive, especially those Novel Compounds which are O-substituted at C40, and these Novel Compounds are particularly useful in indications a and b, but not in indication i. Those of the Novel Compounds which are less immunosuppressive, especially those which are O-substituted at C28 only, are particularly useful in indications h and i, but are less preferred in indications a or b.

The Novel Compounds are utilized by administration of a pharmaceutically effective dose in pharmaceutically acceptable form to a subject in need of treatment. Appropriate dosages of the Novel Compounds will of course vary, e.g. depending on the condition to be treated (for example the disease type or the nature of resistance), the effect desired and the mode of administration.

In general however satisfactory results are obtained on administration orally at dosages on the order of from 0.05 to 5 or up to 10mg/kg/day, e.g. on the order of from 0.1 to 2 or up to 7.5 mg/kg/day administered once or, in divided doses 2 to 4x per day, or on administration parenterally, e.g. intravenously, for example by i.v. drip or infusion, at dosages on the order of from 0.01 to 2.5 up to 5 mg/kg/day, e.g. on the order of from 0.05 or 0.1 up to 1.0 mg/kg/day. Suitable daily dosages for patients are thus on the order of 500

mg p.o., e.g. on the order of from 5 to 100 mg p.o., or on the order of from 0.5 to 125 up to 250 mg i.v., e.g. on the order of from 2.5 to 50 mg i.v..

Alternatively and even preferably, dosaging is arranged in patient specific manner to provide pre-determined trough blood levels, e.g. as determined by RIA technique. Thus patient dosaging may be adjusted so as to achieve regular on-going trough blood levels as measured by RIA on the order of from 50 or 150 up to 500 or 1000ng/ml, i.e. analogously to methods of dosaging currently employed for Ciclosporin immunosuppressive therapy.

The Novel Compounds may be administered as the sole active ingredient or together with other drugs. For example, in immunosuppressive applications such as prevention and treatment of graft vs. host disease, transplant rejection, or autoimmune disease, the Novel Compounds may be used in combination with Ciclosporin, FK-506, or their immunosuppressive derivatives; corticosteroids; azathioprene; immunosuppressive monoclonal antibodies, e.g., monoclonal antibodies to CD3, CD4, CD25, CD28, or CD45; and7or other immunomodulatory compounds. For anti-inflammatory applications, the Novel Compounds can be used together with anti-inflammatory agents, e.g., corticosteroids. For anti-infective applications, the Novel Compounds can be used in combination with other anti-infective agents, e.g., anti-viral drugs or antibiotics.

The Novel Compounds are administered by any conventional route, in particular enterally, e.g. orally, for example in the form of solutions for drinking, tablets or capsules or parenterally, for example in the form of injectable solutions or suspensions. Suitable unit dosage forms for oral administration comprise, e.g. from 1 to 50 mg of a compound of the invention, usually 1 to 10 mg. Pharmaceutical compositions comprising the novel compounds may be prepared analogously to pharmaceutical compositions comprising rapamycin, e.g., as described in EPA 0 041 795, which would be evident to one skilled in the art.

The pharmacological activity of the Novel Compounds are demonstrated in, e.g., the following tests:

1. Mixed lymphocyte reaction (MLR)

The Mixed Lymphocyte Reaction was originally developed in connection with allografts, to assess the tissue compatibility between potential organ donors and recipients, and is one of the best established models of immune reaction in vitro. A murine model MLR, e.g., as described by T.Meo in "Immunological Methods", L. Lefkovits and B. Peris, Eds., Academic Press, N.Y. pp. 227-239 (1979), is used to demonstrate the immunosuppressive effect of the Novel Compounds. Spleen cells (0.5×10^6) from Balb/c mice (female, 8-10 weeks) are co-incubated for 5 days with 0.5×10^6 irradiated (2000 rads) or mitomycin C treated spleen cells from CBA mice (female, 8-10 weeks). The irradiated allogeneic cells induce a proliferative response in the Balb/c spleen cells which can be measured by labeled precursor incorporation into the DNA. Since the stimulator cells are irradiated (or mitomycin C treated) they do not respond to the Balb/c cells with proliferation but do retain their antigenicity. The antiproliferative effect of the Novel Compounds on the Balb/c cells is measured at various dilutions and the concentration resulting in 50% inhibition of cell proliferation (IC_{50}) is calculated. The inhibitory capacity of the test sample may be compared to rapamycin and expressed as a relative IC_{50} (i.e. IC_{50} test sample/IC_{50} rapamycin).

2. IL-6 mediated proliferation

The capacity of the Novel Compounds to interfere with growth factor associated signalling pathways is assessed using an interleukin-6 (IL-6)-dependent mouse hybridoma cell line. The assay is performed in 96-well microtiter plates. 5000 cells/well are cultivated in serum-free medium (as described by M. H. Schreier and R. Tees in Immunological Methods, I. Lefkovits and B. Pernis, eds., Academic Press 1981, Vol. II, pp. 263-275), supplemented with 1 ng recombinant IL-6/ml. Following a 66 hour incubation in the absence or presence of a test sample, cells are pulsed with 1 µCi (3-H)-thymidine/well for

another 6 hours, harvested and counted by liquid scintillation. (3-H)-thymidine incorporation into DNA correlates with the increase in cell number and is thus a measure of cell proliferation. A dilution series of the test sample allows the calculation of the concentration resulting in 50% inhibition of cell proliferation (IC_{50}). The inhibitory capacity of the test sample may be compared to rapamycin and expressed as a relative IC_{50} (i.e. IC_{50} test sample/IC_{50} rapamycin).

3. Macrophilin binding assay

Rapamycin and the structurally related immunosuppressant, FK-506, are both known to bind in vivo to macrophilin-12 (also known as FK-506 binding protein or FKBP-12), and this binding is thought to be related to the immunosuppressive activity of these compounds. The Novel Compounds also bind strongly to macrophilin-12, as is demonstrated in a competitive binding assay.

In this assay, FK-506 coupled to BSA is used to coat microtiter wells. Biotinylated recombinant human macrophilin-12 (biot-MAP) is allowed to bind in the presence or absence of a test sample to the immobilized FK-506. After washing (to remove non-specifically bound macrophilin), bound biot-MAP is assessed by incubation with a streptavidin-alkaline phosphatase conjugate, followed by washing and subsequent addition of p-nitrophenyl phosphate as a substrate. The read-out is the OD at 405nm. Binding of a test sample to biot-MAP results in a decrease in the amount of biot-MAP bound to the FK-506 and thus in a decrease in the OD405. A dilution series of the test sample allows determination of the concentration resulting in 50% inhibition of the biot-MAP binding to the immobilized FK-506 (IC_{50}). The inhibitory capacity of a test sample is compared to the IC_{50} of free FK-506 as a standard and expressed as a relative IC_{50} (i.e., IC_{50}-test sample/ IC_{50}-free FK-506).

4. Localized Graft-Versus-Host (GvH) Reaction

In vivo efficacy of the Novel Compounds is proved in a suitable animal model, as

described, e.g., in Ford et al, TRANSPLANTATION 10 (1970) 258. Spleen cells (1×10^7) from 6 week old female Wistar/Furth (WF) rats are injected subcutaneously on day 0 into the left hind-paw of female (F344 x WF)F_1 rats weighing about 100g. Animals are treated for 4 consecutive days and the popliteal lymph nodes are removed and weighed on day 7. The difference in weight between the two lymph nodes is taken as the parameter for evaluating the reaction.

5. Kidney Allograft Reaction in Rat

One kidney from a female fisher 344 rat is transplanted onto the renal vessel of a unilaterally (left side) nephrectomized WF recipient rat using an end-to-end anastomosis. Ureteric anastomosis is also end-to-end. Treatment commences on the day of transplantation and is continued for 14 days. A contralateral nephrectomy is done seven days after transplantation, leaving the recipient relying on the performance of the donor kidney. Survival of the graft recipient is taken as the parameter for a functional graft.

6. Experimentally Induced Allergic Encephalomyelitis (EAE) in Rats

Efficacy of the Novel Compounds in EAE is measured, e.g., by the procedure described in Levine & Wenk, AMER J PATH 47 (1965) 61; McFarlin et al, J IMMUNOL 113 (1974) 712; Borel, TRANSPLANT. & CLIN. IMMUNOL 13 (1981) 3. EAE is a widely accepted model for multiple sclerosis. Male Wistar rats are injected in the hind paws with a mixture of bovine spinal cord and complete Freund's adjuvant. Symptoms of the disease (paralysis of the tail and both hind legs) usually develop within 16 days. The number of diseased animals as well as the time of onset of the disease are recorded.

7. Freund's Adjuvant Arthritis

Efficacy against experimentally induced arthritis is shown using the procedure described, e.g., in Winter & Nuss, ARTHRITIS & RHEUMATISM 9 (1966) 394; Billingham & Davies, HANDBOOK OF EXPERIMENTAL PHARMACOL (Vane & Ferreira Eds, Springer-Verlag, Berlin) 50/II (1979) 108-144. OFA and Wistar rats (male or

female, 150g body weight) are injected i.c. at the base of the tail or in the hind paw with 0.1 ml of mineral oil containing 0.6 mg of lyophilized heat-killed Mycobacterium smegmatis. In the developing arthritis model, treatment is started immediately after the injection of the adjuvant (days 1 - 18); in the established arthritis model treatment is started on day 14, when the secondary inflammation is well developed (days 14-20). At the end of the experiment, the swelling of the joints is measured by means of a micro-caliper. ED_{50} is the oral dose in mg/kg which reduces the swelling (primary or secondary) to half of that of the controls.

8. Antitumor and MDR activity

The antitumor activity of the Novel Compounds and their ability to enhance the performance of antitumor agents by alleviating multidrug resistance is demonstrated, e.g., by administration of an anticancer agent, e.g., colchicine or etoposide, to multidrug resistant cells and drug sensitive cells in vitro or to animals having multidrug resistant or drug sensitive tumors or infections, with and without co-administration of the Novel Compounds to be tested, and by administration of the Novel Compound alone.

Such in vitro testing is performed employing any appropriate drug resistant cell line and control (parental) cell line, generated, e.g. as described by Ling et al., J. Cell. Physiol. 83, 103-116 (1974) and Bech-Hansen et al. J. Cell. Physiol. 88, 23-32 (1976). Particular clones chosen are the multi-drug resistant (e.g. colchicine resistant) line CHR (subclone C5S3.2) and the parental, sensitive line AUX B1 (subclone AB1 S11).

In vivo anti-tumor and anti-MDR activity is shown, e.g., in mice injected with multidrug resistant and drug sensitive cancer cells. Ehrlich ascites carcinoma (EA) sub-lines resistant to drug substance DR, VC, AM, ET, TE or CC are developed by sequential transfer of EA cells to subsequent generations of BALB/c host mice in accordance with the methods described by Slater et al., J. Clin. Invest, 70, 1131 (1982).

Equivalent results may be obtained employing the Novel Compounds test models of comparable design, e.g. in vitro, or employing test animals infected with drug-resistant and drug sensitive viral strains, antibiotic (e.g. penicillin) resistant and sensitive bacterial strains, anti-mycotic resistant and sensitive fungal strains as well as drug resistant protozoal strains, e.g. Plasmodial strains, for example naturally occurring sub-strains of Plasmodium falciparum exhibiting acquired chemotherapeutic, anti-malarial drug resistance.

9. FKBP binding

Certain of the Novel Compounds are not immunosuppressive, particularly those which are O-substituted at C28 only, such as 28-O-methyl-rapamycin. This can be shown in standard in vitro assays in comparison to FK506 and rapamycin. FK506, for example, is known to be a potent inhibitor of IL-2 transcription, as can be shown in an IL-2 reporter gene assay. Rapamycin, although not active in the IL-2 reporter gene assay, strongly inhibits IL-6 dependent T-cell proliferation. Both compounds are very potent inhibitors of the mixed lymphocyte reaction. Nonimmunosuppressivity can also be shown in the in vivo models 1-7 above. Even those Novel Compounds which are not immunosuppressive, however, bind to macrophilin, which confers certain utilities in which nonimmunosuppressivity is an advantage.

Those of the Novel Compounds which bind strongly to macrophilin and are not themselves immunosuppressive can be used in the treatment of overdoses of macrophilin-binding immunosuppressants, such as FK506, rapamycin, and the immunosuppressive Novel Compounds.

10. Steroid potentiation

The macrophilin binding activity of the Novel Compounds also makes them useful in enhancing or potentiating the action of corticosteroids. Combined treatment with the compounds of the invention and a corticosteroid, such as dexamethasone, results in greatly enhanced steroidal activity. This can be shown, e.g., in the murine mammary tumor virus-

chloramphenicol acetyltransferase (MMTV-CAT) reporter gene assay, e.g., as described in Ning, et al., *J. Biol. Chem.* (1993) **268**: 6073. This synergistic effect allows reduced doses of corticosteroids, thereby reducing the risk of side effects in some cases.

11. Mip and Mip-like factor inhibition

Additionally, the Novel Compounds bind to and block a variety of Mip (macrophage infectivity potentiator) and Mip-like factors, which are structurally similar to macrophilin. Mip and Mip-like factors are virulence factors produced by a wide variety of pathogens, including those of the genera Chlamidia, e.g., Chlamidia trachomatis; Neisseria, e.g., Neisseria meningitidis; and Legionella, e.g., Legionella pneumophilia; and also by the obligately parasitic members of the order Rickettsiales. These factors play a critical role in the establishment of intracellular infection. The efficacy of the Novel Compounds in reducing the infectivity of pathogens which produce Mip or Mip-like factors can be shown by comparing infectivity of the pathogens in cells culture in the presence and absence of the macrolides, e.g., using the methods described in Lundemose, et al., *Mol. Microbiol.* (1993) 7: 777. The nonimmunosuppressive compounds of the invention are preferred for use in this indication for the reason that they are not immunosuppressive, thus they do not compromise the body's natural immune defenses against the pathogens.

The Novel Compounds are also useful in assays to detect the presence or amount of macrophilin-binding compounds, e.g., in competitive assays for diagnostic or screening purposes. Thus, in another embodiment, the invention provides for use of the Novel Compounds as a screening tool to determine the presence of macrophilin-binding compounds in a test solution, e.g., blood, blood serum, or test broth to be screened. Preferably, a Novel Compound is immobilized in microtiter wells and then allowed to bind in the presence and absence of a test solution to labelled macrophilin-12 (FKBP-12). Alternatively, the FKBP-12 immobilized in microtiter wells and allowed to bind in the presence and absence of a test solution to a Novel Compound which has been labelled, e.g., fluoro-, enzymatically- or radio-labelled, e.g., a Novel Compound which has been O-substituted at C40 and/or C28

with a labelling group. The plates are washed and the amount of bound labelled compound is measured. The amount of macrophilin-binding substance in the test solution is roughly inversely proportional to the amount of bound labelled compound. For quantitative analysis, a standard binding curve is made using known concentrations of macrophilin bind compound.

EXAMPLES:

In the following examples, characteristic spectroscopic data is given to facilitate identification. Peaks which do not differ significantly from rapamycin are not included. Biological data is expressed as a relative IC_{50}, compared to rapamycin in the case of the mixed lymphocyte reaction (MLR) and IL-6 dependent proliferation (IL-6 dep. prol.) assays, and to FK-506 in the macrophilin binding assay (MBA). A higher IC_{50} correlates with lower binding affinity.

Example 1: 40-O-Benzyl-rapamycin

To a stirred solution of 183 mg (0.200 mmol) of rapamycin in 2.1 mL of 2:1 cyclohexane-methylene chloride is added 75 µL (0.402 mmol) of benzyl-trichloroacetimidate, followed by 2.6 µL (29 µmol 15 mol%) of trifluoromethanesulfonic acid whereupon the mixture turned immediately yellow. After 3h the mixture is diluted with ethyl acetate and quenched with 10% aqueous sodium bicarbonate. The layers are separated and the aqueous layer is extracted twice with ethyl acetate. The combined organic solution is washed with 10% aqueous sodium bicarbonate, dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The residue is purified by column chromatography on silica gel (50:50 hexane-ethyl acetate) to afford 40-O-benzyl-rapamycin as a white amorphous solid: ^{1}H NMR ($CDCl_3$) δ 0.73 (1H, dd), 1.65 (3H, s), 1.73 (3H, s), 3.12 (4H, s and m), 3.33 (3H, s), 3.49 (3H, s), 4.15 (1H, bd), 4.65 (1H, d), 4.71 (1H, d), 7.22-7.38 (5H, m); MS (FAB) m/z 1026 ($[M+Na]^+$), 972 ($[M-OCH_3)]^+$), 954 ($[M-(OCH_3+H_2O)]^+$).

MBA (rel. IC50)	1.8
IL-6 dep. prol. (rel. IC50)	10
MLR (rel. IC50)	110

Example 2: 40-O-(4'-Hydroxymethyl)benzyl-rapamycin

a) 40-O-[4'-(t-Butyldimethylsilyl)oxymethyl]benzyl-rapamycin

To a stirred, cooled (-78°C) solution of 345 µL (2.0 mmol) of triflic anhydride in 5 mL of methylene chloride is added a solution of 504 mg (2.0 mmol) of 4-(t-

butyldimethylsilyl)oxymethyl-benzyl alcohol and 820 mg (4.0 mmol) of 2,6-di-t-butyl-4-methyl-pyridine in 5 mL of methylene chloride. The resulting mixture is warmed to -20°C and stirring is continued at this temperature for 0.5h. The mixture is then cooled back to -78°C and a solution of 914 mg (1.0 mmol) of rapamycin in 5 mL of methylene chloride is added. This mixture is allowed to warm to room temperature overnight and is then quenched with 10% aqueous sodium bicarbonate. The layers are separated and the aqueous layer is extracted with ethyl acetate. The combined organic solution is washed with saturated brine, dried over sodium sulfate, filtered under reduced pressure and concentrated. The residue is purified by column chromatography on silica gel (50:50 hexane-ethyl acetate) to afford 40-O-[4'-(t-butyldimethylsilyl)oxymethyl]benzyl-rapamycin a white foam: MS (FAB) m/z 1170 ($[M+Na]^+$), 1098 ($[M-(OCH_3+H_2O)]^+$).

b) 40-O-(4'-Hydroxymethyl)benzyl-rapamycin

To a stirred, cooled (0°C) solution of 98 mg (0.093 mmol) of the compound obtained in example 2 in 2 mL of acetonitrile is added 0.2 mL of HF-pyridine. The resulting mixture is stirred for 2h and quenched with aqueous sodium bicarbonate, then extracted with ethyl acetate. The organic solution is washed with brine, dried over sodium sulfate, filtered and concentrated. The residue is purified by column chromatography on silica gel (20:80 hexane-ethyl acetate) to afford the title compound as a white foam: ^1H NMR ($CDCl_3$) δ 0.73 (1H, dd), 1.65 (3H, s), 1.74 (3H, s), 3.22 (1H, m), 4.67 (4H, m), 7.35 (4H, m); MS (FAB) m/z 1056 ($[M+Na]^+$), 1002 ($[M-OCH_3]^+$), 984 ($[M-(OCH_3+H_2O)]^+$), 966 ($[M-(OCH_3+2H_2O)]^+$), 934 ($[M-(OCH_3+CH_3OH+2H_2O)]^+$).

MBA (rel. IC50)	2.7
IL-6 dep. prol. (rel. IC50)	3.9
MLR (rel. IC50)	3

Example 3: 40-O-[4'-(1,2-Dihydroxyethyl)]benzyl-rapamycin

a) 40-O-[4'-(2,2-Dimethyl-1,3-dioxolan-4-yl)]benzyl-rapamycin

In 10 mL of 1:1 cyclohexane-methylene chloride is dissolved 452 mg (1.24 mmol) of 4-(2,2-dimethyl-1,3-dioxolan-4-yl)benzyl trichloroacetimidate, followed by 0.14 mL (0.64

mmol) of 2,6-di-t-butylpyridine and 56 µL (0.64 mmol) of trifluoromethanesulfonic acid. To this mixture is added a solution of 587 mg (0.64 mmol) of rapamycin in 2 mL of methylene chloride. The reaction is stirred overnight at room temperature and quenched with aqueous sodium bicarbonate. The layers are separated and the aqueous layer is extracted twice with ethyl acetate. The combined organic solution is wshed with saturated brine, dried over anhydrous sodium sulfate, filtered and concentrated. The residue is purified by column chromatography on silica gel (50:50 hexane-ethyl acetate) to give 40-O-[4'-(2,2-Dimethyl-1,3-dioxolan-4-yl)]benzyl-rapamycin as a white, amorphous solid: ^{1}H NMR ($CDCl_3$) δ 0.73 (1H, dd), 1.48 (3H, s), 1.55 (3H, s), 1.65 (3H, s), 1.74 (3H, s), 3.67 (3H, m), 4.28 (1H, dd), 4.62 (1H, d), 4.69 (1H, d), 5.06 (1H, dd), 7.33 (4H, m); MS (FAB) m/z 1126 ([M+Na]$^+$), 1072 ([M-OCH_3]$^+$), 1054 ([M-(OCH_3+H_2O)]$^+$), 1014 ([M-(OCH_3+CH_3COCH_3)]$^+$), 996 ([M-(OCH_3+H_2O+CH_3COCH_3)]$^+$), 978 ([M-(OCH_3+2H_2O+ CH_3COCH_3)]$^+$).

b) 40-O-[4'-(1,2-Dihydroxyethyl)]benzyl-rapamycin

To a solution of 90.7 mg (0.08 mmol) of 40-O-[4'-(2,2-Dimethyl-1,3-dioxolan-4-yl)]benzyl-rapamycin in 4 mL of methanol is added 1 mL of 1N aqueous HCl. After 2h the mixture is quenched with aqueous sodium bicarbonate and extracted twicw with ethyl acetate. The organic solution is washed with brine, dried over anhydrous sodium sulfate and concentrated. The residue is purified by column chromatography on silica gel (ethyl acetate) and the title compound is obtained as a white foam: ^{1}H NMR ($CDCl_3$) δ 0.73 (1H, dd), 1.65 (3H, s), 1.74 (3H, s), 3.70 (4H, m), 4.63 (1H, d), 4.69 (1H, d), 4.80 (1H, dd), 7.33 (4H, m); MS (FAB) m/z 1086 ([M+Na]$^+$), 1032 ([M-OCH_3]$^+$), 1014 ([M-(OCH_3+H_2O)]$^+$), 996 ([M-(OCH_3+2H_2O)]$^+$).

MBA (rel. IC50)	0.92
IL-6 dep. prol. (rel. IC50)	10.5
MLR (rel. IC50)	22

Example 4: 40-O-Allyl-rapamycin

To a stirred, cooled (-78°C) solution of 0.33 mL (2.01 mmol) of triflic anhydride in 10 mL of methylene chloride is slowly added a solution of 0.14 mL (2.06 mmol) of allyl

alcohol and 0.42 g (2.04 mmol) of 2,6-di-t-butyl-4-methyl-pyridine in 5 mL of methylene chloride. The resulting greenish solution is stirred for 1.5h and a solution of 915 mg (1.00 mmol) of rapamycin and 0.42 g (2.04 mmol) of 2,6-di-t-butyl-4-methyl-pyridine in 5 mL of methylene chloride is added. Stirring is continued for 0.5h at -78°C and then the mixture is warmed to room temperature. After one more hour the mixture is quenched with aqueous sodium bicarbonate and the layers are separated. The aqueous layer is extracted twice with ethyl acetate. The combined organic solution is washed with aqueous sodium bicarbonate and brine, dried over anhydrous sodium sulfate, filtered and concentrated. The resulting green oil is purified by column chromatography on silica gel (60:40 hexane-ethyl acetate) to afford the title compound as a colorless, amorphous solid: ^{1}H NMR ($CDCl_3$) δ 0.72 (1H, dd), 1.65 (3H, s), 1.74 (3H, s), 3.05 (1H, m), 4.13 (2H, bd), 5.14 (2H, m), 5.27 (2H, m), 5.92 (2H, m); MS (FAB) m/z 976 ($[M+Na]^+$), 922 ($[M-OCH_3]^+$), 904 ($[M-(OCH_3+H_2O)]^+$), 886 ($[M-(OCH_3+2H_2O)]^+$), 872 ($[M-(2CH_3OH+OH)]^+$), 854 ($[M-(OCH_3+CH_3OH+2H_2O)]^+$).

MBA (rel. IC50)	1
IL-6 dep. prol. (rel. IC50)	8
MLR (rel. IC50)	260

Example 5: 40-O-[3'-(2,2-Dimethyl-1,3-dioxolan-4(S)-yl)-prop-2'-en-1'-yl]-rapamycin

To a stirred, cooled (-78°C) solution of 0.64 g (4.00 mmol) of E-(4S)-4,5-O,O-isopropylidene-pent-2-en-1,4,5-triol and 1.26 g (6.00 mmol) of 2,6-di-t-butyl-4-methyl-pyridine in 20 mL of methylene chloride is added 0.82 mL (5.00 mmol) of triflic anhydride. The resulting mixture is stirred at this temperature for 2h and a solution of 1.82 g (2.00 mmol) of rapamycin and 1.26 g (6.00 mmol) of 2,6-di-t-butyl-4-methyl-pyridine in 5 mL of methylene chloride is added. The mixture is allowed to gradually warm to room temperature overnight and is then quenched with aqueous sodium bicarbonate. The layers are separated and the aqueous layer is extracted three times with ethyl acetate. The organic solution is washed with aqueous sodium bicarbonate and brine, dried over anhydrous sodium sulfate, filtered and concentrated. The residue is purified by column chromatography on silica gel

(40:60 hexane-ethyl acetate) to afford the title compound as a white solid: ^{1}H NMR ($CDCl_3$) δ 0.72 (1H, dd), 1.38 (3H, s), 1.42 (3H, s), 1.65 (3H, s), 1.73 (3H, s), 3.06 (1H, m), 3.58 (2H, m), 4.08 (1H, dd), 4.15 (2H, m), 4.52 (1H, bdd), 5.72 (1H, m), 5.88 (1H, m); MS (FAB) m/z 1076 ($[M+Na]^+$), 1022 ($[M-OCH_3]^+$), 1004 ($[M-(OCH_3+H_2O)]^+$), 964 ($[M-(OCH_3+CH_3COCH_3)]^+$), 946 ($[M-(OCH_3+H_2O+CH_3COCH_3)]^+$), 946 ($[M-(OCH_3+2H_2O+CH_3COCH_3)]^+$).

MBA (rel. IC50)	0.64
IL-6 dep. prol. (rel. IC50)	11
MLR (rel. IC50)	8

Example 6: (2'E, 4'S)-40-O-(4',5'-Dihydroxypent-2'-en-1'-yl)-rapamycin

The conditions described in example 3, step b) applied to the compound obtained in in the previous example, followed by purification through column chromatography on silica gel (95:5 ethyl acetate-methanol) afford the title compound as a white foam: ^{1}H NMR ($CDCl_3$) δ 0.68 (1H, dd), 3.04 (1H, m), 4.18 (5H, m), 5.75 (1H, dd), 5.88 (1H, m); MS (FAB) m/z 1036 ($[M+Na]^+$), 1013 (M^+), 995 ($[M-H_2O]^+$), 982 ($[M-OCH_3]^+$), 964 ($[M-(OCH_3+H_2O)]^+$), 946 ($[M-(OCH_3+2H_2O)]^+$), 832 ($[M-(2CH_3OH+OH)]^+$), 914 ($[M-(OCH_3+CH_3OH+2H_2O)]^+$).

MBA (rel. IC50)	1.7
IL-6 dep. prol. (rel. IC50)	12
MLR (rel. IC50)	3.5

Example 7: 40-O-(2-Hydroxy)ethoxycarbonylmethyl-rapamycin

a) 40-O-[2-(t-Butyldimethylsilyl)oxy]ethoxycarbonylmethyl-rapamycin

To a stirred solution of 2.74 g (3.00 mmol) of rapamycin and 30 mg (0.06 mmol) of dirhodium tetraacetate dihydrate in 30 mL of methylene chloride is added a solution of 0.38 mL (3.60 mmol) of 2-(t-butyldimethylsilyl)oxyethyl diazoacetate in 10 mL of methylene chloride over 5h. After the addition is complete stirring is continued for one more hour, then the reaction is quenched with 1N aq. HCl. The layers are separated and the aqueous layer is

extracted with ethyl acetate. The combined organic solution is washed with aq. sodium bicarbonate and brine, dried over anhydrous sodium sulfate, filtered and concentrated. The residue is purified by column chromatography on silica gel (40:60 hexane-ethyl acetate) yielding 40-O-[2-(t-butyldimethylsilyl)oxy]ethoxycarbonylmethyl-rapamycin: ^{1}H NMR ($CDCl_3$) δ 0.06 (6H, s), 0.68 (1H, dd), 0.88 (9H, s), 1.64 (3H, s), 1.73 (3H, s), 3.12 (5H, s and m), 3.81 (2H, dd), 4.19 (2H, dd), 4.32 (2H, s); MS (FAB) m/z 1152 ([M+Na]$^+$), 1080 ([M-(OCH_3+H_2O)]$^+$).

b) 40-O-(2-Hydroxy)ethoxycarbonylmethyl-rapamycin

To a stirred, cooled (0°C) solution of 81 mg (0.07 mmol) of 40-O-[2-(t-butyldimethylsilyl)oxy]ethoxycarbonylmethyl-rapamycin in 1.5 mL of acetonitrile is added 0.15 mL of HF-pyridine. After 2h the reaction is quenched with aq. sodium bicarbonate. The mixture is extracted with ethyl acetate. The organic solution is washed with brine, dried over anhydrous sodium sulfate, filtered and concentrated. The residue is purified by PTLC (ethyl acetate) to afford the title compound as a white solid: ^{1}H NMR ($CDCl_3$) δ 0.70 (1H, dd), 1.65 (3H, s), 1.75 (3H, s), 3.13 (5H, s and m), 3.85 (3H, m), 4.25 (5H, m); MS (FAB) m/z 1038 ([M+Na]$^+$), 984 ([M-OCH_3]$^+$), 966 ([M-(OCH_3+H_2O)]$^+$), 948 ([M-(OCH_3+2H_2O)]$^+$).

MBA (rel. IC50)	4
IL-6 dep. prol. (rel. IC50)	9.7
MLR (rel. IC50)	2.1

Example 8: 40-O-(2-Hydroxy)ethyl-rapamycin

a) 40-O-[2-(t-Butyldimethylsilyl)oxy]ethyl-rapamycin

A solution of 9.14 g (10 mmol) of rapamycin and 4.70 mL (40 mmol) of 2,6-lutidine in 30 mL of toluene is warmed to 60°C and a solution of 6.17 g (20 mmol) of 2-(t-butyldimethylsilyl)oxyethyl triflate and 2.35 mL (20 mmol) of 2,6-lutidine in 20 mL of toluene is added. This mixture is stirred for 1.5h. Then two batches of a solution of 3.08 g (10 mmol) of triflate and 1.2 mL (10 mmol) of 2,6-lutidine in 10 mL of toluene are added in a 1.5h interval. After addition of the last batch, stirring is continued at 60°C for 2h and the resulting brown suspension is filtered. The filtrate is diluted with ethyl acetate and washed

with aq. sodium bicarbonate and brine. The organic solution is dried over anhydrous sodium sulfate, filtered and concentrated. The residue is purified by column chromatography on silica gel (40:60 hexane-ethyl acetate) to afford 40-O-[2-(t-butyldimethylsilyl)oxy]ethyl-rapamycin as a white solid: ^{1}H NMR ($CDCl_3$) δ 0.06 (6H, s), 0.72 (1H, dd), 0.90 (9H, s), 1.65 (3H, s), 1.75 (3H, s), 3.02 (1H, m), 3.63 (3H, m), 3.72 (3H, m); MS (FAB) m/z 1094 ($[M+Na]^+$), 1022 ($[M-(OCH_3+H_2O)]^+$).

b) 40-O-(2-Hydroxy)ethyl-rapamycin

To a stirred, cooled (0°C) solution of 4.5 g (4.2 mmol) of 40-O-[2-(t-butyldimethylsilyl)oxy]ethyl-rapamycin in 20 mL of methanol is added 2 mL of 1N HCl. This solution is stirred for 2h and neutralized with aq. sodium bicarbonate. The mixture is extracted with three portions of ethyl acetate. The organic solution is washed with aq. sodium bicarbonate and brine, dried over anhydrous sodium sulfate, filtered and concentrated. Purification by column chromatography on silica gel (ethyl acetate) gave the title compound as a white solid: ^{1}H NMR ($CDCl_3$) δ 0.72 (1H, dd), 1.65 (3H, s), 1.75 (3H, s), 3.13 (5H, s and m), 3.52-3.91 (8H, m); MS (FAB) m/z 980 ($[M+Na]^+$), 926 ($[M-OCH_3]^+$), 908 ($[M-(OCH_3+H_2O)]^+$), 890 ($[M-(OCH_3+2H_2O)]^+$), 876 ($[M-(2CH_3OH+OH)]^+$), 858 ($[M-(OCH_3+CH_3OH+2H_2O)]^+$).

MBA (rel. IC50)	2.2
IL-6 dep. prol. (rel. IC50)	2.8
MLR (rel. IC50)	3.4

Example 9: 40-O-(3-Hydroxy)propyl-rapamycin

a) 40-O-[3-(t-Butyldimethylsilyl)oxy]propyl-rapamycin

The same procedure as described in example 8, step a) using 3-(t-butyldimethylsilyl)oxyprop-1-yl triflate affords 40-O-[3-(t-butyldimethylsilyl)oxy]propyl-rapamycin: ^{1}H NMR ($CDCl_3$) δ 0.05 (6H, s), 0.72 (1H, dd), 0.90 (9H, s), 1.65 (3H, s), 1.74 (3H, s), 1.77 (2H, m), 3.03 (1H, m), 3.52-3.73 (7H, m); MS (FAB) m/z 1108 ($[M+Na]^+$), 1036 ($[M-(OCH_3+H_2O)]^+$).

b) 40-O-(3-Hydroxy)propyl-rapamycin

Treatment of the compound obtained in step a) in the conditions described in example 8, step b) yields the title compound: ^{1}H NMR ($CDCl_3$) δ 0.72 (1H, dd), 1.65 (3H, s), 1.75 (3H, s), 1.80 (2H, m), 3.05 (1H, m), 3.55-3.91 (8H, m); MS (FAB) m/z 994 ($[M+Na]^+$), 940 ($[M-OCH_3]^+$), 922 ($[M-(OCH_3+H_2O)]^+$), 904 ($[M-(OCH_3+2H_2O)]^+$), 872 ($[M-(OCH_3+CH_3OH+2H_2O)]^+$).

MBA (rel. IC50)	1.6
IL-6 dep. prol. (rel. IC50)	2.7
MLR (rel. IC50)	11

Example 10: 40-O-(6-Hydroxy)hexyl-rapamycin

a) 40-O-[6-(t-Butyldimethylsilyl)oxy]hexyl-rapamycin

The same procedure as described in example 8, step a) using 6-(t-butyldimethylsilyl)oxyhex-1-yl triflate affords 40-O-[6-(t-Butyldimethylsilyl)oxy]hexyl-rapamycin: MS (FAB) m/z 1150 ($[M+Na]^+$).

b) 40-O-(6-Hydroxy)hexyl-rapamycin

Treatment of the compound obtained in step a) in the conditions described in example 8, step b) yields the title compound: ^{1}H NMR ($CDCl_3$) δ 0.72 (1H, dd), 1.38 (2H, m), 1.57 (4H, m), 1.65 (3H, s), 1.74 (3H, s), 3.02 (1H, m), 3.49-3.72 (8H, m); MS (FAB) m/z 1036 ($[M+Na]^+$), 982 ($[M-OCH_3]^+$), 964 ($[M-(OCH_3+H_2O)]^+$), 946 ($[M-(OCH_3+2H_2O)]^+$), 914 ($[M-(OCH_3+CH_3OH+2H_2O)]^+$).

MBA (rel. IC50)	0.8
IL-6 dep. prol. (rel. IC50)	8.5
MLR (rel. IC50)	18

Example 11: 40-O-[2-(2-Hydroxy)ethoxy]ethyl-rapamycin

a) 40-O-[2-(t-Butyldimethylsilyl)oxyethoxy]ethyl-rapamycin

The same procedure as described in example 8, step a) using 2-[2-(t-butyldimethylsilyl)oxy-ethoxy]ethyl triflate affords 40-O-[2-(t-butyldimethylsilyl)oxyethoxy]ethyl-rapamycin: ^{1}H NMR ($CDCl_3$) δ 0.06 (6H, s), 0.71 (1H, dd), 0.88 (9H, s), 1.65 (3H, s), 1.74 (3H, s), 3.07

(1H, m), 3.51-3.79 (11H, m); MS (FAB) m/z 1138 ([M+Na]$^+$), 1115 (M$^+$), 1097 ([M-H_2O]$^+$), 1084 ([M-OCH_3]$^+$), 1066 ([M-(OCH_3+H_2O)]$^+$), 1048 ([M-(OCH_3+2H_2O)]$^+$), 1034 ([M-(2CH_3OH+OH)]$^+$), 1016 ([M-(OCH_3+CH_3OH+2H_2O)]$^+$).

b) 40-O-[2-(2-Hydroxy)ethoxy]ethyl-rapamycin

Treatment of the compound obtained in step a) in the conditions described in example 8, step b) yields the title compound: ^{1}H NMR ($CDCl_3$) δ 0.72 (1H, dd), 1.65 (3H, s), 1.74 (3H, s), 3.05 (1H, m), 3.51-3.77 (11H, m); MS (FAB) m/z 1024 ([M+Na]$^+$), 1001 (M$^+$), 983 ([M-H_2O]$^+$), 970 ([M-OCH_3]$^+$), 952 ([M-(OCH_3+H_2O)]$^+$), 934 ([M-(OCH_3+2H_2O)]$^+$), 920 ([M-(2CH_3OH+OH)]$^+$), 902 ([M-(OCH_3+CH_3OH+2H_2O)]$^+$).

MBA (rel. IC50)	1.2
IL-6 dep. prol. (rel. IC50)	3.2
MLR (rel. IC50)	2

Example 12: 40-O-[(3S)-2,2-Dimethyldioxolan-3-yl]methyl-rapamycin

The same procedure as described in example 8, step a) using the triflate of glycerol acetonide affords the title compound: ^{1}H NMR ($CDCl_3$) δ 0.72 (1H, dd), 1.36 (3H, s), 1.42 (3H, s), 1.65 (3H, s), 1.75 (3H, s), 3.06 (1H, m), 3.55 (2H, m), 3.69 (3H, m), 4.06 (1H, dd), 4.26 (1H, m); MS (FAB) m/z 1050 ([M+Na]$^+$), 996 ([M-OCH_3]$^+$), 978 ([M-(OCH_3+H_2O)]$^+$), 960 ([M-(OCH_3+2H_2O)]$^+$).

MBA (rel. IC50)	0.9
IL-6 dep. prol. (rel. IC50)	8
MLR (rel. IC50)	290

Example 13: 40-O-[(2S)-2,3-Dihydroxyprop-1-yl]-rapamycin

Treatment of the compound obtained in the previous example in the conditions described in example 3 yields the title compound: ^{1}H NMR ($CDCl_3$) δ 0.72 (1H, dd), 1.65 (3H, s), 1.75 (3H, s), 3.07 (1H, m), 3.68 (8H, m); MS (FAB) m/z 1010 ([M+Na]$^+$), 956 ([M-OCH_3]$^+$), 938 ([M-(OCH_3+H_2O)]$^+$), 920 ([M-(OCH_3+2H_2O)]$^+$), 888 ([M-(OCH_3+CH_3OH 2H_2O)]$^+$).

MBA (rel. IC50)	0.67
IL-6 dep. prol. (rel. IC50)	9
MLR (rel. IC50)	10

Example 14: 40-O-(2-Acetoxy)ethyl-rapamycin

To a stirred, cooled (0°C) solution of 53 mg (0.055 mmol) of 40-O-hydroxyethyl-rapamycin in 2 mL of methylene chloride is added 0.2 mL of pyridine followed by 0.02 mL (0.281 mmol) of acetyl chloride. The mixture is stirred for 3h and diluted with ethyl acetate, then washed with aq. sodium bicarbonate, cold 1N HCl and again with aq. sodium bicarbonate. The organic solution is dried over anhydrous sodium sulfate, filtered and concentrated. The residue is purified by column chromatography on silica gel (30:70 hexane-ethyl acetate) to afford the title compound as a white solid: ^{1}H NMR ($CDCl_3$) δ 0.72 (1H, dd), 1.65 (3H, s), 1.75 (3H, s), 2.08 (3H, s), 3.07 (1H, m), 3.78 (2H, dd), 4.20 (2H, dd); MS (FAB) m/z 1022 ($[M+Na]^+$), 999 (M^+), 982 ($[M-OH]^+$), 968 ($[M-OCH_3]^+$), 950 ($[M-(OCH_3+H_2O)]^+$), 932 ($[M-(OCH_3+2H_2O)]^+$), 918 ($[M-(2CH_3OH+OH)]^+$), 900 ($[M-(OCH_3+CH_3OH+2H_2O)]^+$).

MBA (rel. IC50)	2
IL-6 dep. prol. (rel. IC50)	7.6
MLR (rel. IC50)	3.6

Example 15: 40-O-(2-Nicotinoyloxy)ethyl-rapamycin

The same procedure as described in the previous example using nicotinoyl chloride hydrochloride affords the title compound: ^{1}H NMR ($CDCl_3$) δ 0.72 (1H, dd), 1.65 (3H, s), 1.75 (3H, s), 3.07 (1H, m), 3.94 (2H, dd), 4.49 (2H, t), 7.39 (1H, dd), 8.31 (1H, ddd), 8.78 (1H, ddd), 9.24 (1H, dd); MS (FAB) m/z 1085 ($[M+Na]^+$), 1063 ($[M+H]^+$), 1045 ($[M-OH]^+$), 1031 ($[M-OCH_3]^+$), 1013 ($[M-(OCH_3+H_2O)]^+$).

MBA (rel. IC50)	1.1
IL-6 dep. prol. (rel. IC50)	6.9
MLR (rel. IC50)	5

Example 16: 40-O-[2-(N-Morpholino)acetoxy]ethyl-rapamycin

a) 40-O-(2-Bromoacetoxy)ethyl-rapamycin

The same procedure as described in example 14 using bromoacetyl chloride affords 40-O-(2-bromoacetoxy)ethyl-rapamycin: ^{1}H NMR ($CDCl_3$) δ 0.72 (1H, dd), 1.67 (3H, s), 1.76 (3H, s), 3.03 (1H, m), 3.82 (2H, m), 3.87 (2H, s), 4.31 (2H, m); MS (FAB) m/z 1100, 1102 ([M+Na]$^+$), 1077 (M$^+$), 1061 ([M-H_2O]$^+$), 1046, 1048 ([M-OCH_3]$^+$), 1028, 1030 ([M-(OCH_3+H_2O)]$^+$), 1012 ([M-(OCH_3+2H_2O)]$^+$), 996 ([M-(2CH_3OH+OH)]$^+$), 980 ([M-(OCH_3+CH_3OH+2H_2O)]$^+$).

b) 40-O-[2-(N-Morpholino)acetoxy]ethyl-rapamycin

To a stirred, cooled (-45°C) solution of 54 mg (0.05 mmol) of 40-O-(2-bromoacetoxy)ethyl-rapamycin in 0.5 mL of DMF is added a solution of 0.022 mL (0.25 mmol) of morpholine in 0.2 mL of DMF and the resulting mixture is stirred at that temperature for 1h, then treated with aq. sodium bicarbonate. This mixture is extracted three times with ethyl acetate. The organic solution is washed with brine, dried over anhydrous sodium sulfate, filtered and concentrated. The residue is purified by column chromatography on silica gel (95:5 ethyl acetate-methanol) yielding the title compound as an amorphous white solid: ^{1}H NMR ($CDCl_3$) δ 0.72 (1H, dd), 1.67 (3H, s), 1.76 (3H, s), 2.60 (3H, m), 3.07 (1H, m), 3.24 (2H, s), 3.78 (8H, m), 4.27 (2H, t); MS (FAB) m/z 1107 ([M+Na]$^+$), 1085 ([M+H]$^+$), 1067 ([M-OH]$^+$), 1053 ([M-OCH_3]$^+$), 1035 ([M-(OCH_3+H_2O)]$^+$).

MBA (rel. IC50)	1.3
IL-6 dep. prol. (rel. IC50)	4
MLR (rel. IC50)	3.5

Example 17: 40-O-(2-N-Imidazolylacetoxy)ethyl-rapamycin

The same procedure as described in example 16, step b) using imidazole affords the title compound: ^{1}H NMR ($CDCl_3$) δ 0.72 (1H, dd), 1.67 (3H, s), 1.78 (3H, s), 3.06 (1H, m), 3.80 (2H, m), 4.32 (2H, m), 4.73 (2H, s), 6.97 (1H, dd), 7.09 (1H, dd), 7.52 (1H, dd); MS (FAB) m/z 1066 ([M+H]$^+$), 1048 ([M-OH]$^+$), 1034 ([M-OCH_3]$^+$), 1016 ([M-(OCH_3+ H_2O)]$^+$).

MBA (rel. IC50)	1

IL-6 dep. prol. (rel. IC50)	7.6
MLR (rel. IC50)	3.4

Example 18: 40-O-[2-(N-Methyl-N'-piperazinyl)acetoxy]ethyl-rapamycin

The same procedure as described in example 16, step b) using N-methylpiperazine affords the title compound: ^{1}H NMR ($CDCl_3$) δ 0.72 (1H, dd), 1.67 (3H, s), 1.77 (3H, s), 2.78 (4H, s and m), 3.02 (4H, bs), 3.08 (1H, m), 3.32 (2H, s), 3.80 (2H, dd), 4.27 (2H, t); MS (FAB) m/z 1098 ($[M+H]^+$), 1066 ($[M-OCH_3]^+$).

MBA (rel. IC50)	2.6
IL-6 dep. prol. (rel. IC50)	10.3
MLR (rel. IC50)	5

Example 19: 39-O-Desmethyl-39,40-O,O-ethylene-rapamycin

To a stirred, cooled (-20°C) solution of 48 mg (0.05 mmol) of 40-O-hydroxyethyl-rapamycin and 0.023 mL (0.20 mmol) of 2,6-lutidine in 0.5 mL of methylene chloride is added 0.008 mL (0.05 mmol) of triflic anhydride. The mixture is stirred at this temperature for 2h, then allowed to warm to room temperature and stirred for one more hour. The reaction is quenched with aq. sodium bicarbonate and the resulting mixture is extracted with three portions of ethyl acetate. The organic solution is washed with brine, dried over anhydrous sodium sulfate, filtered and concentrated. The residue is purified by column chromatography on silica gel (30:70 hexane-ethyl acetate) to afford the title compound as a white solid: ^{1}H NMR ($CDCl_3$) δ 1.66 (3H, s), 1.75 (3H, s), 3.14 (3H, s), 3.35 (3H, s), 3.76 (4H, s); MS (FAB) m/z 948 ($[M+Na]^+$), 925 (M^+), 908 ($[M-OH]^+$), 894 ($[M-OCH_3]^+$), 876 ($[M-(OCH_3+H_2O)]^+$), 858 ($[M-(OCH_3+ 2H_2O)]^+$), 844 ($[M-(2CH_3OH+OH)]^+$), 826 ($[M-(OCH_3+CH_3OH+2H_2O)]^+$).

MBA (rel. IC50)	1.6
IL-6 dep. prol. (rel. IC50)	22.9
MLR (rel. IC50)	16

Example 20: (26R)-26-Dihydro-40-O-(2-hydroxy)ethyl-rapamycin

a) (26R)-26-Dihydro-40-O-[2-(t-Butyldimethylsilyloxy)]ethyl-rapamycin

In 4.5 mL of 2:1 acetonitrile-acetic acid is dissolved 315 mg (1.2 mmol) of tetramethylammonium-triacetoxyborohydride. The resulting solution is stirred for 1h at room temperature and cooled to -35°C, then 161 mg (0.15 mmol) of 40-O-[2-(t-butyldimethylsilyl)oxy]ethyl-rapamycin is added. The resulting mixture is stirred at the same temperature overnight and is quenched by the addition of aq. sodium bicarbonate. The mixture is extracted with three portions of ethyl acetate. The organic solution is washed with aq. sodium bicarbonate, two portions of 30% aq. Rochelle's salt and brine, dried over anhydrous sodium sulfate, filtered and concentrated. The residue is purified by column chromatography on silica gel (40:60 hexane-ethyl acetate) to afford the title compound as a white solid: ^{1}H NMR ($CDCl_3$) δ 0.06 (6H, s), 0.73 (1H, dd), 0.90 (9H, s), 1.64 (3H, s), 1.67 (3H, s), 3.02 (1H, m), 3.15 (1H, m), 3.64 (3H, m), 3.71 (2H, dd), 3.91 (1H, s); MS (FAB) m/z 1096 ([M+Na]$^+$), 1041 ([M-$HOCH_3$]$^+$), 1024 ([M-(OCH_3+H_2O)]$^+$), 1006 ([M-(OCH_3+2H_2O)]$^+$), 974 ([M-(OCH_3+CH_3OH+2H_2O)]$^+$).

b) (26R)-26-Dihydro-40-O-(2-hydroxy)ethyl-rapamycin

Treatment of the compound obtained in step a) in the conditions described in example 8, step b) yields the title compound: ^{1}H NMR ($CDCl_3$) δ 0.75 (1H, dd), 1.66 (3H, s), 1.70 (3H, s), 3.18 (1H, m), 3.52-3.84 (7H, m); MS (FAB) m/z 982 ([M+Na]$^+$), 928 ([M-OCH_3]$^+$), 910 ([M-(OCH_3+H_2O)]$^+$), 892 ([M-(OCH_3+2H_2O)]$^+$).

MBA (rel. IC50)	3.9
IL-6 dep. prol. (rel. IC50)	53
MLR (rel. IC50)	18

Example 21: 28-O-Methyl-rapamycin

To a stirred solution of 103 mg (0.1 mmol) of 40-O-TBS-rapamycin (obtained by silylation of rapamycin with 1 eq. of TBS triflate in methylene chloride in the presence of 2 eq. of 2,6-lutidine at 0°C) in 0.5 mL of methylene chloride is added 85.8 mg (0.40 mmol) of proton sponge followed by 44 mg (0.30 mmol) of trimethyloxonium tetrafluoroborate. The

resulting brown heterogeneous mixture is stirred overnight, quenched with aq. sodium bicarbonate and extracted with ethyl acetate. The organic solution is washed with 1N HCl, aq. sodium bicarbonate and brine, then dried over anhydrous sodium sulfate, filtered and concentrated. The residue is purified by column chromatography on silica gel (60:40 hexane-ethyl acetate) to afford 40-O-t-butyldimethylsilyl-28-O-methyl-rapamycin. The latter compound is desilylated in the conditions described in example 10, step b) to afford, after PTLC (ethyl acetate), the title compound as a white solid: ^{1}H NMR ($CDCl_3$) δ 0.70 (1H, dd), 1.68 (6H, 2s), 2.95 (1H, m), 3.13 (3H, s), 3.14 (3H, s), 3.28 (3H, s), 3.41 (3H, s); MS (FAB) m/z 950 ($[M+Na]^+$), 927 (M^+), 909 ($[M-H_2O]^+$), 896 ($[M-OCH_3]^+$), 878 ($[M-(OCH_3+H_2O)]^+$), 864 ($[M-(OCH_3+ CH_3OH)]^+$), 846 ($[M-(2CH_3OH+OH)]^+$), 832 ($[M-(OCH_3+2CH_3OH)]^+$), 814 ($[M-(3CH_3OH+ OH)]^+$).

MBA (rel. IC50)	1.58
IL-6 dep. prol. (rel. IC50)	1240
MLR (rel. IC50)	1300

Example 22: 40-O-(2-aminoethyl)-rapamycin

a) 40-O-(2-bromoethyl)-rapamycin

A solution of 914 mg rapamycin in 5 mL toluene containing 0.64 ml of 2,6-lutidine and 1.28 g of 2-bromoethyl triflate is heated at 65 C for 18 h. The reaction mixture is then cooled to room temperature, poured on 20 ml of a saturated bicarbonate solution and extracted with 3x 20 mL ethyl acetate. The organic phases are dried over sodium carbonate and the solvent removed at reduced pressure on the rotatory evaporator. The residue is chromatographed on 100 g silica gel, eluting with hexane/ethyl acetate 3/2 to afford 40-O-(2-bromoethyl)-rapamycin as an amorphous solid: MS (FAB) m/z 1044 and 1042 (100%; M+Na); 972 and 970 (55%, M-(MeOH+H2O)).

H-NMR (CDCl3) d: 0.72 (1H, q, J=12 Hz); 3.13 (3H, s); 3.33 (3H, s); 3.45 (3H,s); 3.9 (4H, m); 4.78 (1H, s)

b) 40-O-(2-azidoethyl)-rapamycin

A solution of 2.4 g of 40-O-(2-bromoethyl)-rapamycin in 40 mL DMF is treated with 0.19 g sodium azide at room temperature. After 2h, the mixture is poured on 100 mL of saturated sodium bicarbonate and extracted with 3x 100 mL ethyl acetate. The organic phases are combined, dried over sodium sulfate and the solvent removed under reduced pressure. The crude product is purified by chromatography on silica gel eluting with hexane/ethyl acetate to afford 40-O-(2-azidoethyl)-rapamycin: MS (FAB): 1005 (100%, M+Na); 951 (24%, M-MeOH); 933 (57%, M-(MeOH+H2O)

c) 40-O-(2-aminoethyl)-rapamycin

To a solution of 230 mg 40-O-(azidoethyl)-rapamycin in 3 mL of THF/water 5/1 at room temperature are added 307 mg of triphenylphosphine. The reaction mixture is becomes yellow. After 7 h, the reaction mixture is loaded on x g silical gel and chromatographed with ethyl acetate/methanol/acetic acid 50/50/0.5 to afford the title product in the form of its acetate: MS (FAB) m/z 979 (45%, M+Na); 957 (100%, MH); 925 (63%, M-MeOH); 907 (25%, M-(MeOH+H2O)

MBA (rel. IC50): 0.7

IL-6 dep. prol. (rel. IC50): 10

Example 23: 40-O-(2-acetaminoethyl)-rapamycin

To a solution of 101 mg of the acetate of 40-O-(2-aminoethyl)-rapamycin in 2 mL THF are added 0.02 mL pyridine and 0.07 mL acetyl chloride. The reaction mixture is kept at room temperature for 18h and then poured on 7 mL saturated sodium bicarbonate. The aqueous phase is extracted 3x with 5 mL ethyl acetate, the organic phases are combined and dried over sodium sulfate. The solvent is evaporated and the residue chromatographed on 10 g silica gel eluting first with ethyl acetate followed by ethyl acetate/methanol/acetic acid 50/50/0.5 to afford the title product: MS (FAB) m/z 1021 (20%, M+Na); 967 (28%, M-MeOH); 949 (100%, M-(MeOH+H2O)

H-NMR (CDCl3) d: 071 (1H, q, J=12 Hz); 1.98 (3H, s); 3.13 (3H, s); 3.34 (3H, s); 3.44 (3H, s); 4.75 (1H, s)

MBA (rel. IC50): 1.1

IL-6 dep. prol. (rel. IC50): 2.3

Example 24: 40-O-(2-nicotinamidoethyl)-rapamycin

101 mg of 40-(2-aminoethyl)-rapamycin acetate are dissolved in 5 ml ethyl acetate and extracted 2x with saturated sodium bicarbonate. The organic phase is dried over sodium sulfate and the solvent evaporated. The residue is dissolved in 2 mL THF and treated with 22 mg DCC and 15 mg nicotinic acid. After 15h at room temperature the reaction mixture is evaporated and the residue chromatogrphed on silica gel, eluting with ethyl acetate followed by ethyl acetate/methanol 9/1, to afford the title product: MS (FAB) m/z 1084 (80%, M+Na); 1062 (40%, MH); 1038 (100%, M-MeOH); 1012 (50%, M-(MeOH+H2O)
H-NMR (CDCl3) d: 0.72 (1H, q, J=12 Hz); 3.13 (3H, s); 3.33 (3H, s); 3.37 (3H, s); 7.39 (1H, dd; J=6 Hz, J=8 Hz), 8.19 (1H, d, J=8 Hz); 8.75 (1H, d, J=6 Hz); 9.04 (1H, broad s)
MBA (rel. IC50): 1.2
IL-6 dep. prol. (rel. IC50): 2.8

Example 25: 40-O-(2-(N-Methyl-imidazo-2'-ylcarbethoxamido)ethyl)-rapamycin

To a solution of 30 mg N-methyl-imidazol-2-carboxylic acid in 1 mL DMF are added 58 mg DCC and 58 mg HOBT. After 2h, 150 mg 40-O-(2-aminoethyl)-rapamycin are added and the reaction mixture is stirred for 18h at room temperature. The suspension is then filtered, the filtrate diluted with 5 mL ethyl acetate and washed with 2x 2 mL of a saturated aqueous bicarbonate solution. The organic phase is dried over sodium sulfate and the solvent evaporated under reduced pressure. The residue is chromatographed over 10 silica gel, eluting with hexane/ethyl acetate 1/4 and then ethyl acetate to afford the title product:
MS (FAB) m/z 1087 (36%, M+Na); 1065 (57%,MH); 1033 (100%, M-MeOH); 1015 (46%, M-(MeOH+H2O))
H-NMR (CDCl3) d: 0.72 (1H, q, J=12 Hz); 3.13 (3H, s); 3.33 (3H, s); 3.46 (3H, s); 4.03 (3H, s); 6.93 (1H, broad s); 6.98 (1H, broad s); 7.78 (1H, m)
MBA (rel. IC50): 1.1

IL-6 dep. prol. (rel. IC50): 7

Example 26: 40-O-(2-ethoxycarbonylaminoethyl)-rapamycin

A solution of 200 mg 40-O-(2-azidoethyl)-rapamycin in 3 mL THF/water 5/1 is treated with 267 mg triphenylphosphine for 7h at room temperature. Then 0.4 mL pyridine are added followd by 194 µL ethyl chloroformiate. After 2 h, the reaction mixture is poured on 5 mL ethyl acetate and washed successively with 10 mL saturated sodium bicarbonate, 5 mL water and 5 ml 10% citric acid. The organic phase is dried over sodium sulfate and the solvent evaporated. The residue is chromatographed over 20 g silica gel, eluting with ethyl acetate followed by ethyl acetate/methanol 9/1, to afford the title product.: MS (FAB) m/z 1051 (35%, M+Na); 997 (30%, M-MeOH); 979 (100%, M-(MeOH+H2O)
H-NMR (CDCl3) d: 0.71 (1H, q, J=12 Hz); 1.24 (3H, t, J=8 Hz); 3.13 (3H, s); 3.34 (3H, s); 3.43 (3H, s); 4.10 (2H, q, J=8 Hz); 5.48 (1H, m)
MBA (rel. IC50): 1.1
IL-6 dep, prol. (rel. IC50): 1.7

Example 27: 40-O-(2-tolylsulfonamidoethyl)-rapamycin

A solution of 200 mg 40-O-(2-aminoethyl)-rapamycin in 3 mL THF is treated with 0.4 mL pyridine and 390 mg tosyl chloride and the reaction mixture is stirred for 12h at room temperature. The solution is then poured onto 5 ml of a saturated bicarbonate solution and the aqueous phase is extracted with 2x 5 mL ethyl acetate. The combined organic phases are washed with 5 mL of 10% citric acid and 5mL water. After drying on sodium sulfate the solvent is evaporated and the residue chromatographed on 20 g silica gel, eluting with hexane/ethyl acetate 1/1 to afford the title product as a white foam: MS (FAB) m/z 1133 (100%, M+Na); 1078 (25%, M-MeOH); 1061 (85%, M-(MeOH+H2O))
H-NMR (CDCL3) d: 0.68 (1H, q, J=12Hz); 2,43 (3H, s); 3,13 (3H, s); 3,35 (3H, s); 3,41 (3H, s); 4.76 (1H, s); 5.85 (1H, t, J=6Hz); 7.30 (2H, d, J=8 Hz); 7.75 (2H, d, J=8Hz).
MBA (rel. IC50): 15.9
IL-6 dep. prol. (rel. IC50): 14

Example 28: 40-O-[2-(4',5'-dicarboethoxy-1',2',3'-triazol-1'-yl)-ethyl]-rapamycin

98 mg of 40-O-(2-azidoethyl)-rapamycin and 32 mg diethylacetylene dicarboxylate are suspended in 0.5 ml toluene and heated at 65 C for 5h. The reaction mixture is then cooled at room temperature, loaded on 10 g silica gel and eluted with hexane/ethyl acetate 1/1 to afford the title product: MS (FAB) m/z 1175 (20%,M+Na); 1121 (15%, M-MeOH); 1103 (60%, M-(MeOH+H2O))

H-NMR (CDCl3) d: 0.62 (1H, q, J=12 Hz); 1.40 (3H, t, J=8 Hz); 1.42 (3H, t, J=8 Hz); 3.13 (3H, s); 3.25 (3H, s); 3.33 (3H, s)

MBA (rel. IC50): 2.7

IL-6 dep. prol. (rel. IC50): 12

The previous examples may also be made using as starting material instead of rapamycin, 9-deoxo-rapamycin, 26-dihydro rapamycin, or 9-deoxo-, 26-dihydro-rapamycin. Alternatively, and preferably, as described e.g., in example 20, the rapamycin compounds of the above examples may be hydrogenated or reduced, using suitable protecting groups where necessary. The following novel methods for reducing the keto at C9, or hydrogenating the keto at C26 are provided:

Example 29: Removal of keto at C9

A stream of hydrogen sulfide is passed at room temperature through a stirred solution of 3.2 g (3.5 mmol) of rapamycin in 50 ml pyridine and 2.5 ml DMF. The solution turns from colorless to yellow. After two hours, the introduction of hydrogen sulfide is stopped and stirring is continued for five days, during which time the solution turns gradually orange. TLC and HPLC analysis verifies complete consumption of the starting material and the presence of a single new compound. The solution is purged with nitrogen for one hour and concentrated under reduced pressure. The residue is taken up in ethyl acetate, washed with cold 1N HCl solution (3x), saturated sodium bicarbonate solution and saturated brine. The organic layer is dried over anhydrous sodium sulfate and filtered and concentrated under reduced pressure. The residue is taken up in ether and the precipitated

sulfur is filtered off. Concentration of the ethereal solution followed by column chromatography on silica gel (10:4:1 CH_2Cl_2/i-Pr_2O/MeOH) yields 9-deoxorapamycin as a colorless foam. The identity of the product is confirmed by nuclear magnetic resonance spectroscopy (NMR), mass spectrometry (MS), and/or infrared spectrosopy (IR). 9-deoxorapamycin is found to exhibit the following characteristic physical data: ^{1}H NMR ($CDCL_3$) δ 1.61 (3H,d,J = 1 Hz, C17-CH_3), 1.76 (3H,d,J = 1.2 Hz,C29-CH_3), 2.42 (1H,d,J = 14.5 Hz, H-9), 2.74 (1H,d,J = 14.5 Hz, H-9), 3.13 (3H,s,C16-OCH_3) 3.5 (3H,s,C27-OCH_3), 3.40 (3H,s,C39-OCH_3), 5.40 (1H,d,J = 10 Hz, H-30), 5.57 (1H,dd,J_1 = 8.6 Hz, J_2 = 15 Hz, H-22), 5.96 (1H,d,J = 9 Hz, H-18), 6.09 (1H,d,J = 1.7 Hz, 10-OH), 6.15 (1H,dd,J_1 = 10 Hz, J_2 = 15Hz, H-21), 6.37 (1H,dd,J_1 = 1.5 Hz, J_2 = 5 Hz, H-19), 6.38 (1H,J = 9.5 Hz, H-20). ^{13}C NMR ($CDCl_3$) δ 38.5 (C-9), 98.0 (C-10), 170.7 (C-1), 173.0 (C-8), 208.8 (C-32), 216.9 (C-26).

MS(FAB) m/z 922 8[M+Na^+]), 899 (M^+), 881 ([M-H_2O]$^+$), 868 ([M-OCH_3]$^+$), 850 ([M-(H_2O+OCH_3)]$^+$).

IR (major peaks)(cm^{-1}) 987, 1086, 1193, 1453, 1616, 1717, 1739, 3443.

MBA (rel. IC_{50}): 1

MLR (rel. IC_{50}): 14

IL-6 dep. prol. (rel. IC_{50}): 9

Example 30: Dihydrogenation of keto at C26

To a stirred solution of 421 mg (1.6 mmol) of tetramethylammonium triacetoxyborohydride in 2 ml of acetonitrile is added 2 ml of acetic acid. The resulting mixture is stirred for 30 minutes at room temperature and cooled to -35°C. At this temperature a solution of 180 mg (0.2 mmol) of 9-deoxo-rapamycin in 1 ml of acetonitrile is added and the resulting mixture is allowed to stir for 24 hours. The mixture is quenched with a saturated sodium potassium tartrate solution and allowed to warm to room temperature. Stirring is continued until both layers are clear and ethyl acetate is added. The layers are separated and the aqueous layer is extracted twice with ethyl acetate. The resulting organic solution is washed once with a 10% sodium bicarbonate solution and twice with

saturated brine, then dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The residue is purified by column chromatography on silica gel (90:10 AcOEt-hexane). As the starting material in this case was 9-deoxorapamycin, the final compound is 9-deoxorapamycin, 26-dihydrorapamycin is produced as a colorless foam, having the following characteristic spectroscopic data: ^{1}H NMR ($CDCl_3$) (major isomer) δ.9 (3H,d,J = 6.9 Hz, CH$\underline{CH_3}$), 0.93 (3H,d,J = 6.9 Hz, CH$\underline{CH_3}$), 1.00 (3H,d,J = 6.9 Hz CH$\underline{CH_3}$), 1.07 (3H,d,J = 6.9 Hz, CH$\underline{CH_3}$), 1.17 (3H,d,J = 6.9 Hz, CH$\underline{CH_3}$), 1.61 (3H,d,J = 1Hz, C17-CH_3), 1.73 (3H,d,J = 1.2 Hz, C29-CH_3), 2.43 (1H,dd,J = 4.1 and 16.0 Hz, H-33), 2.46 (1H,d,J = 13.8 Hz, H-9), 2.58 (1H,m,H-25), 2.77 (1H,d,J = 13.8 Hz, H-9), 2.82 (1H,dd,J = 8.3 and 16.0 Hz, H-33), 3.17 (1H,dd,J = 4.1 and 9.2 Hz, H-27), 3.61 (2H,m, H-14 and H28), 5.19 (1H,ddd,J = 4.1, 4.6 and 8.3 Hz, H-34), 5.49 (1H, broad d,J = 5.0 Hz, H-2), 5.56 (1H,d,J = 9.1 Hz, H-30), 5.75 (1H,dd,J = 6.9 and 14.7 Hz, H-22), 5.76 (1H,s,10-OH), 5.99 (1H,broad d,J = 9.2 Hz, H-18), 6.10 (1H,m,H-21), 6.36 (2H,m,H-19 and H-20);
MS (FAB) m/z 924 ([M + Na]), 852 ([M-(H_2O + CH_3O)]$^+$).
MBA (rel. IC_{50}): 47
MLR (rel. IC_{50}): 134
IL-6 dep. prol. (rel. IC_{50}): 78

26-dihydrorapamycin is prepared in the same manner, using rapamycin in place of 9-deoxorapamycin. This product has the following characteristic spectroscopic data: ^{13}C-NMR ($CDCl_3$) (major isomer) d = 208.3 (C-32); 194.0 (C-9); 169.3 (C-1); 166.6 (C-8); 140.9 (C-22); 136.5 (C-29); 136.2 (C-17); 133.5 (C-20); 129.1 (C-21); 128.7 (C-18); 126.2 (C-30); 125.3 (C-19); 98.6 (C-10); 84.4 (C-39); 83.9 (C-16; 81.6 (C-27); 75.4 (C-34); 74.3 (C-28); 73.9 (C-40); 72.9 (C-26); 67.4 (C-14); 59.1 (27-O$\underline{C}H_3$); 56.6 (39-O$\underline{C}H_3$); 55.9 (16-O$\underline{C}H_3$); 51.3 (C-2); 46.8 (C-31); 44.3 (C-6); 40.4 (C-33); 40.4 (C-25); 39.5 (C-24); 38.8 (C-15); 38.0 (C-36); 34.3 (C-23); 34.2 (C-38); 33.5 (C-11); 33.3 (C-37); 33.2 (C-35); 31.5 (C-42); 31.3 (C-41); 30.9 (C-13); 27.1 (C-12); 27.0 (C-3); 25.2 (C-5); 21.4 (23-$\underline{C}H_3$); 20.7 (C-4); 17.3 (11-$\underline{C}H_3$); 16.1 (31-$\underline{C}$H3); 15.9 (35-$\underline{C}H_3$); 14.4 (25-$\underline{C}H_3$); 14.2 (29-$\underline{C}H_3$); 10.3 (17-$\underline{C}H_3$).

MS (FAB) m/z : 884 (M-OCH_3, 35%); 866 (M-[OCH_3 + H_2O], 100%; 848 (M-[OCH_3 + 2 H_2O], 40%).

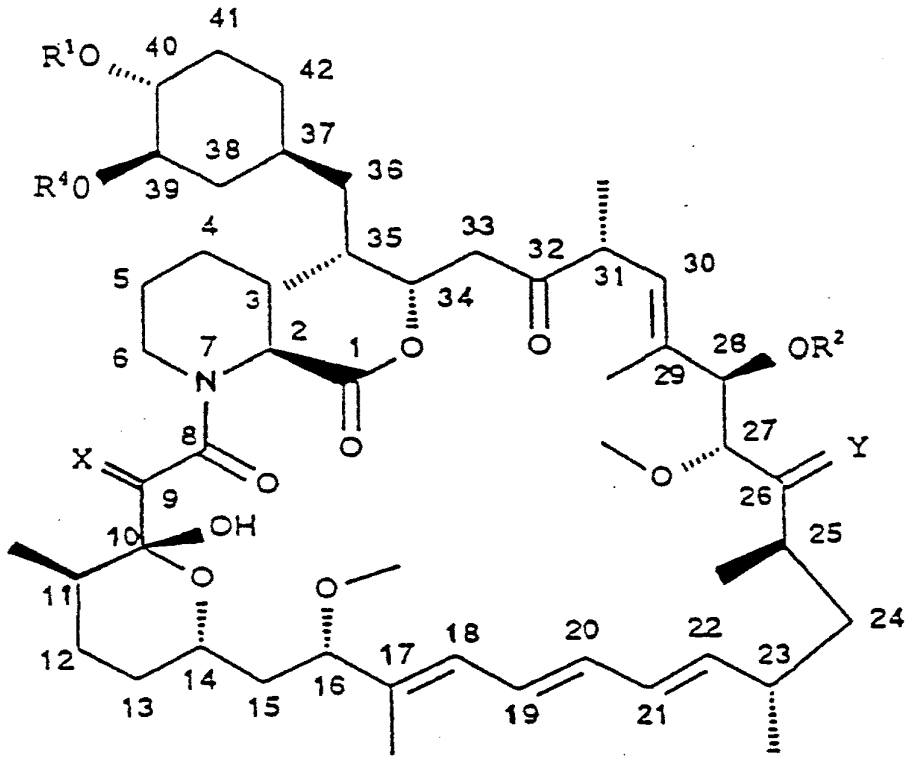
MBA (rel. IC_{50}): 1.7

MLR (rel. IC_{50}): 1

IL-6 dep. prol. (rel. IC_{50}): 7.5

CLAIMS

1. A compound of Formula I



(I)

X is (H,H) or O;

Y is (H,OH) or O;

R^1 and R^2 are independently selected from

H, alkyl, thioalkyl, arylalkyl, hydroxyalkyl, dihydroxyalkyl, hydroxyalkylarylalkyl, dihydroxyalkylarylalkyl, alkoxyalkyl, acyloxyalkyl, aminoalkyl, alkylaminoalkyl, alkoxycarbonylaminoalkyl, acylaminoalkyl,

arylsulfonamidoalkyl, allyl, dihydroxyalkylallyl, dioxolanylallyl, caralkoxyalkyl, and $(R^3)_3Si$ where each R^3 is independently selected from H, methyl, ethyl, isopropyl, t-butyl, and phenyl; wherein "alk-" or "alkyl" refers to C_{1-6} alkyl, branched or linear, preferably C_{1-3} alkyl, in which the carbon chain may be optionally interrupted by an ether (-O-) linkage; and

R^4 is methyl or R^4 and R^1 together form C_{2-6} alkylene;

provided that R^1 and R^2 are not both H; and
provided that where R^1 is carbalkoxyalkyl or $(R^3)_3Si$, X and Y are not both O.

2. Compounds according to claim 1 selected from the following:

1. 40-O-Benzyl-rapamycin
2. 40-O-(4'-Hydroxymethyl)benzyl-rapamycin
3. 40-O-[4'-(1,2-Dihydroxyethyl)]benzyl-rapamycin
4. 40-O-Allyl-rapamycin
5. 40-O-[3'-(2,2-Dimethyl-1,3-dioxolan-4(S)-yl)-prop-2'-en-1'-yl]-rapamycin
6. (2'E, 4'S)-40-O-(4',5'-Dihydroxypent-2'-en-1'-yl)-rapamycin
7. 40-O-(2-Hydroxy)ethoxycarbonylmethyl-rapamycin
8. 40-O-(2-Hydroxy)ethyl-rapamycin
9. 40-O-(3-Hydroxy)propyl-rapamycin
10. 40-O-(6-Hydroxy)hexyl-rapamycin
11. 40-O-[2-(2-Hydroxy)ethoxy]ethyl-rapamycin
12. 40-O-[(3S)-2,2-Dimethyldioxolan-3-yl]methyl-rapamycin
13. 40-O-[(2S)-2,3-Dihydroxyprop-1-yl]-rapamycin
14. 40-O-(2-Acetoxy)ethyl-rapamycin
15. 40-O-(2-Nicotinoyloxy)ethyl-rapamycin
16. 40-O-[2-(N-Morpholino)acetoxy]ethyl-rapamycin

17. 40-O-(2-N-Imidazolylacetoxy)ethyl-rapamycin
18. 40-O-[2-(N-Methyl-N'-piperazinyl)acetoxy]ethyl-rapamycin
19. 39-O-Desmethyl-39,40-O,O-ethylene-rapamycin
20. (26R)-26-Dihydro-40-O-(2-hydroxy)ethyl-rapamycin
21. 28-O-Methyl-rapamycin
22. 40-O-(2-Aminoethyl)-rapamycin
23. 40-O-(2-Acetaminoethyl)-rapamycin
24. 40-O-(2-Nicotinamidoethyl)-rapamycin
25. 40-O-(2-(N-Methyl-imidazo-2'-ylcarbethoxamido)ethyl)-rapamycin
26. 40-O-(2-Ethoxycarbonylaminoethyl)-rapamycin
27. 40-O-(2-Tolylsulfonamidoethyl)-rapamycin
28. 40-O-[2-(4',5'-Dicarboethoxy-1',2',3'-triazol-1'-yl)-ethyl]-rapamycin

3. Compounds according to claim 1 where X and Y are both O, R^2 is H, R^4 is methyl, and R^1 is other than H.

4. 40-O-(2-Hydroxy)ethyl-rapamycin.

5. Compounds according to any one of claims 1 through 4 obtained or obtainable by (i) reacting a rapamycin, deoxorapamycin, or dihydrorapamycin (optionally in O-protected form) with an organic radical attached to a leaving group under suitable acidic or neutral reaction conditions, and (ii) optionally reducing the product..

6. A compound according to any one of claims 1-5 for use as a pharmaceutical.

7. A pharmaceutical composition comprising a compound according to any one of claims 1-5 together with a pharmaceutically acceptable diluent or carrier.

8. Use of a compound according to claims 1-5 in the manufacture of a medicament for

treating or preventing any of the following conditions:

(i) autoimmune disease,

(ii) allograft rejection,

(iii) graft vs. host disease,

(iv) asthma,

(v) multidrug resistance,

(vi) tumors or hyperproliferative disorders, or

(vii) fungal infections,

(viii) inflammation,

(ix) infection by pathogens having Mip or Mip-like factors, or

(x) overdose of macrophilin-binding immunosuppressants.

9. Novel products, processes, and utilities substantially as described herein.

INTERNATIONAL SEARCH REPORT

Internal Application No
PCT/EP 93/02604

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 C07D498/18 C07F7/18 A61K31/435 //C07D498/18,311:00, 273:00,221:00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 5 C07D C07F A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US,A,5 151 413 (C. E. CAUFIELD ET AL) 29 September 1992 see claims 1,13 ---	1,7
X	US,A,5 120 842 (A. A. FAILLI ET AL) 9 June 1992 see claim 1 -----	1

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

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"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

1

Date of the actual completion of the international search	Date of mailing of the international search report
14 December 1993	28. 12. 93

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Voyiazoglou, D

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Information on patent family members

Interna[illegible]nal Application No
PCT/EP 93/02604

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
US-A-5151413	29-09-92	NONE		
US-A-5120842	09-06-92	AU-A-	1389392	08-10-92
		EP-A-	0507556	07-10-92
		JP-A-	5078377	30-03-93

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BIB DATA SHEET

CONFIRMATION NO. 8586

SERIAL NUMBER	FILING or 371(c) DATE	CLASS	GROUP ART UNIT	ATTORNEY DOCKET NO.
13/546,686	07/11/2012 RULE	514	1611	031671-US-CNT03 167-62 C3

APPLICANTS

Heidi Lane, Basel, SWITZERLAND;
Terence O'Reilly, Basel, SWITZERLAND;
Jeanette Marjorie Wood, Biel-Benken, SWITZERLAND;

**** CONTINUING DATA **************************

This application is a CON of 10/468,520 01/27/2004
which is a 371 of PCT/EP02/01714 02/18/2002

**** FOREIGN APPLICATIONS **************************

UNITED KINGDOM 0104072.4 02/19/2001
UNITED KINGDOM 0124957.2 10/17/2001

**** IF REQUIRED, FOREIGN FILING LICENSE GRANTED ****

07/24/2012

Foreign Priority claimed	☑ Yes ☐ No	☐ Met after Allowance	STATE OR COUNTRY	SHEETS DRAWINGS	TOTAL CLAIMS	INDEPENDENT CLAIMS
35 USC 119(a-d) conditions met	☑ Yes ☐ No		SWITZERLAND	0	7	1
Verified and Acknowledged	/KORTNEY L KLINKEL/ Examiner's Signature	Initials				

ADDRESS

DILWORTH & BARRESE, LLP
1000 WOODBURY ROAD
SUITE 405
WOODBURY, NY 11797
UNITED STATES

TITLE

TREATMENT OF SOLID TUMORS WITH RAPAMYCIN DERIVATIVES

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1250	FEES: Authority has been given in Paper No._____ to charge/credit DEPOSIT ACCOUNT No._____ for following:	☐ All Fees ☐ 1.16 Fees (Filing) ☐ 1.17 Fees (Processing Ext. of time) ☐ 1.18 Fees (Issue) ☐ Other _____ ☐ Credit

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Search	Add to builder	Query	Items found	Time
#2	Add	Search **rapamycin brain (tumor or carcinoma)**	342	12:22:32
#1	Add	Search **everolimus brain**	44	12:14:42

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APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
13/546,686	07/11/2012	Heidi Lane	031671-US-CNT03 167-62 C3

CONFIRMATION NO. 8586

28249
DILWORTH & BARRESE, LLP
1000 WOODBURY ROAD
SUITE 405
WOODBURY, NY 11797

PUBLICATION NOTICE

Title:TREATMENT OF SOLID TUMORS WITH RAPAMYCIN DERIVATIVES

Publication No.US-2012-0283285-A1
Publication Date:11/08/2012

NOTICE OF PUBLICATION OF APPLICATION

The above-identified application will be electronically published as a patent application publication pursuant to 37 CFR 1.211, et seq. The patent application publication number and publication date are set forth above.

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
13/546,686	07/11/2012	Heidi Lane	031671-US-CNT03 167-62 C3	8586

28249 7590 02/19/2013

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WOODBURY, NY 11797

EXAMINER	
KLINKEL, KORTNEY L	
ART UNIT	**PAPER NUMBER**
1611	
MAIL DATE	**DELIVERY MODE**
02/19/2013	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Applicant-Initiated Interview Summary	Application No.	Applicant(s)	
	13/546,686	LANE ET AL.	
	Examiner	Art Unit	
	Kortney L. Klinkel	1611	

All participants (applicant, applicant's representative, PTO personnel):

(1) *Kortney L. Klinkel*. (3)_____.

(2) *Ann Pokalsky*. (4)_____.

Date of Interview: *12 February 2013*.

Type: ☒ Telephonic ☐ Video Conference
☐ Personal [copy given to: ☐ applicant ☐ applicant's representative]

Exhibit shown or demonstration conducted: ☐ Yes ☒ No.
If Yes, brief description: _____.

Issues Discussed ☐101 ☐112 ☐102 ☒103 ☐Others
(For each of the checked box(es) above, please describe below the issue and detailed description of the discussion)

Claim(s) discussed: *all pending*.

Identification of prior art discussed: *Geoerger et al. (Cancer Research, 61, 2/15/2001, 1527-1532) in view of Cottens (WO 94/09010)*.

Substance of Interview
(For each issue discussed, provide a detailed description and indicate if agreement was reached. Some topics may include: identification or clarification of a reference or a portion thereof, claim interpretation, proposed amendments, arguments of any applied references etc...)

Applicant proposed antidating the Geoerger et al. reference in order to overcome the 103 rejection over Geoerger et al. in view of Cottens et al. The Examiner noted that the Geoerger et al. reference was published 2/15/2001 and was available on line 2/1/2001. The effective filing date of the instant application is 2/18/2002. Therefore, Geoerger et al. is a 102b dated reference and therefore applicant cannot antidate the Geoerger et al. reference.

Applicant recordation instructions: The formal written reply to the last Office action must include the substance of the interview. (See MPEP section 713.04). If a reply to the last Office action has already been filed, applicant is given a non-extendable period of the longer of one month or thirty days from this interview date, or the mailing date of this interview summary form, whichever is later, to file a statement of the substance of the interview

Examiner recordation instructions: Examiners must summarize the substance of any interview of record. A complete and proper recordation of the substance of an interview should include the items listed in MPEP 713.04 for complete and proper recordation including the identification of the general thrust of each argument or issue discussed, a general indication of any other pertinent matters discussed regarding patentability and the general results or outcome of the interview, to include an indication as to whether or not agreement was reached on the issues raised.

☐ Attachment

/Kortney L. Klinkel/ Primary Examiner, Art Unit 1611	

Summary of Record of Interview Requirements

Manual of Patent Examining Procedure (MPEP), Section 713.04, Substance of Interview Must be Made of Record
A complete written statement as to the substance of any face-to-face, video conference, or telephone interview with regard to an application must be made of record in the application whether or not an agreement with the examiner was reached at the interview.

Title 37 Code of Federal Regulations (CFR) § 1.133 Interviews
Paragraph (b)

In every instance where reconsideration is requested in view of an interview with an examiner, a complete written statement of the reasons presented at the interview as warranting favorable action must be filed by the applicant. An interview does not remove the necessity for reply to Office action as specified in §§ 1.111, 1.135. (35 U.S.C. 132)

37 CFR §1.2 Business to be transacted in writing.
All business with the Patent or Trademark Office should be transacted in writing. The personal attendance of applicants or their attorneys or agents at the Patent and Trademark Office is unnecessary. The action of the Patent and Trademark Office will be based exclusively on the written record in the Office. No attention will be paid to any alleged oral promise, stipulation, or understanding in relation to which there is disagreement or doubt.

The action of the Patent and Trademark Office cannot be based exclusively on the written record in the Office if that record is itself incomplete through the failure to record the substance of interviews.

It is the responsibility of the applicant or the attorney or agent to make the substance of an interview of record in the application file, unless the examiner indicates he or she will do so. It is the examiner's responsibility to see that such a record is made and to correct material inaccuracies which bear directly on the question of patentability.

Examiners must complete an Interview Summary Form for each interview held where a matter of substance has been discussed during the interview by checking the appropriate boxes and filling in the blanks. Discussions regarding only procedural matters, directed solely to restriction requirements for which interview recordation is otherwise provided for in Section 812.01 of the Manual of Patent Examining Procedure, or pointing out typographical errors or unreadable script in Office actions or the like, are excluded from the interview recordation procedures below. Where the substance of an interview is completely recorded in an Examiners Amendment, no separate Interview Summary Record is required.

The Interview Summary Form shall be given an appropriate Paper No., placed in the right hand portion of the file, and listed on the "Contents" section of the file wrapper. In a personal interview, a duplicate of the Form is given to the applicant (or attorney or agent) at the conclusion of the interview. In the case of a telephone or video-conference interview, the copy is mailed to the applicant's correspondence address either with or prior to the next official communication. If additional correspondence from the examiner is not likely before an allowance or if other circumstances dictate, the Form should be mailed promptly after the interview rather than with the next official communication.

The Form provides for recordation of the following information:
- Application Number (Series Code and Serial Number)
- Name of applicant
- Name of examiner
- Date of interview
- Type of interview (telephonic, video-conference, or personal)
- Name of participant(s) (applicant, attorney or agent, examiner, other PTO personnel, etc.)
- An indication whether or not an exhibit was shown or a demonstration conducted
- An identification of the specific prior art discussed
- An indication whether an agreement was reached and if so, a description of the general nature of the agreement (may be by attachment of a copy of amendments or claims agreed as being allowable). Note: Agreement as to allowability is tentative and does not restrict further action by the examiner to the contrary.
- The signature of the examiner who conducted the interview (if Form is not an attachment to a signed Office action)

It is desirable that the examiner orally remind the applicant of his or her obligation to record the substance of the interview of each case. It should be noted, however, that the Interview Summary Form will not normally be considered a complete and proper recordation of the interview unless it includes, or is supplemented by the applicant or the examiner to include, all of the applicable items required below concerning the substance of the interview.

A complete and proper recordation of the substance of any interview should include at least the following applicable items:
1) A brief description of the nature of any exhibit shown or any demonstration conducted,
2) an identification of the claims discussed,
3) an identification of the specific prior art discussed,
4) an identification of the principal proposed amendments of a substantive nature discussed, unless these are already described on the Interview Summary Form completed by the Examiner,
5) a brief identification of the general thrust of the principal arguments presented to the examiner,
   (The identification of arguments need not be lengthy or elaborate. A verbatim or highly detailed description of the arguments is not required. The identification of the arguments is sufficient if the general nature or thrust of the principal arguments made to the examiner can be understood in the context of the application file. Of course, the applicant may desire to emphasize and fully describe those arguments which he or she feels were or might be persuasive to the examiner.)
6) a general indication of any other pertinent matters discussed, and
7) if appropriate, the general results or outcome of the interview unless already described in the Interview Summary Form completed by the examiner.

Examiners are expected to carefully review the applicant's record of the substance of an interview. If the record is not complete and accurate, the examiner will give the applicant an extendable one month time period to correct the record.

Examiner to Check for Accuracy

If the claims are allowable for other reasons of record, the examiner should send a letter setting forth the examiner's version of the statement attributed to him or her. If the record is complete and accurate, the examiner should place the indication, "Interview Record OK" on the paper recording the substance of the interview along with the date and the examiner's initials.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s): Lane et al.

Examiner: Klinkel, Kortney L.

U.S. Appl. No.: 13/546,686

Group Art Unit:

Filed: July 11, 2012

Docket: 031671-US-CNT03 (167-62 CON III)

For: **TREATMENT OF SOLID TUMORS WITH RAPAMYCIN DERIVATIVES**

Confirmation No.: 8586

Dated: March 11, 2013

Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

AMENDMENT

In response to the Office Action of October 9, 2012, please amend the above-identified application as follows:

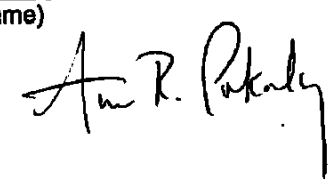
Amendments to the Claims are reflected in the listing of claims, which begins on page 2 of this paper.

Remarks / Arguments begin on page 4 of this paper.

Certificate of EFS-Web Transmission

I hereby certify that this correspondence is being transmitted to the U.S. Patent and Trademark Office via the Office's electronic filing system on March 11, 2013.

Ann R. Pokalsky
(Printed Name)

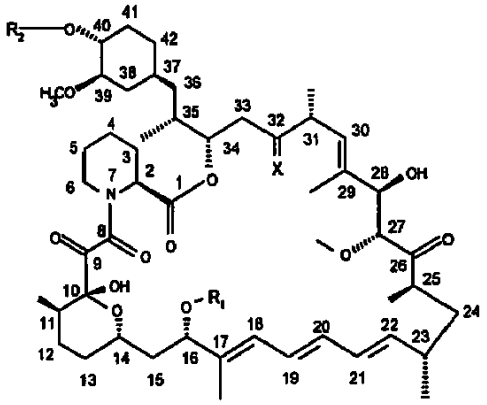
Signature: 

Amendments to the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application.

Listing of Claims:

Claim 1 (currently amended): A method for inhibiting growth of solid tumors of the brain in a subject, wherein the solid tumor of the brain is a carcinoma, said method comprising administering to said subject a therapeutically effective amount of a compound of formula I



I

wherein

R_1 is CH_3,

R_2 is $-CH_2-CH_2-OH$, and

X is =O.

Claim 2 (canceled).

Claim 3 (previously presented): The method of claim 1 wherein the compound of formula I is administered at a daily dose range of from about 0.1 to 25 mg, as a single dose or in divided doses.

Claim 4 (previously presented): The method of claim 1 wherein the compound of formula I is administered in a unit dosage form of from about 0.05 to 12.5 mg.

Claim 5 (previously presented): The method of claim 1 wherein the compound of formula I is administered in a unit dosage form of from about 0.25 to 10 mg.

Claim 6 (previously presented): The method of claim 1 wherein the compound of formula I is administered in a unit dosage form of 10 mg.

Claim 7 (previously presented): The method of claim 1 wherein the compound of formula I is administered orally.

REMARKS / ARGUMENTS

In response to the Office Action of October 9, 2012, Applicants have amended claim 1 and canceled claim 2 without prejudice, which when considered with the following remarks, is deemed to place the present application in condition for allowance. Favorable consideration of the claims is respectfully requested.

Claims 1-7 have been rejected under 35 U.S.C. §103(a) as allegedly unpatentable over Geoerger et al. ("Antitumor Activity of the Rapamycin Analog CCI-779 in Human Primitive Neuroectodermal Tumor/Medulloblastoma Models as Single Agent and in Combination Chemotherapy", Cancer Research, 61 2/15/2001, 1527-1532 in view of Cottens et al. (WO 94/090101).

Geoerger et al. has been cited for allegedly teaching that rapamycin has antitumor activity; that co-administration of rapamycin with cisplatin, or 5-fluoroacil and cyclophosphamide exhibited enhanced apoptosis inhuman cell lines and cytotoxicity in colon tumor models respectively. The reference has also been cited for allegedly teaching: (i) Rapamycin and its 40-O substituted analog CCI-779 are effective brain tumor therapeutics both alone and in combination with chemotherapeutics such as cisplatin and camptothecin; (ii) brain tumor cell lines are exquisitely sensitive to rapamycin (p. 1527, 2nd column, first full paragraph); (iii) rapamycin in combination with cisplatin or camptothecin has an additive effect in cell lines resistant to rapamycin; (iv) antitumor activity of rapamycin has been demonstrated in tumors, human rhabdomyosarcoma and neuroblastoma tumor cell lines *in vitro* and in B16 melanocarcinoma, Colon 38 tumors, CD8F1 mammary tumors, EM ependymoblastoma, and U251 glioblastoma brain tumors *in vivo*, (v) tumor toxicity can be increased by using combination chemotherapy with a rapamycin without the risk of increased systemic cytotoxicity; (vi) that cisplatin, camptothecin, CPT 11 and topotecan are effective agents in the chemotherapeutic treatment of brain tumors but that dosages of these agents are limited due to their toxicity; (vii) that because rapamycin and the 40-O-substituted derivative CCI-779 show at least an additive effect when combined with chemotherapeutics and they have low toxicity, they are good adjuvants for these toxic chemotherapeutics; (viii) that CCI-779 exhibits an enhanced antitumor effect when combined with cisplatin in vivo.

The Examiner has acknowledged that the teachings of Geoerger et al. differ from the present claims in that rapamycin or the 40-O substituted rapamycin derivative CCI-779 are administered either alone or in combination with other chemotherapeutics for the treatment of brain tumors *inter alia*, rather than the claimed rapamycin derivative 40-O-(2-hydroxyethyl) rapamycin (everolimus). Geoerger et al. also fail to teach explicit dosages in terms of mg administered, but rather teaches dosages in terms of mg/kg. The dosages described by Geoerger et al. are all administered intraperiotoneally rather than orally as required by instant claim 7.

Cottens et al. has been cited for allegedly teaching compounds of formula I, including the presently claimed compound i.e. 40-O-(2-hydroxyethyl) rapamycin (compound 8, last line; 21-22; Example 8 p. 21-22; claim 2, compound 8) and that these
derivatives of rapamycin have an improved pharmacologic profile over rapamycin,
exhibit greater stability and bioavailability and allow for greater ease in producing
gelenic formulations (p. 2, first full paragraph). The Cottens et al. reference is also cited for allegedly teaching that the use of rapamycin as an antitumor agent is restricted by its low and variable bioavailability.

Cottens et al. is further relied upon for allegedly teaching that compounds of formula I have demonstrated antitumor activity and the ability to enhance performance of antitumor agents by alleviating multidrug resistance e.g. by administration with anticancer agent e.g. colchicine or etoposide, to multidrug resistant cells and drug sensitive cells in vitro or to animals having multidrug resistant or drug sensitive tumors. Cottens et al. also allegedly teach that the compounds may be administered as the sole active ingredient or together with other drugs e.g. corticosteroids, azathioprine, immunosuppressive monoclonal antibodies (page 8, second full para.).

The Cottens et al. reference has also been cited for allegedly teaching a method of treating tumors or hyperproliferative disorders comprising administering a compound of formula I.

It is the position of the Examiner, that it would have been *prima facie* obvious to one of ordinary skill in the art at the time of the instant invention to substitute rapamycin or CCI-779 of Geoerger et al. for the claimed rapamycin derivative 40-O-(2-hydroxyethyl)rapamycin of Cottens et al. with the reasonable expectation that solid tumors, including brain tumors or brain carcinoma would be treated when administered alone or in combination with other chemotherapeutics such as cisplatin, 5-fluoruracil, and topotecan. According to the Examiner, one would have been

motivated to do so because it is well known in the art that 40-O-(2-hydroxyethyl)rapamycin is useful for treating tumors and hyperproliferative disorders and that it exhibits an improved pharmacologic profile over rapamycin, exhibits greater stability and bioavailability and allows for greater ease in formulating. According to the Examiner, one of ordinary skill in the art would be imbued with the reasonable expectation that the combination of 40-O-(2-hydroxyethyl)rapamycin with the chemotherapeutics 5-fluorouracil and topotecan would exhibit at least an additive effect as this is what is observed for the combination of rapamycin or CCI-779 with these agents. Further according to the Examiner, one would be imbued with the reasonable expectation that the combination of 40-O-(2-hydroxyethyl)rapamycin with cisplatin would exhibit an enhanced antitumor effect, as this is what is observed for the 40-O-substituted rapamycin derivative CCI-779.

In response to the rejection, and in order to advance prosecution of this application, claim 1 has been amended to recite a solid tumor of the brain which is a carcinoma. Support for the amendment to claim 1 may be found throughout the specification, e.g., page 3, which teaches: "where hereinbefore and subsequently a tumor, a tumor disease, a carcinoma, or a cancer is mentioned, also metastasis in the original organ or tissue and/or in any other location are implied alternatively or in addition, whatever the location of the tumor and/or metastasis is."

Carcinomas are a type of cancer arising from the epithelial (outer layer, coverings) cells of lung, breast, skin, etc. *See* http://en.wikipedia.org/wiki/carcinoma. Brain metastasis consists of complex biological processes by which the cells of the primary carcinoma (most commonly, lung, breast and melanoma) travel through the blood stream and established residence in the brain, often growing more aggressively than the primary site. *See*: Steeg, 2006 page 899, p5 left column, provided herewith as **Exhibit A.**

Non-small cell lung carcinoma is the most common primary carcinoma causing carcinoma in the brain (*See*: Lassman and DeAngelis, 2003; page 4, p3, provided herewith as **Exhibit B**). Reyes et al (1999) (provided herewith as **Exhibit C**) observed that in a large scale study of patients presenting brain metastasis, 20-50% had presented with primary lung carcinoma and that this was the source of the brain carcinoma (Reyes et al, 1999).

Pages 12-13, under section B.1 of the present specification provide an example where fragments of A549 tumors were transplanted subcutaneously into the left flank of BALB/c nude mice. 40-O-(2-hydroxyethyl)rapamycin (also referred to as compound A in the present application), when

administered at a dose of 2.5 mg/kg resulted in persisting regressions (41%); a dose of 0.5 mg/kg resulted in transient regressions (38% on day 17), with a final T/C of 16%, and a dose of 0.1 mg/kg slowed tumor growth resulting in a final T/C of 43% (T/C for control animals is 100%).

As set forth in the specification on page 13, the A549 tumors were derived from Cell line CCL-185, American Type Culture Collection (ATCC). As set forth in the ATTC product sheet, provided herewith as **Exhibit D,** Cell line A549 was derived from a 58- year old patient suffering from lung adenocarcinoma. A549 cells grown in culture are rapidly growing, highly invasive and show epithelial morphology.

Geoerger et al. does not teach or suggest anything about administration of rapamycin or rapamycin derivatives for the treatment of brain carcinomas. As discussed above and supported by the attached exhibits, brain carcinomas are distinct from primary brain tumors. The human primitive Neuroectodermal tumor and medullobalstoma, which Geoerger et al. studied are considered primary brain tumors, and not brain carcinomas.

Cottens et al. also does not teach or suggest anything about administration of 40-O-(2-hydroxyethyl)rapamycin for the treatment of brain carcinomas. Although Cottens et al. teach on page 6 that the compounds disclosed therein may be used in the "treatment of proliferative disorders, e.g., tumors, hyperproliferative skin disorder and the like," there is no teaching or suggestion that such compounds, including 40-O-(2-hydroxyethyl)rapamycin, are useful in the treatment of brain carcinoma. The Ehrlich ascites carcinoma (EA) utilized as a model on page 12 of Cottens et al., is derived from mouse, and there is no evidence that it metastasizes to the brain, even in mouse. *See* **Exhibit E.**

Applicants respectfully submit that prior to the present application, it was *not* well known that 40-O-(2-hydroxyethyl)rapamycin is useful for treating tumors and hyperproliferative disorders. Cottens et al. teach at pages 3-4, twenty eight different "Preferred Novel Compounds", one of which is 40-O-(2-hydroxy)ethyl-rapamycin, presently recited in Applicants' claims. Page 4 of Cottens et al. also teaches that 40-O-(2-hydroxy)ethyl-rapamycin is especially preferable for *immunosuppressive use* and page 7 of Cottens et al. teaches that 25 of the 28 compounds taught at pages 3-4 (i.e., those which are O-substituted at C40, which would include 40-O-(2-hydroxy)ethyl-rapamycin as recited in Applicants' claims) are particularly useful in indications (a) and (b) as set forth on pages 5-6 therein. The conditions set forth in (a) on pages 5 of Cottens

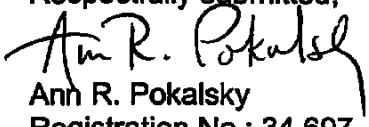
et al. include organ or tissue transplant rejection, and graft-versus-host disease. The conditions set forth in (b) on pages 5-6 of Cottens et al. comprise at least 40 different inflammatory diseases with an etiology including an autoimmune component.

Since neither Geoerger et al., nor Cottens et al., even mention treatment of brain carcinomas, there would have been no motivation to combine the two references in the first instance. *Pro arguendo*, even if there was motivation to combine the two references, one of skill in the art would not have had a reasonable expectation of success that 40-O-(2-hydroxy)ethyl-rapamycin would be useful in treating brain carcinoma as presently recited in the claims.

A proper obviousness determination requires two distinct elements: (1) motivation and (2) reasonable expectation of success. *Takeda Chem. Indus., Ltd. v. Alphapharm Pty., Ltd.*, 492 F3d 1350, 83 USPQ2d 1169 (Fed. Cir. 2007). Neither element is present in the obviousness rejection set forth in the office action with respect to the presently amended claims. Withdrawal of the rejection of claims 1 and 3-7 under 35 U.S.C. §103(a) as allegedly unpatentable over Geoerger et al. in view of Cottens et al. is therefore warranted.

In view of the foregoing amendments, remarks and exhibits, it is firmly believed that claims 1 and 3-7 are in condition for allowance, which action is earnestly solicited.

Respectfully submitted,



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ARP/ml

EXHIBIT A

nature medicine

REVIEW

Tumor metastasis: mechanistic insights and clinical challenges

Patricia S Steeg

Metastatic disease is the primary cause of death for most cancer patients. Complex and redundant pathways involving the tumor cell and the microenvironment mediate tumor invasion at the primary site, survival and arrest in the bloodstream, and progressive outgrowth at a distant site. Understanding these pathways and their dynamic interactions will help identify promising molecular targets for cancer therapy and key obstacles to their clinical development.

Although surgery and radiation therapy effectively control many cancers at the primary site, the development of metastatic disease signals a poor prognosis. Most metastatic lesions are not treated by surgery, as the presence of one lesion often signals wider systemic disease. Chemotherapy, hormonal therapy and radiation serve palliative purposes in the metastatic setting, and some offer a modest but statistically significant extension of survival. Morbidity and mortality arising from metastatic disease can result from direct organ damage by the growing lesions, paraneoplastic syndromes, or from the complications of treatment. It is hoped that a mechanistic understanding of metastasis will help develop better therapies and improve patient outcome.

Tumor metastasis consists of a series of discrete biological processes that move tumor cells from the primary neoplasm to a distant location (Fig. 1). Tumor cells must invade the tissue surrounding the primary tumor, enter either the lymphatics or the bloodstream, survive and eventually arrest in the circulation, extravasate into a tissue and grow at the new site. The term 'colonization' is used herein to reflect the combined influences of tumor cell proliferation, apoptosis, dormancy and angiogenesis in the formation of a progressively growing lesion in a distant site. One of the most enduring observations in metastasis research was published in 1889 by Stephen Paget[1]. Describing tumor cells as the "seed" and the host environment as the "soil," Paget hypothesized that their interaction determines metastatic outcome: "When a plant goes to seed, its seeds are carried in all directions; but they can only live and grow if they fall on congenial soil." This observation predicted that the tissue environment, composed of a myriad of specialized cell types, extracellular matrices and cells recruited to the site, may facilitate tumor metastasis and contribute to the organ selectivity sometimes seen in metastatic colonization.

These theoretical steps are practically analyzed in metastasis assays. In humans, only the end stages of the metastatic process are observed, when a distant lesion is sufficiently large to be imaged. To associate a molecular event with human metastasis, its occurrence in primary tumors or disseminated cells is correlated with patient survival or other indicators such as disease-free survival or the presence of regional lymph node metastases. Most mechanistic insights into metastasis are derived from xenograft studies in rodents (reviewed in ref. 2). Typically, a tumor cell line known to metastasize *in vivo* is manipulated to change the expression or mutation status of a single gene. In spontaneous assays, the tumor cells are injected into a site, a primary tumor forms and metastases develop. It is preferable to inject cells into an orthotopic location, the tissue of origin. This assay measures the complete metastatic process but suffers from poor quantification and slow completion. In experimental metastasis assays, tumor cells are injected into the bloodstream from the tail vein or other sites. Metastases form more quickly than in spontaneous assays and in greater numbers, facilitating statistical analysis. A drawback of experimental metastasis assays is that only part of the metastatic process, the postintravasation stage, is modeled. Several transgenic mouse strains develop both primary tumors and spontaneous metastases[3] and are crossed to other genetically engineered mice to determine effects on metastasis. In preclinical studies, a compound is administered to animals either just after injection of the tumor cells or after metastases have formed, constituting prevention and treatment studies, respectively. Veterinary animals including pet dogs and cats are increasingly used to test therapeutics in the metastatic setting, and for a subset of cancers their pathophysiology may more closely resemble that of humans[4,5].

THE STEPS OF METASTASIS

Invasion

Invasion, which initiates the metastatic process, consists of changes in tumor cell adherence to cells and to the extracellular matrix (ECM), proteolytic degradation of surrounding tissue and motility to physically propel a tumor cell through tissue. Tumor cell adherence to the ECM is mediated by integrins. Integrins are heterodimers of 1 of 18 α and 1 of 8 β transmembrane proteins. Each heterodimer binds to specific proteins in the ECM and can transmit signals into or out of the cell[6]. Other tumor cell receptors for ECM proteins include CD44, a highly polymorphic receptor for hyaluronan,

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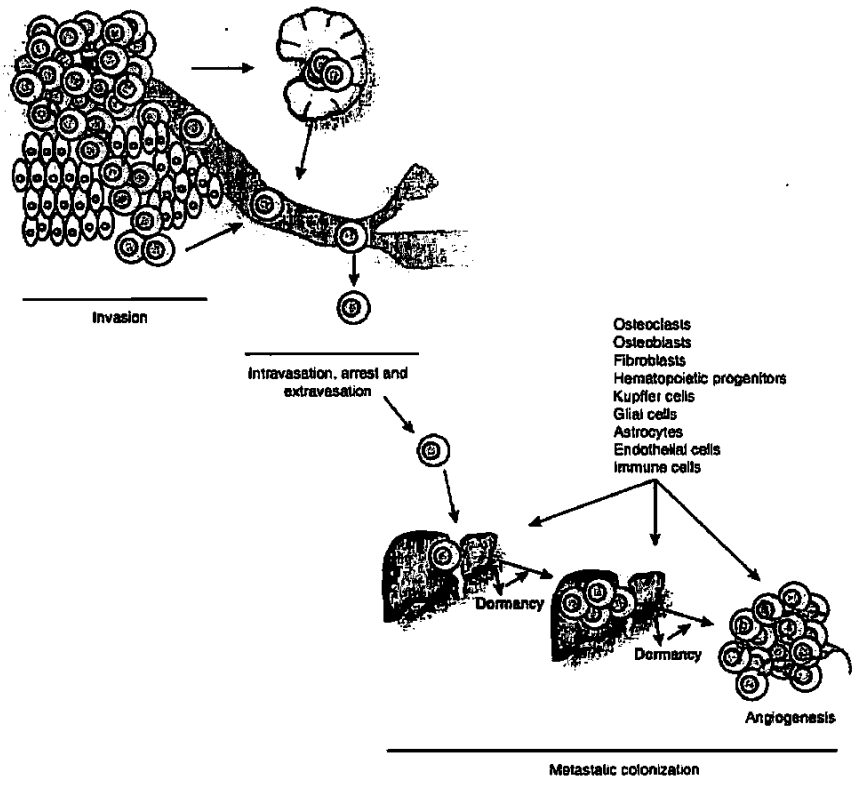

Figure 1 The tumor metastatic process. At the primary tumor site, tumor cells invade into the lymphatics or directly into the circulation. Once in the bloodstream, tumor cells must survive and avoid immune attack to extravasate. Arrest is most often by size restriction in capillary beds but can involve specific adhesive interactions. The process by which tumor cells form micrometastases and then progressively growing, vascularized macrometastases in a distant organ is termed metastatic colonization. Metastatic colonization involves reciprocal interactions between tumor cells and cells in the microenvironment of the distant organ, and can pause for periods of dormancy.

immunoglobulin superfamily receptors and surface proteoglycans. Cell-cell adhesions are mediated by cadherins (reviewed in ref. 7), which bind cells through homophilic protein-protein interactions of their extracellular domains; intracellularly, cadherins signal to catenins and the actin cyotskeleton. Invasion is accompanied by a 'switch' in tumor cell cadherin expression, for instance from E-cadherin, which promotes tumor cell–tumor cell adherence (and blocks invasion), to N-cadherin, which is normally expressed on mesenchymal cells and facilitates tumor cell binding to the stroma during invasion.

The engagement of integrins and other attachment molecules is accompanied by the recruitment of proteases to degrade the ECM, providing a pathway for invasion[8]. Matrix metalloproteinases, plasmin, urokinase plasminogen activator, cathepsins and heparanases, when transfected into a tumor cell line, augment invasion. Besides the destruction of ECM, proteases liberate embedded growth factors and chemokines, activate latent proteins on the cell surface and may serve protective roles in tumorigenesis[9,10].

Most tumor cell movement in invasion is dynamic, involving the formation of adhesions to the ECM at the leading edge of the cell, detachment from the ECM at the trailing edge and a ratcheting of the cell forward. Virtually every 'growth factor' stimulates tumor cell motility *in vitro*. Pathways may be tumor cell autonomous or may involve paracrine loops with cells in the environment[11]. Chemokines, chemotactic cytokines that bind G-protein-coupled receptors, represent another important class of motility-inducing proteins (reviewed in ref. 12). Chemokines also contribute to tumor cell invasion by inducing infiltration of tumors by macrophages and lymphocytes, which release proteases and other inflammatory stimuli.

Growth factors can stimulate motility and invasion by mechanisms distinct from those involved in mitogenesis. In the case of receptor tyrosine kinase (RTK) activation, proliferation is mediated by the phosphatidylinositol-3 kinase (PI3K) and extracellular signal–regulated kinase (Erk) pathways, whereas invasion can occur by RTK interaction with integrins to stimulate formation of a focal adhesion kinase (Fak)-Src complex (reviewed in refs. 13–15; Fig. 2). Sequential binding of protein cascades to Fak triggers many of the downstream cellular changes in invasion. Activation of Rac leads to the formation of lamellipodia, membrane protrusions in the direction of forward movement. Rac activity activates matrix metalloproteinase production and is held in check by Tiam1. The binding of N-WASP to Fak leads to activation of the GTPase CDC42, engagement of Arp2/3 and actin cytoskeletal contraction to push the lamellipodia forward. FAK binding of p190RhoGEF leads to Rho activation, the formation of cytoplasmic actin stress fibers and mature focal adhesions, and stabilization of the nascent adhesion. Adhesion at the leading edge must be coupled to de-adhesion at other sites so that the cell can be pulled forward by cytoskeletal contraction. Several mechanisms limit Fak-mediated adhesion, including the direct dephosphorylation of Fak by protein tyrosine phosphatases (PTPs), and activation of an intracellular cysteine protease, calpain-2, which degrades Fak and its associated protein paxillin. Other proteins can limit Fak activity by competing for binding to its protein partners or downstream effectors.

A second example of signaling distinguishing proliferation and invasion stems from the c-met growth factor RTK (reviewed in ref. 16). Hepatocyte growth factor (HGF), also known as scatter factor, binds and activates c-met. A unique adapter, GAB1, in turn binds to the intracellular portion of activated c-met, is phosphorylated and recruits multiple effector proteins. Activation of effectors, such as the PTP Shp2, leads to Rac activation, altered expression of cell-cell adhesion molecules and, eventually, motility. A recent report suggests that HGF may also stimulate a proliferative response by first inducing c-Myc expression through a post-transcriptional mechanism[17].

A second type of invasion, ameboid, results from loose attachment to the ECM and loss of cell polarity, resulting in rapid movement in the path of least resistance, dictated by cell shape and tissue barriers. Found in three-dimensional models of lymphoma migration, amoeboid invasion does not involve firm integrin attachments but permits the cell to glide, using the cortical rather than stress fiber actin machinery (reviewed in ref. 8).

Survival and arrest in the bloodstream

The bloodstream is a harsh environment for metastasizing tumor cells because of velocity-induced shear forces, lack of a substratum and the presence of immune cells. A simple observation that most cell biologists take for granted is that tumor cells grow best when attached to a plate or a substratum. Death upon detachment is described as anoikis, and this is hypothesized to contribute to metastatic inefficiency while cells are bloodborne (reviewed in ref. 18). Expression of multiple RTKs and invasion signaling components induces tumor cell resistance to anoikis *in vitro* and may contribute to survival in the circulation.

But beyond survival, tumor cells must arrest in the circulatory system. The generally accepted notion of arrest and extravasation is that a proportion of cells nonspecifically arrest by binding coagulation factors and by size restriction in the capillary beds[19], although specific adhesive interactions also occur. Tumor cells extravasate by inducing endothelial retraction, leading to the attachment of tumor cells to the subendothelial ECM and reformation of the capillary. Although many studies find that single tumor cells rapidly arrest and extravasate from the circulatory system or die while within it, in other model systems tumor cells complete initial proliferation steps while inside the vasculature and attached to the endothelium[20].

Tumor cell binding to coagulation factors including tissue factor, fibrinogen, fibrin and thrombin creates an embolus facilitating arrest in capillary beds. The endothelial cell E- and P-selectins also contribute to tumor cell arrest. In lymphocyte trafficking, selectins mediate the initial tethering and rolling weak adhesion to the endothelium, followed by firm endothelial–tumor cell adhesion involving cadherins or immunoglobulin-like cell adhesion molecules[21,22]. Other potential mediators of tumor cell arrest are tumor derived, including glycosylation patterns and integrins. Expression of vascular attachment factors may be dynamic: injection of metastatic but not nonmetastatic tumor cell lines into the liver triggered rapid production of tumor necrosis factor (TNF)-α by liver macrophage-like Kupffer cells, followed by increased expression of E- and P-selectins and adhesion molecules on the sinusoidal endothelial cells[23]. Thus the tumor cell and vascular microenvironment interact to facilitate arrest, and functional targets in arrest may not be ubiquitously present in model systems.

One of the many surprises in metastasis research is that not all capillaries are alike. Endothelial cells from different organs express distinct surface proteins that can be identified by biopanning using phage display. Studies have identified numerous peptides that selectively bind the endothelia of target organs and may be pertinent to determining the site of tumor cell arrest (reviewed in ref. 24). One protein identified by biopanning, metadherin, mediated tumor cell homing to the lung, as opposed to the skin, kidney and other organs. Antibodies or siRNA to metadherin reduced experimental metastases, indicating that such approaches can impact the metastatic process[25].

Metastatic colonization

Colonization is an inefficient business. In a melanoma experimental metastasis model, the majority (>80%) of injected tumor cells survived the circulation and successfully extravasated into the liver. Only 1 in 40 cells formed micrometastases by day 3, however, and only 1 in 100 micrometastases progressed to form macroscopic metastases 10 days later[26]. Successful colonization crucially depends on interaction with the microenvironment or "soil" of the distant tissue. Molecular characterization of the microenvironments from the major sites of metastases indicate both similarities and distinct differences, the latter of which may contribute to the development of site-specific therapeutic approaches.

Our understanding of the word "microenvironment" is changing, to include both the stable cellular architecture of the tissue as well as an influx of cells from other sites. Recent results indicate that a 'premetastatic niche' may be formed by bone marrow–derived hematopoietic progenitor cells that attract tumor cells and support a developing metastasis[27]. If this finding is confirmed and extended, characterization of these bone marrow cells and interruption of the bone marrow–tumor cell interaction may hold promise for the prevention of metastatic colonization.

Angiogenesis and vascular permeability. In each tissue, expansion of the blood supply is required for the growth of metastases beyond the limits of diffusion, and provides oxygen, growth factors, nutrients and metabolites. Angiogenesis is the formation of a new blood supply from preexisting vasculature (reviewed in refs. 28–32) and is stimulated by an angiogenic 'switch' that occurs when the ratio of inducers to inhibitors tips in favor of inducers. Many inhibitors of angiogenesis are ECM proteins such as thrombospondin[33,34] or ECM protein fragments such as endostatin[35]. Bone marrow–derived circulating endothelial precursor cells may also have a role in tumor angiogenesis, although the extent of their involvement is still under investigation[36]. The tumor blood supply is also influenced by vasculogenic mimicry, the formation of blood-conducting pathways by tumor cells[37], and by intrassuception,

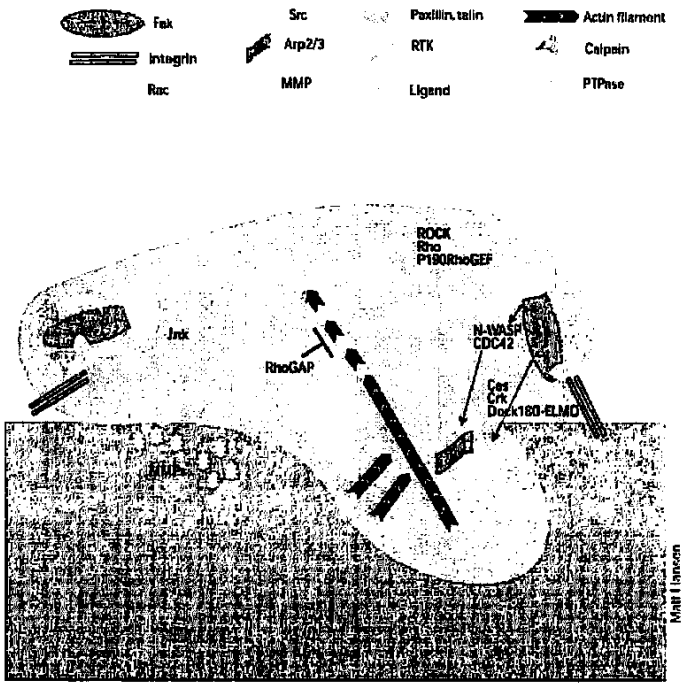

Figure 2 Dynamic signaling in invasion. A prototypical receptor tyrosine kinase (RTK) associates with integrins, activating a Fak-Src complex which then initiates focal adhesions on the leading edge of the tumor cell; binding of Fak to integrins is mediated by paxillin and talin. Cascades of proteins interacting with Fak mediate many of the component processes of invasion. Sequential binding of p130CAS, CRK and the DOCK180-ELMO complex leads to localized RAC1 activation, creating lamellipodia, membrane protrusions in the direction of forward movement. Binding of N-WASP to Fak leads to CDC42 activation, engagement of Arp2/3 and actin filament formation. Fak binding of p190RhoGEF leads to Rho activation and the formation of stable actin stress fibers and mature focal adhesions. Src activates Jnk, resulting in matrix metalloproteinase (MMP) production and proteolysis of the extracellular matrix (ECM). At the trailing edge of the cell, adhesions are broken, so that the cell can be ratcheted forward by actin contraction. The protease calpain degrades paxillin and Fak, unlinking integrins from Fak. Protein phosphotyrosine phosphatases (PTPases) limit Fak activation. Src activation of p190 RhoGAP limits stress fiber stability.

the formation of interstitial tissue columns in the vascular lumen that participate in a vascular network.

Multiple factors stimulate endothelial cells to induce angiogenesis including vascular endothelial growth factor (VEGF), angiopoietin, ephrin (Eph), platelet-derived growth factor (PDGF), transforming growth factor (TGF)-β and basic fibroblast growth factor (bFGF) families. VEGF[38,39] is the best studied and has advanced as a molecular target through clinical approval. VEGF also stimulates the mobilization of endothelial progenitor cells and the outgrowth of pericytes that line mature vessels. The VEGF family consists of six members and their variants, of which the 165-amino-acid–long form of VEGFA is predominant. VEGF is expressed by tumor cells and is also bound to the ECM and liberated by protease cleavage. Bevacizumab, a recombinant humanized monoclonal antibody that binds VEGFA, has clinical activity in metastatic cancers in combination with cytotoxic agents[40–42]. Three VEGF receptors (VEGFRs) bind the VEGFs, in both overlapping and distinct patterns, and soluble forms of VEGFR can function as decoy receptors. VEGFR2 is thought to mediate most of the angiogenic effects of VEGFA. The neuropilins (NRP)-1 and NRP-2 function as coreceptors for the VEGFRs, in addition to their roles as receptors for semaphorins and collapsins in chemotactic repulsion signaling. Small-molecule inhibitors of VEGFR have been developed, which inhibit its receptor tyrosine kinase activity and often other kinases as well(for example, refs. 43–45).

VEGF is a multifunctional protein: (i) in addition to its role in stimulating angiogenesis, it induces vascular permeability to circulating macromolecules[38]. VEGF-induced vascular permeability involves both intraendothelial macromoleulcar transport, resulting from caveolae and chains of vesiculovacuolar organelles, as well as interendothelial leakage resulting from reduced endothelial cell-cell adherence[46]. Fluid movement can contribute to edema, extracellular fibrin deposition and alterations in interstitial pressure, which can influence drug delivery. VEGF signaling has been linked to Src; in Src knockout mice, metastasis but not primary tumor formation was reduced as compared to wild-type mice and correlated with decreased vascular permeability but unchanged microvessel density[47], suggesting that VEGF alterations in vascular permeability can influence metastasis. (ii) VEGFR-positive tumor cells have been identified, leading to the hypothesis that an autocrine loop affects nonangiogenic aspects of tumor cell progression. Survival, proliferation and invasive responses of tumor cell lines have been shown to be mediated by VEGF and VEGFR through the Erk1/2 and PI3K pathways[48–50]. (iii) Bone marrow–derived hematopoietic progenitor cells that formed premetastatic niches were positive for VEGFR[27]. It will be of interest to determine the relative contribution of angiogenesis versus other functions to the clinical efficacy of bevacizumab.

Colonization of the bone. Breast and prostate carcinomas and multiple myeloma mestastasize to bone (reviewed in refs. 51–55). The two basic types of bone metastases are osteoblastic, found in prostate cancer, and osteoclastic, found in breast cancer and multiple myeloma. Osteoblastic metastases stimulate osteoblasts, the bone-forming cells, to lay down new, flawed 'woven' bone, along with some bone resorption. Osteoblast function is stimulated by at least two transcription factors, osterix and Runx-2, which are activated by tumor cell or microenvironmental signals such as bone morphogenic protein (BMP), IGF-1R, FGFRs and endothelins (ET1)[52].

Osteoclastic lesions evolve through interactions between tumor cells and the bone microenvironment known as the 'vicious cycle' (**Fig. 3**). Tumor cells secrete parathyroid hormone–related protein (PTHrP), which stimulates osteoblasts to produce both a membrane bound RANK ligand (RANKL) and osteoprotegerin (OPG), a soluble decoy receptor for RANKL and member of the TNF receptor family. It is the ratio of

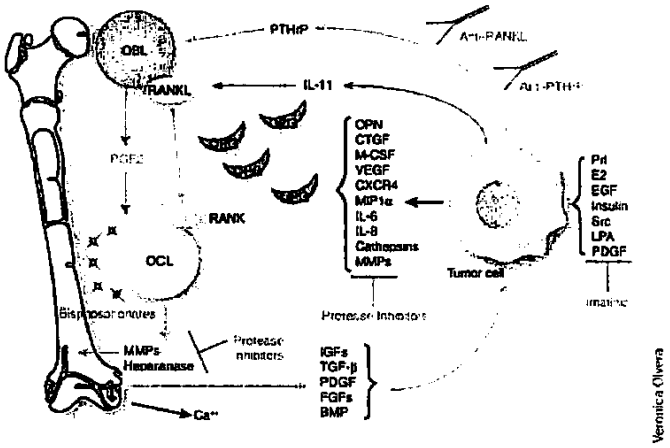

Figure 3 Tumor cell–microenvironment interactions in osteoclastic bone metastases. In the "vicious cycle" (green lines), tumor cells produce PTHrP, which stimulates osteoblasts to secrete RANKL and its decoy binding protein OPG. The ratio of RANKL to OPG determines osteoclast activation, initiating bone resorption and releasing embedded growth factors. The growth factors then stimulate the tumor cells, restarting the cycle. Additional cycles have been reported, including tumor cell production of IL-11, resulting in osteoblast secretion of PGE2 and osteoclast activation (blue lines). Other factors released by bone metastatic tumor cells that may influence the microenvironment include proteases, growth factors, inflammatory stimuli and angiogenic factors. Potential palliative and therapeutic strategies are shown in red.

RANKL to OPG that determines osteoclast activation, through its receptor for RANKL. Activated osteoclasts degrade the bone matrix–releasing embedded growth factors including the IGFs and TGF-β, which in turn stimulate tumor cells to produce more PTHrP. But the vicious cycle is not the only regulator of osteoclastic bone metastases; several interleukins augment PTHrP pathways and exert PTHrP-independent roles[56]. Molecular profiling of bone metastatic breast cancer cells identified a combination of IL-11, matrix metalloproteinase 1 (MMP1), the chemokine receptor CXCR4 and the connective tissue growth factor (CTGF) that augmented bone metastases in quadruple transfection experiments[57].

Therapeutic strategies directed toward bone metastases have focused on the tumor cells and the microenvironment (**Fig. 3**). In preclinical models, imatinib mesylate inhibited the PDGFR activity of tumor and local endothelial cells, and reduced osteolytic metastases[58]. Protease (MMP) inhibitors reduced breast osteolytic metastases in both therapy and prevention preclinical models[59]. Surprisingly, the Hsp90 chaperone inhibitor 17-allylamino-17-demethoxygeldanamycin (17-AAG) enhanced osteoclast activation and osteolytic bone metastasis in a mouse model[60]. These data contrast with other beneficial effects of 17-AAG[61], suggesting the importance of testing in metastatic preclinical models. A monoclonal antibody against PTHrP is in clinical development[51,62]. Clinically, bisphosphonates, which coat the osteoclast to reduce its resorptive effects, are approved for the treatment of bone metastases, although they do not extend patient survival (reviewed in ref. 55). Atrasentan, an inhibitor of the endothelin receptor (ET-A) involved in bone remodeling has completed phase 1 testing[63]. Denosumab, a human monoclonal antibody to RANKL, decreased bone turnover in resorption in individuals with multiple myeloma and breast cancer who have bone metastases[64].

An emerging concept is that the reciprocal, 'vicious' interactions between tumor and cells in the bone microenvironment result in the

local upregulation of tumor 'survival' or antiapoptotic factors, including OPG, IGFs, IL-6 and others. Production of IL-6 by bone stromal cells or the promotion of specific adhesive events protected multiple myeloma cells from apoptosis inducers or cytotoxic drugs[65]. OPG, the decoy receptor for RANKL, is produced by bone marrow stromal cells from individuals with breast cancer at concentrations sufficient to block TRAIL-mediated apoptosis[66].

Colonization of the liver. Liver metastases are a major contributor to the mortality of individuals with colorectal cancer. The liver is the first capillary bed encountered by colon cancer cells traversing the hepatic artery. The liver environment, including ECM and stromal cells, may facilitate metastatic colonization. ECM extracted from primary rat hepatocytes stimulated greater proliferation of metastatic colorectal carcinoma cell lines than ECM from fetal rat fibroblast cultures[67]. Moreover, conditioned medium from cultures of liver metastasis–associated fibroblasts stimulated the growth of a colon carcinoma cell line to a greater extent than that of liver fibroblasts distant from the metastasis or skin fibroblasts from patients[68].

The role of angiogenesis in liver metastasis is complex. Using stained sections of resected metastases, a nonangiogenic 'replacement' type metastasis was described, in which tumor cells replaced hepatocytes at the tumor-liver interface, preserving tissue architecture and co-opting the sinusoidal blood vessels[69]. In contrast, a 'pushing' type liver metastasis contains greater numbers of proliferative endothelial cells and is thought to be influenced by angiogenic regulatory pathways[70]. Strategies directed against VEGF and its receptors inhibited liver metastases of multiple cancer histologies in preclinical models[71–74], and bevacizumab has shown activity in metastatic colorectal carcinoma in combination with cytotoxic agents[42,75]. Angiogenesis of liver metastases may also involve Cox-2, polyunsaturated fatty acids, interferons, 2-methoxyestradiol, apolipoprotein(a) kringles, integrin antagonists, heparanase, matrix metalloproteinases, EGFR tyrosine kinase inhibitors and retinoids. Angiogenesis inducers other than the traditional VEGF and FGF have been reported for liver metastases including platelet-derived endothelial cell growth factor[76].

New candidate molecular targets for liver metastases have been reported. A tyrosine phosphatase, PRL-3, was identified by SAGE analysis as overexpressed in liver metastases of colorectal carcinoma[77]. Transfection of tumor cells with siRNAs to either PRL-3 or PRL-1 reduced liver metastases in an orthotopic model[78,79]. The D6.1A tetraspanin, a cell-surface organizer, interacted with the $\alpha_6\beta_4$ integrin and facilitated liver colonization by pancreatic carcinoma cells injected intraperitoneally[80]. Other targets include Cox-2, the synthesis of hyaluronin in the ECM, plasminogen activator and nitric oxide synthetase. Several tyrosine kinase signaling pathways have been connected to liver metastasis. A Src kinase inhibitor abrogated lymph node and liver metastases in an orthotopic model of pancreatic cancer when combined with gemcitabine[81]. Colonization of the liver was thought to involve a signaling pathway distinct from those described for invasion involving the signal transducer and activator of transcription 3 (Stat3), which upregulates the expression of antiapoptotic and growth-promoting genes. Blockade of IGF-I and IGF-II using neutralizing antibodies reduced liver metastases after intrasplenic injection of colorectal carcinoma cells in both prevention and treatment models[82]. Few of these strategies have advanced to clinical testing.

Colonization of the brain. Brain metastases are most common in individuals with lung and breast cancer, and are also frequent in individuals with melanoma[83]. For people with metastatic breast cancer whose tumors overexpress Her-2 and are treated with trastuzumab, the incidence of brain metastases may be twice that of other breast cancer patients and often occurred when the patients were responding to therapy at other sites or had stable disease[84,85]. Similar trends were reported in a limited cohort of individuals with advanced non–small-cell lung carcinoma treated with the EGFR kinase inhibitor gefitinib[86]. The brain is thought to represent a 'sanctuary' site as systemic control improves. Metastases occur in the brain parenchyma or in the leptomeninges, the coverings of the brain, or the cerebral spinal fluid (CSF) fluid in between them.

The brain constitutes one of the most unique microenvironments for metastasis. The blood-brain barrier (BBB) describes the endothelium surrounding the brain, which is continuously connected by tight junctions, loaded with efflux pumps and surrounded by a basement membrane, pericytes and astrocytes. Access to macromolecules in the bloodstream is severely curtailed. Once tumor cells traverse the BBB, a blood-tumor barrier remains, which is poorly characterized. Within the brain parenchyma, tumor cells encounter glial cells and astrocytes which can synthesize a host of cytokines, chemokines and growth factors. The catecholamine neurotransmitters norepinephrine, dopamine, histamine, angiotensin and substance P have all been reported to induce tumor cell motility[87].

Stat3, already thought to be involved in liver metastasis, represents a new molecular target of interest for brain metastases. Resected human melanoma brain metastases exhibited higher Stat3 immunostaining than a cohort of primary tumors, and overexpression of Stat3 in a melanoma cell line increased brain metastasis[88]. Both angiogenesis and invasion were elevated by Stat3 expression. The role of angiogenesis in brain metastasis was also observed using antisense constructs to VEGF165 or a kinase inhibitor[89,90]. The treatment of brain metastases may require the development of drugs selected for both efficacy and BBB permeability (lipid solubility or facilitated transport).

Colonization of the lung. As the majority of metastasis assays measure lung metastases, the list of contributing pathways is extensive. Many of the traditional invasion pathway components modulate lung metastasis. Little is known, however, about whether these pathways are specific to lung versus other organs. Gene expression profiling of lung metastatic sublines of a human breast cancer cell line identified several membrane-localized or secreted proteins that, together, could induce lung metastasis, but could not when expressed singly[91]. These studies provided a first cross-comparison of lung versus bone metastases, finding some functionally involved genes in common and others distinct, a strategy that can be applied to the remainder of lung metastasis–associated genes.

Numerous pathways involved in cell survival and resistance to cell death have been shown to promote lung metastasis and/or survival of cells after extravasation into the lungs, including ezrin, TGF-β and apoptotic signaling intermediates[92–98]. Nuclear factor κB (NF-κB), which mediates inflammatory and antiapoptotic pathways, is also linked to lung metastasis through the inhibitory NF-κB binding protein IκB and pharmacologic inhibitors such as parthenolide[99,100]. The lung microenvironment may induce changes in expression of antiapoptotic genes, such as Bcl-2, in lung-colonizing tumor cells and enhance the survival of metastatic tumor cells after treatment with proapoptotic drugs[101]. Suppression of Fas ligand in melanoma cells resulted in enhanced lung metastases due to inhibition of granulocytic infiltration and tumor cell killing[102]. Thus, a complex and dynamic interplay between tumor cells and the lung tissue is available for therapeutic development.

GENETIC INSIGHTS

Although traditional metastasis research has identified several required steps and a rational list of proteins mediating each, genetics has uncovered new, unexpected insights. For instance, genes downstream of ini-

tiating oncogenes are thought to contribute to progression, but are the 'background' genes of the host contributory? A series of crosses between FVB/N-TgN (MMTV-PyMT) transgenic mice, which develop both primary mammary tumors and lung metastases, and other inbred mouse strains investigated this point. When the F_1 progeny were examined, parameters of tumorigenicity were comparable but metastatic dissemination varied widely[103]. Using a multi-cross mapping strategy, a candidate host metastasis-regulatory gene was identified, the signal-induced proliferation-associated gene 1 (Sipa1). Transfection of Sipa1 promoted metastasis, and a polymorphism was discovered that affected its Ras-GTPase activating protein (Ras-GAP) function, which is involved in cell-cell adherence[104]. These data indicate that the host background functionally contributes to metastasis. These findings also raise an important question: are some individuals, as a result of their genetic background, programmed to develop highly metastatic disease once cancer occurs?

The identification of metastasis suppressor genes (MSGs) provided another unexpected series of insights from genetics. Although a number of genes inhibit both primary tumor formation and metastasis in experimental models, the MSGs are a distinct class. MSGs, upon re-expression at physiologic levels in a metastatic cell line, reduced metastasis without a significant effect on tumorigenicity. Further study showed that MSGs regulated metastasis at many stages, but several were documented to have roles in metastatic colonization[105,106]. Twelve MSGs have been confirmed (reviewed in refs. 107,108). Few of the MSGs were known contributors to metastasis, and many exhibited functions previously unlinked with this process. An example is the MKK4 MSG. MKK4, as a component of the p38 and Jnk mitogen-activated protein kinase pathways, is hypothesized to facilitate stress-induced apoptosis. MKK4 was preferentially activated in lung metastases of prostate cancer as opposed to primary tumor cells; abrogation of MKK4 activation may prevent the apoptotic response of metastatic tumor cells stressed by the new environment, thus facilitating colonization[109]. Moreover, the metastasis suppressors commonly have more than one validated role in metastasis and may serve to integrate these pathways: BRMS1 shuts down PI3K signaling and also facilitates cell-cell communication via gap junctions, for example[110,111]. Nm23 has many reported functions; those associated with metastasis include inhibiting the Erk pathway, regulating cell adhesion and influencing cell metabolism[112–116].

At least two translational approaches to the MSGs have been reported. For Nm23, mutation is rare, and 'turning on' the wild-type protein in micrometastatic tumor cells was hypothesized to limit their subsequent colonization. Through analysis of the promoter of the gene encoding Nm23, medroxyprogesterone acetate (MPA) was identified as an unconventional glucocorticoid that increased expression of Nm23 in metastatic breast carcinoma cell lines[117]. MPA inhibited the incidence, number and size of pulmonary metastases in a treatment model system of breast cancer lung colonization[118]. A second translational approach is to target genes regulated by MSGs. In a microarray analysis of control and MSG-transfected bladder carcinoma transfectants, elevated endothelin-1 (ET-1) expression correlated with low RhoGDI2 levels. Atrasentan, an ET-1 receptor antagonist, reduced the lung metastasis of bladder carcinoma cells expressing a low level of RhoGDI2 (ref. 119). In other words, if one cannot turn the MSG back on, can one identify a molecular correlate that can be targeted?

INSIGHTS FROM MICROARRAYS

Gene expression profiling has served a hypothesis-generating role in metastasis research. Reasoning that, when the transfection of a single oncogene or MSG alters the *in vivo* metastatic activity of a tumor cell line, changes in the expression of a series of downstream genes are involved, expression profiling has identified candidate genes. Comparisons of gene expression profiles from human tumors with functional activities in mice have generated the hypothesis that a 17-gene expression signature measures the influence of host genetics[120,121]. Profiling of cell lines with organ-specific metastatic patterns has identified new signatures, for which some of the component genes have been functionally demonstrated in metastasis assays and validated as prognostic factors in human tumor cohorts[57,91]. The similarity of matched primary tumors and metastases upon gene expression profiling has questioned the origins of metastasis, whether it results from the occasional tumor cell that has all the required functions or, alternatively, results from cells exhibiting oncogene-induced gene expression profiles that dominate a primary tumor[122–124]. Rather than supporting any single conclusion, each of these studies adds a layer of understanding to a complex process.

BURNING QUESTIONS

1. What parts of the metastatic process are most amenable to therapeutic intervention? Data collected by the Surveillance, Epidemiology, and End Result (SEER) program of the US National Cancer Institute (NCI) illustrate the portions of the metastatic cascade available for intervention at the time of cancer diagnosis. Patients were classified into those with localized disease (no sign of progression), regional disease (typically lymph node involvement) and distant disease (distant metastases detected). The proportion of US patients in each category for the years 1988–2001 are graphed in **Figure 4**. Of the four most prevalent cancers, less than 10% of breast and prostate cancer patients, 20% of colorectal cancer patients and 40% of lung cancer patients had detectable distant metastases at diagnosis. These facts argue that interruption of the metastatic process could be useful for a majority of individuals with cancer.

But is the entire metastatic process open to intervention? Here, the regional disease incidence is informative. For individuals with breast, colorectal and lung cancer (local and regional data were combined for those with prostate cancer), another 29–37% already had tumor cells in lymph nodes. These individuals are at the highest risk of metastasis. Invasion has already happened. What is unclear, because we lack imaging of a sufficient sensitivity to detect single cells, is whether intravasation and extravasation of the circulatory system and colonization as an occult micrometastasis has occurred. These data argue that the last steps in metastasis, angiogenesis and colonization to form a detectable metastasis represent priority steps for therapeutic intervention.

2. Are we studying the right cells? Are there subpopulations of metastatic tumor cells that we ignore at our own peril, because their molecular makeup and consequently their therapeutic sensitivities are distinct?

Dormant cells

Clinically, dormancy has long been recognized, particularly in breast and prostate cancers and melanoma[125,126]. It refers to the prolonged survival of single cells or small micrometastases without apparent progression. Where are dormant cells? Several studies identified dormant tumor cells in liver and lungs from animals injected with melanoma and breast carcinoma cells[26,127,128]. Resection of the organ resulted in the outgrowth of the 'dormant' cells *ex vivo*, and the breast cancer culture formed a primary tumor upon reinjection into the mammary fat pad. Thus, the dormant cells in this model system were viable and the microenvironment was thought to regulate dormancy. In other studies, tumor cells were identified in the blood or bone marrow, and correlated with poor patient outcome (reviewed in ref. 129). Tumor cells in the bone marrow could represent ongoing dissemination or could be a site of dormancy. The finding that colorectal carcinoma cells occur in the bone marrow, a histology that rarely metastasizes to the bone, lends further support to the latter hypothesis[130].

Different pictures have emerged of dormant tumor cells . Lewis

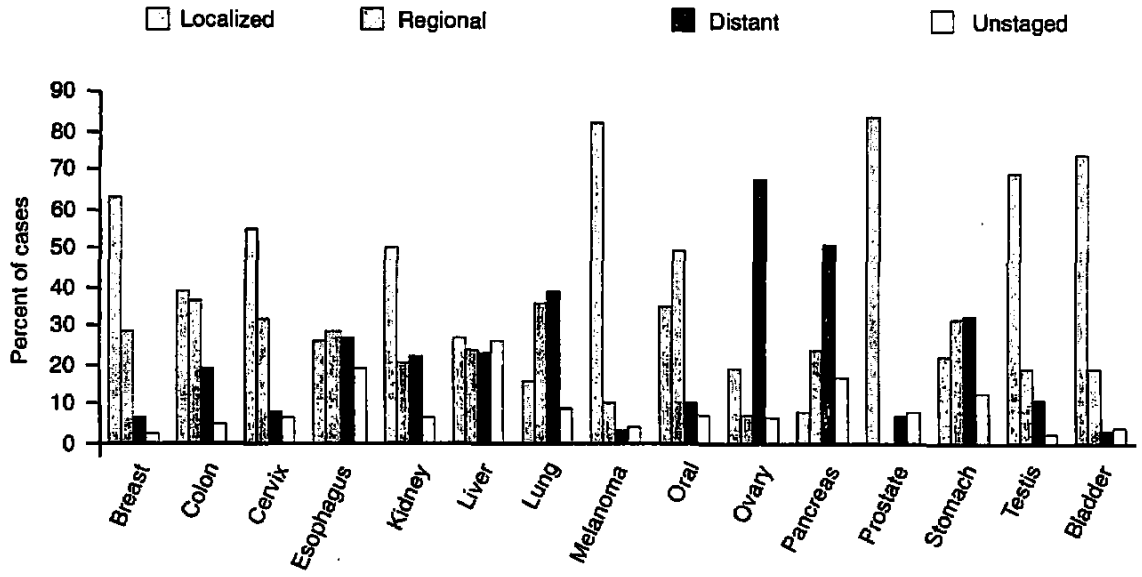

Figure 4 Surveillance Epidemiology and End Result (SEER) program stage distribution of cancer at diagnosis. The percentage of individuals at diagnosis with localized, regional (lymph node metastases) or metastatic cancer (distant metastases), by cancer histology for solid tumors. Data represent the results of nine SEER registries for the years 1988–2001, including all races and ages (http://www.seer.cancer.gov, SEER*StatDatabase:Incidence-SEER 9 Regs Public-Use, Nov. 2004 Sub (1973–2002), National Cancer Institute, DCCPS, Surveillance Research Program, Cancer Statistics Branch, released April 2005, based on the November 2004 submission).

lung carcinoma cells developed dormant lung metastases that could be activated by angiogenesis and by the removal of the primary tumor[131]. In this case, tumor cell dormancy represented a balance of proliferation and apoptosis. In other models, dormancy was caused by nonproliferating, viable cells[26,127,128]. Genes thought to be involved in dormancy include those encoding the MKK4 and Kiss1 metastasis suppressors, Bcl-xL, p38 and, for bone marrow dormancy, integrin $\alpha_5\beta_1$.

The conditions under which dormant cells can be reactivated to progressive growth are not well understood. Likewise, the best strategies for eliminating dormant tumor cells, particularly those that are not dividing, are not entirely obvious. In one study, mice were injected with metastatic and dormant breast carcinoma cell lines and treated with doxorubicin, a standard cytotoxic drug used in breast cancer chemotherapy, at a dose that reduced the metastatic burden of the aggressive line. This dose did not reduce the density of nondividing dormant cells[132]. In a separate model system, angiostatin induced dormancy in T241 fibrosarcoma cells[133]. The testing of compounds that target multiple aspects of a nonproliferative pathway may be fruitful, such as the Hsp90 inhibitors, proteasome inhibitors or multi-kinase inhibitors targeting VEGFR. Clinical testing would require long, costly studies. Nevertheless, these studies highlight our dearth of knowledge with respect to targeting dormant cells.

Stem cells

Stem cells give rise to all tissues during embryonic development and control tissue homeostasis in the adult. They are capable of asymmetric division to generate a daughter cell with distinct proliferative and differentiation capacity, and regenerate a stem cell. Cells with stem cell features have been identified in leukemias and a few solid tumors including breast cancer and brain tumors (reviewed in refs. 134–136). Eight of nine of the breast cancer specimens from which stem cells were identified were metastases, indicating that this population exists in metastatic lesions. The stem cell compartment is of critical importance to metastasis if distinct regulatory pathways are operative as compared to primary tumor cells[137], as distinct drugs would be needed to target both populations. One observation, that stem cell populations express multidrug resistance transporters[138], suggests that new treatment strategies may be needed. Validating whether a compound affects the minority stem cell population in preclinical experiments will be difficult, but experiments could test whether metastases regrow with time after being 'eradicated', indicating the potential presence of a stem cell population.

Chemoresistant cells

Phase 1 clinical trials are routinely conducted with individuals who have failed multiple therapies and, by definition, have resistant disease. Yet we infrequently test potential compounds on chemoresistant tumor cells. *In vitro* treatment of a nasal carcinoma cell line with melphalan increased its invasiveness[139], indicating that chemotherapeutics can alter some metastatic properties. A role for Bcl-xL in the resistance of lymph node and visceral metastases to docetaxel was reported and was accompanied by increased drug-induced genetic instability[140]. Tumor cell–microenvironment interactions, discussed in the section on bone metastases, may also have a significant role in mediating resistance to chemotherapy[65].

3. How are antimetastatic therapies best developed? Relatively few parts of the metastatic process have been successfully developed as therapeutic targets, although momentum is building for several pathways. Bevacizumab has shown activity in combination with cytotoxic compounds in the metastatic setting of several cancer histologies, and multi-kinase inhibitors including VEGFR are in trials. Trastuzumab, a recombinant monoclonal antibody to Her-2, is approved for metastatic breast cancer in combination with cytotoxics[141], and successful data in the adjuvant (lymph node–positive) setting have been reported[142,143]. Inhibitors of the EGFR have shown limited activity in advanced lung cancer as single agents (reviewed in ref. 144). These pathways and drugs function in both tumorigenesis and metastasis. The matrix metalloproteinase (MMP) inhibitors went through clinical development and failed. In examining the MMP inhibitors, it became obvious that MMPs have complex, sometimes conflicting roles in invasion and metastasis. Fundamental clinical data to validate that the target was expressed in metastatic lesions and that the compounds hit the target *in vivo* were also lacking[145].

Given this paucity of success, are we doing something wrong? One potential factor is our reliance on primary tumor biology for drug development. *In vivo* studies of MSGs, Src-mediated vascular permeability, EGFR, Bcl-xL and the insulin receptor substrate 2 indicate that metastasis can be modified in the absence of a change in the primary tumor[47,96,108,146,147]. Furthermore, preclinical studies report differential effects of drugs on primary and metastatic disease[148–153]. Despite these data, drug development continues to rely heavily on short-term reductions in the size of primary tumors. These data suggest that compounds validated in this manner may not work on metastatic disease, and that compounds with antimetastatic efficacy may not be validated in tests based on reduction of primary tumor size.

For the preclinical validation of metastasis-directed compounds we may also need to 'raise the bar'. Many agents are tested in a metastasis prevention setting. Yet phase 1 and 2 trials examine a compound for activity against an already-developed metastasis. The answer, in part, is to also test compounds in a treatment setting where metastatic colonization begins before a treatment is started.

4. How can the clinical trials process be optimized for antimetastatic drugs? One potential trend that makes theoretical sense is that the earlier in cancer progression that drugs are given, the better. For trastuzumab, the magnitude of clinical response data in the metastatic setting, although statistically significant[141], is dwarfed by that recently reported in the adjuvant setting (lymph node positive, distant metastasis negative)[142,143]. A logical extension of this trend is that compounds that interrupt metastatic colonization may not have conventional efficacy (complete and partial responses) in phase 1 clinical trial settings where they are asked to 'melt' an already established metastasis. Antimetastatic compounds may elicit stable disease, usually defined over a six-month period. Considerable resources and fortitude will be needed to advance a compound into expensive adjuvant setting trials, where angiogenesis and metastatic colonization have not been completed, particularly in cases where standard clinical responses in the metastatic setting have not been amassed[154]. It will therefore be critical to establish that the compound hits the metastasis target in early clinical testing, using sequential biopsies of metastatic tissue, a surrogate assay or imaging. Molecular imaging holds great promise in this regard. Imaging probes are being developed for angiogenesis (reviewed in ref. 155), multidrug resistance[156], apoptosis[157], proteolytic activity[158–160] and gene expression[161].

Additional facets of drug testing may require revision (reviewed in refs. 162,163). Combinations of drugs targeting multiple relevant pathways may be needed to overcome pathway redundancy or resistance. Given the presumed need for chronic dosing, it may also be critical to define a biologically effective dose rather than a maximum tolerated dose, to limit adverse effects of long-term dosing. Niche trials, rather than trials with individuals who all have the same cancer histology, may become more common. For instance, atrasentan may be of value to two subsets of patients such as prostate cancer patients at high risk for osteoblastic bone metastases as well as bladder cancer patients whose primary tumors exhibit low expression of the RhoGDI2 metastasis suppressor. Identification of individuals whose tumor expresses the target of interest will be critical for success. A vigorous dialogue between academic, pharmaceutical and biotechnology researchers on these topics may lead to increased investment in metastasis targets and logical clinical testing schemas.

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COMPETING INTERESTS STATEMENT

The author declares that she has no competing financial interests.

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EXHIBIT B

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Brain metastases

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Brain metastases are one of the most feared complications of cancer, because even small tumors may cause incapacitating neurologic symptoms. Surgical resection, often the major treatment modality in other cancers, is unavailable for many patients and also can cause neurologic morbidity. Furthermore, slight growth of a brain metastasis can kill patients by compressing normal brain against a nonexpansible skull, herniating the intracranial contents across compartmental precincts. The main contrast to disease in other organs is the inhomogeneity of the brain that leads to focal neurologic deficits often caused by small but seemingly strategically placed metastases.

Over a century ago, the eminent physician Gowers wrote: "It is probable that, in most forms of [brain] tumor, arrest of growth now and then occurs, but these are exceptions too few and far between to justify, in any given case, more than the dimmest ray of hope" [1]. Since that time vast improvements in the diagnosis and treatment of brain metastases have led to significant improvements in prognosis. This article reviews the epidemiology, clinical features, treatment, and prognosis of brain metastases from systemic malignancies.

Brain anatomy

Before discussing "brain metastases," it is important to define the CNS compartments. The cerebral cortex forms the outer brain layer and consists of neuronal cell bodies (gray matter) that communicate synaptically with deeper structures. White-matter tracts are myelinated

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axons that carry information between the cortex and the deep gray structures, such as the basal ganglia and thalami. At the base of the brain, the brain stem is divided into three parts: the midbrain, pons (from Latin for "bridge"), and medulla (from Latin for "inmost part," also called medulla oblongata) that caudally becomes the cervical spinal cord. The 12 cranial nerves arise from nuclei within the brain stem to control the motor and sensory functions of the face and head. Finally, the cerebellum (Latin for "little brain") lies behind the brain stem in the posterior fossa of the skull. "Brain metastases" refers, therefore, to metastatic lesions anywhere within the brain parenchyma: the cerebral hemispheres, brain stem, or cerebellum.

Surrounding the brain are three membranous coverings, or meninges (Greek "meningo," for membrane): the pia, arachnoid, and dura. The pia (Latin for "tender mother") is the innermost covering that follows the undulations of the cortical gyri and sulci. The arachnoid (Greek "arachne," for "spider," as the filamentous connections between the arachnoid and pia resemble a spider's web) composes the middle layer. The subarachnoid space, a real space, lies between the arachnoid and pia and is filled with cerebrospinal fluid. Most of the cranial nerves travel a significant distance within the subarachnoid space. The pia and arachnoid together compose the leptomeninges (from Greek "lepto," for slender).

The dura (Latin for "hard mother"), also called the pachymeninges, is the outermost, tough fibrous covering of the brain that lies immediately adjacent to the skull. The potential space between the dura and arachnoid is the subdural space; the potential space between the skull and the dura is the epidural space.

Supratentorial lesions are those above the tentorium (or "tent") that separates the hemispheres from the cerebellum and brain stem; infratentorial lesions occur below the tentorium. Supratentorial lesions often cause seizures (if cortically based and especially if hemorrhagic), cognitive dysfunction, and headaches. Infratentorial lesions often cause ataxia, diplopia, dysarthria, and dysphagia. Large infratentorial lesions may cause hydrocephalus and quickly lead to coma and death, as brain compliance is exhausted and herniation of the cerebellar tonsils through the foramen magnum ensues.

Epidemiology

Brain metastases are the most common cerebral tumors [2]. Although any primary systemic tumor may metastasize to the brain, several large clinical and autopsy series have identified the common malignancies (Table 1) [3–12].

Posner and Chernik produced the largest and most comprehensive autopsy series; they studied 3219 patients at Memorial Sloan-Kettering Cancer Center from 1970 to 1976 [9]. Of the 2375 cases that included an

Table 1
Percentage of brain metastases caused by different primary tumors

	Baker [3]	Globus [4]	Tom [5]	Chason et al [7]	Hunter and Rewcastle [8]	Posner and Chernik [9]	Zimm et al [10]	Lagerwaard et al [11]	Nussbaum et al [12]
N =	114	41	82	200	393	572	191	1291	729
Lung	21	46	22	61	34	18	64	56	39
Breast	21	2	16	16	19	17	14	16	17
Colorectal	7	12	11	4	6	2	3		
Melanoma	8 ("skin")	7	9	5	6	16	4		11
Kidney	8	2	1	4	4	2	2	4	6
Thyroid	1	10	1	<1	2				
Leukemia						12			
Lymphoma						10			
Unknown primary	4	2	18	1	4		8	8	5
% with brain mets.	18	14		18	6	24			

autopsy of the brain, approximately one in four (572, 24%) had intracranial metastases; one in five (467, 20%) had intraparenchymal or leptomeningeal metastases (ie, intradural lesions). These overall statistics are similar to other studies in which 18% to 24% of patients have brain metastases at autopsy.

Unfortunately, comparison among these pathologic studies is difficult for several reasons. First, there were differences in patient selection; for example, in one series, the investigators noted that neurologic symptoms led to inclusion of the brain among the organs examined at necropsy, perhaps falsely elevating the rate of brain involvement [13]. Second, some studies included hematologic malignancies (leukemias and lymphomas), whereas others did not. Third, some investigators grouped colon and rectal cancers together with other gastrointestinal malignancies. Finally, some investigators did not distinguish among parenchymal metastases, leptomeningeal metastases, and metastases that arose from bone or dura and involved the leptomeninges or brain by direct extension. This distinction is important for understanding the different biology of CNS metastases from each primary tumor. For example, Posner and Chernik [9] found 15 cases (3%) of intracranial metastases from prostate cancer; however, 14 were dural and only one involved the leptomeninges from direct extension from the dura. None was intraparenchymal; thus, prostate metastases to "brain" are rare.

Despite the methodological differences, lung cancer was universally the most common primary tumor, causing brain metastases in 18% to 64% of cases studied. The next most common cancers in descending order were breast (2%–21%), melanoma (4%–16%), and colorectal cancers (2%–11%). When included, the hematologic malignancies caused approximately 10% of cerebral metastases, primarily to the leptomeninges [9].

In addition, the data in Table 1 are helpful in predicting the primary tumor in a patient without known cancer who presents with brain metastases. When brain metastases are the presenting manifestation of cancer, the search for a primary tumor usually includes a CT scan of the chest, abdomen, and pelvis. Other tests, such as mammography or colonoscopy, are performed only if appropriate. Alternatively, a body positron emission tomography (PET) scan may localize the primary tumor and other systemic metastases, obviating other tests [14]. PET scans are not widely available at present, but are becoming more accessible. Occasionally an exhaustive search, including autopsy, cannot identify the primary tumor.

Biology

Brain metastases occur most commonly in the setting of widely disseminated cancer. In particular, lung metastases are often present when brain metastases are discovered from nonpulmonary primary tumors. For example, one series documented primary or metastatic cancer in the lung in

99.5% (199 of 200) of patients with brain metastases in an autopsy study of 1096 patients with various carcinomas [7]. In another series, 79% of patients with brain metastases suffered from either lung cancer or lung metastases [5]. Similar data regarding the frequent involvement of the lungs were documented by others [4,10]. In fact, the lungs are so frequently involved in patients with brain metastases that investigators from the era preceding modern brain imaging stated: "it seems wise to have a careful roentgenographic study made of the chest ... in all cases of unexplained stupor" to detect a lung cancer as a potential cause of brain metastases with associated neurologic abnormalities [15].

The high rate of primary or metastatic disease in the lung suggests that tumor reaches the brain via hematogenous dissemination as first proposed by Ewing in 1928 [16]. According to this theory, after starting in or reaching the lung, tumor cells eventually circulate in the blood to the left side of the heart and then embolize to other organs including the brain. In support of this theory, metastases to the brain stem, cerebellum, and hemispheres do occur approximately in proportion to the weight of and blood flow to those structures [9]. In addition, arterial border zones ("watershed areas") are an overrepresented site of metastases [17], presumably from tumor emboli. This theoretic mechanism is supported by stroke data documenting embolic phenomena causing ischemic stroke in these areas [18]. In addition, hemispheric metastases frequently occur at the junction of the gray and white matter where arterioles narrow sufficiently to trap tumor emboli [17,19], similar to the localization of brain abscesses from bacterial endocarditis.

Blood flow, however, does not explain all the features of brain metastases. In 1889, Paget proposed the "soil-seed" theory; he likened cancer to a plant, and "when a plant goes to seed, its seeds are carried in all directions; but they can only live and grow if they fall on congenial soil." In other words, the "soil" in this case is the brain and it must be congenial to the circulating tumor cells [20]. Indeed, there is a different distribution of metastases within the brain depending on the tumor type. For example, colorectal and genitourinary primaries metastasize disproportionately to the posterior fossa, and hematologic malignancies metastasize disproportionately to the leptomeninges [19]. In the century since these theories were first proposed, many studies have shown support for Paget's and Ewing's hypotheses, each of which explain some biologic features of brain metastasis.

Symptoms and signs

The presenting symptoms and signs depend on the neuroanatomic structures disrupted by the metastasis. Some lesions present slowly, with progressive headache or cognitive dysfunction. Others present acutely with

seizures. Hemorrhage into metastases may produce sudden severe headache, coma, or stroke-like focal neurologic findings; however, in an older clinicopathologic series of 15 patients with hemorrhagic metastases, the presentation was acute in only three (20%), whereas the onset was gradual in five (33%) and subacute in the remainder (approximately 50%) [21]. Tumors particularly prone to hemorrhage include melanoma, renal cell and thyroid carcinomas, and choriocarcinoma [22]. Lung cancer is not a typically hemorrhagic tumor; however, the high frequency of brain metastases from pulmonary primaries makes lung the most frequent hemorrhagic metastasis.

The "classic" history of brain tumor headache is morning pain from increased intracranial pressure (ICP) exacerbated by lying supine through the night, but in clinical practice only 17% of patients give this history [23]. In a series of 111 patients, only approximately half (49%) of those with metastatic brain tumors presented with headache. Headaches were a more common (78%) presenting complaint in patients with a prior history of benign headache, and approximately one third of these (36%) described the tumor-associated headache as identical to their prior headaches. Furthermore, brain metastases can masquerade as migraine, even causing aura [24].

In the era before neuroimaging, headache was the most common presenting symptom of brain metastases, and papilledema was found in approximately 25% of patients at presentation as a result of increased ICP [25,26]. With the advent of CT and MR scanning, however, metastases are discovered earlier and cognitive disturbances are the most frequent presenting symptom; papilledema is rare. Common presenting symptoms and signs are shown in Table 2 [10,12,27]. Notably, 9% had no symptoms or signs.

Lateralizing symptoms and signs, such as hemiparesis, aphasia, and a visual field disturbance, are common in most patients with brain metastases. When there are many bilateral lesions, however, the clinical picture may

Table 2
Presenting clinical features in 1013 patients with brain metastases

Symptoms and signs	Percentage with feature
Cognitive or mental status change	34
Headache	31
Weakness	24
Seizure	19
Ataxia	11
Visual change	5
Nausea or vomiting	4
Other (includes bulbar symptoms, dizziness and syncope)	4
Sensory change	2
Papilledema	0.5
None	9

Data from references [10,12,27].

resemble a toxic/metabolic encephalopathy from bilateral hemispheric dysfunction. In addition, the authors have seen cases of innumerable metastases above and below the tentorium with few symptoms. Contrast-enhanced MRI from one such patient who presented with mild headache are shown in Fig. 1. A characteristic finding in these patients is bilateral disease that does not cause marked shift of the intracranial structures. It is as if the brain metastases "balance" one another and, therefore, produce few symptoms.

Occasionally, there are rare presentations, such as chorea [28] (Greek "choreia," for dance), a movement disorder characterized by smooth uncontrollable movements of the limbs and trunk. In short, any new cerebral neurologic symptom in a cancer patient should provoke a search for brain metastases.

Methods of detection

At present, contrast-enhanced MRI is the best noninvasive test for evaluating the presence of brain metastases and their response to treatment. Although MR scanning is almost ubiquitous, there are occasions when it is unavailable or contraindicated (as for patients with pacemakers). In these patients, CT scanning usually delineates the lesions. Small metastases or lesions in the posterior fossa may be missed on CT, however [29–32].

Contrast enhancement on MRI or CT scanning identifies the metastases by highlighting disruption of the blood–brain barrier that occurs with tumors. Rarely, small metastases do not enhance but are evident on careful

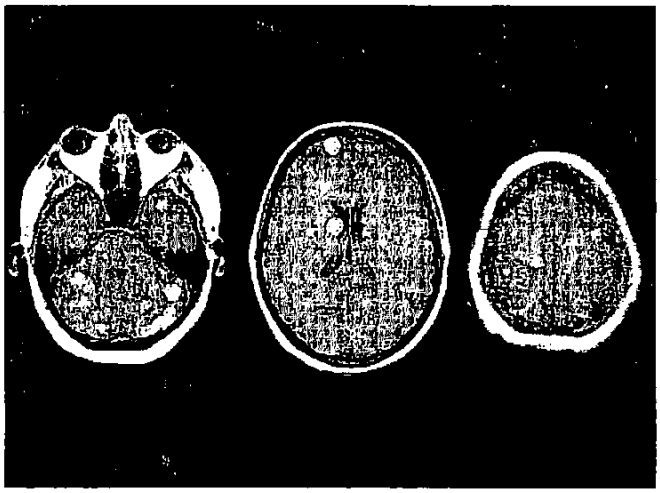

Fig. 1. Contrast-enhanced brain Magnetic Resonance Imaging (MRI) from a woman with breast cancer. Despite the presence of innumerable metastases, she presented without significant focal symptoms, as if the bilateral lesions "balanced" each other.

scrutiny of T2-weighted MR images. For example, miliary perivascular metastases ("carcinomatous encephalitis") may not enhance, but one must also consider inflammatory and infectious processes such as vasculitis or tuberculosis as alternative diagnoses in the appropriate setting [32,33].

Most contrast-enhancing lesions in a cancer patient are metastases, but the common differential diagnosis includes primary brain tumor, abscess, infarction, radiation necrosis (in a previously treated patient), granuloma, and demyelination, as indicated in Box 1.

Cancer and its treatments cause immune suppression; herpetic infections also should be considered in the appropriate clinical setting [34]. In one study, 11% of cancer patients with cerebral lesions were misdiagnosed with metastases; biopsy may be necessary whenever the diagnosis is in question [35].

Frequently, there are radiographic features that suggest the lesion is not a metastasis. For example, the enhancement in demyelination is often C-shaped rather than a complete ring [36]. Recurrent tumor often is indistinguishable on CT and MRI scanning from radiation necrosis, but PET can help differentiate the two. On PET imaging with 18F-fluorodeoxyglucose, neoplasia typically shows increased cellular uptake of glucose, whereas radiation necrosis is usually hypometabolic [37]. Magnetic resonance spectroscopy (MRS) examines the nuclear magnetic resonance pattern of the abnormal tissue seen on MRI. By comparing the different relative intensities of lactate, lipids, choline, and N-acetylaspartate (NAA), one may distinguish normal brain from edema, neoplasia, necrosis, or demyelination noninvasively [32].

Lumbar puncture (LP) is unnecessary in the diagnosis of brain metastasis. It also may be dangerous in patients with impending herniation [38]. Occasionally, LP is performed to diagnose concurrent leptomeningeal metastasis if subarachnoid seeding is suspected clinically or radiographically. Leptomeningeal metastasis may coexist with parenchymal metastases

Box 1. Major differential diagnoses of brain metastases

Primary brain tumor
  Glioma
  Primary CNS lymphoma
  Others
Infection
  Abscess
  Herpes encephalitis
Granuloma
Demyelinating plaque
Infarction
Radiation necrosis in a previously treated metastasis

in patients with multiple small superficial lesions particularly at the base of sulci, or lesions in the choroid plexus or immediately adjacent to the ventricles.

Therapy

Infrequently, patients may be "cured" of their brain metastases and survive many years with good neurologic function. Without treatment, however, most patients succumb quickly. Clinical prognostic factors include performance status, systemic disease burden, age, response to treatment, interval from primary diagnosis until brain metastases, and cognitive function [10,11]. The Karnofsky Performance Status (KPS) was introduced more than 50 years ago [39,40] and remains one of the most commonly used and reliable scores to assess overall clinical function. Several prognostic factors have been grouped together by the Radiation Therapy Oncology Group (RTOG) to form a three-tiered classification scheme to prognosticate survival in patients with brain metastases [41]. This scheme was based on the correlation between survival and clinical features in 1200 patients enrolled in multiple RTOG trials evaluating radiation regimens. Class 1 patients (best prognosis) had a KPS > 70 and were <65 years old with controlled primary tumor and no metastases outside the brain. Class 3 patients (worst prognosis) had a KPS < 70. The remaining patients were Class 2. The median survivals for Class 1, 2, and 3 patients were 7.1 months, 4.2 months, and 2.3 months respectively. Treatment can have a significant impact on the survival curves, however. Treatment for brain metastases is symptomatic and definitive, as indicated in Box 2.

Symptomatic treatment

Corticosteroids

Corticosteroids reduce the vasogenic edema that typically surrounds brain metastases, thereby relieving some of the aggregate mass that raises ICP [42]. The effect is often a dramatic, albeit temporary, clinical improvement allowing

Box 2. Treatment for brain metatases

- Symptomatic
  - Corticosteroids
  - Anticonvulsants
- Definitive
  - Whole-brain radiotherapy
  - Surgery
  - Stereotactic radiosurgery
  - Chemotherapy

time for more definitive therapy to occur. Although the initial effect may become evident within hours, the maximal benefit may not be manifest for several days, and in some cases may take as long as two weeks [43].

Typically, the authors start with a 10- to 24-mg bolus of dexamethasone followed by a similar daily dose divided every six hours (2–6 mg every 6 hours). Frequently, corticosteroids can be tapered or discontinued after completing definitive therapy, such as surgery, radiation, or chemotherapy. The final dose should be the lowest necessary to control the patient's neurologic symptoms. Chronic corticosteroid use may lead to well-known adverse effects, such as hyperglycemia, hemorrhagic gastritis, osteoporosis, poor wound healing, and immune suppression leading to opportunistic infections such as oral candidiasis [44]. Life-threatening side effects also can occur, such as gastrointestinal perforation [45], and the clinician must remain vigilant as steroids may mask pain and infection. Steroid-induced proximal muscle weakness (myopathy) can develop quickly, even within weeks [46], and such weakness also can lead to significant diagnostic confusion when metastases also cause weakness. Steroid myopathy often is first evident when patients develop difficulty arising from a chair or the toilet and need to use their arms for support. Spinal cord compression from metastasis to the vertebral bodies, epidural space, or spinal leptomeninges also must be considered in patients with leg weakness. Patients who remain on corticosteroids longer than six weeks should take prophylactic antibiotics against *Pneuomocystis carinii* pneumonia.

Cerebral herniation

Cerebral herniation occurs when mass lesions severely raise ICP and shift the intracranial contents in a life-threatening manner. If it is recognized and treated quickly, the syndrome often is reversible.

A first step is the administration of hyperosmolar agents, such as a 100-gm bolus of 20% mannitol solution (1–2 gm/kg). By increasing the serum osmolarity, water is drawn out of the brain parenchyma to reduce ICP. When given chronically, however, mannitol may diffuse into damaged brain tissue leading to a rebound increase in ICP as water is drawn back into the brain. Some clinicians follow the initial bolus with additional infusions of mannitol (25 gm or 0.25–0.5 gm/kg) every 4 to 6 hours in cases of severe refractory increased ICP, but such treatment is controversial. Second, hyperventilation to drive the pCO_2 down to approximately 25 mmHg induces cerebral vasoconstriction. This reduces the volume in the cerebral vasculature, thereby reducing ICP. Such treatment usually requires intubation and mechanical ventilation to protect against brain stem dysfunction and subsequent autonomic respiratory failure. Third, raising the head of the bed increases venous outflow and quickly reduces ICP; this is a quick and safe maneuver unless the brain contents are herniating downward through the foramen magnum. Fourth, in a patient with

hydrocephalus, either from parenchymal or leptomeningeal metastases, diversion of the cerebrospinal fluid (CSF) with a ventricular drain rapidly reduces ICP. As an added benefit, a ventricular drain can be connected to a monitor to directly measure ICP. All of these measures, however, only temporize until more definitive therapy is undertaken [47].

Seizure prophylaxis

One issue that frequently arises is whether to give anticonvulsants to all patients with brain metastases. Patients who present with seizures require anticonvulsants [48]. Patients without seizures, however, frequently are given prophylactic anticonvulsants. In a recent meta-analysis of 12 studies in patients with brain tumors, 10 of which included patients with brain metastases, none supported a role for prophylactic anticonvulsants. Prophylactic anticonvulsants did not protect against subsequent seizures; furthermore, antiepileptic drugs frequently are associated with side effects or drug interactions.

Many anticonvulsants stimulate the hepatic cytochrome 450 enzyme system. This may enhance the metabolism of some chemotherapeutic agents, often rendering such therapies less effective [49]. Anticonvulsants also may enhance the metabolism of corticosteroids, thus reducing control of cerebral edema. Furthermore, anticonvulsant side effects, including life-threatening ones, were more frequent in brain tumor patients than in patients with seizures from other etiologies [48]. In particular, the combination of phenytoin [50] or carbamazepine [51] with cranial radiotherapy, especially during a taper of corticosteroids, may predispose to the Stevens-Johnson syndrome. Therefore, prophylactic anticonvulsants are ineffective and may be associated with significant side effects; consequently, they are not recommended for patients with brain metastases.

Definitive treatment

Without treatment, patients with brain metastases survive approximately one month; supportive treatment with corticosteroids lengthens median survival to approximately two months, and almost any treatment prolongs survival [11,52]. More than 20 years ago, a study of 191 patients with brain metastases demonstrated that radiation improved median survival to three to four months, and surgery with radiation improved survival to 8 to 10 months, although there was significant selection bias [10]. Similar data are reported for brain metastases from melanoma [53,54], breast [55], renal [56], colon [57], and other cancers. Although these survival statistics far surpass the prognosis during Gowers' time, the median survivals have not improved appreciably since the advent of modern surgical techniques and radiation. For example, a more recent retrospective study of 1292 patients referred for radiation yielded similar results, with median survivals of approximately one

month, four months, and nine months following treatment with steroids, radiotherapy, and surgery with radiation, respectively [11].

Whole-brain radiotherapy

Chao et al wrote their seminal paper almost 50 years ago describing whole-brain radiotherapy (WBRT) for brain metastases [58], and it remains the main treatment modality; several controlled trials have confirmed that WBRT improves survival and neurologic function. The RTOG conducted several studies comparing multiple time-dose fractionation schemes. In pooling the results of 1812 patients who participated in the first two Phase III trials, 75% to 80% of patients remained neurologically stable or improved, specific symptoms improved in up to 90%, median survival was approximately four months, and there were no significant differences among various treatment regimens [59]. The regimen of 3000 cGy divided into 10 equal 300 cGy fractions over two weeks has been widely adopted as providing safe, rapid palliation for most patients. Not surprisingly, patients who begin WBRT with better overall function respond more favorably. Ultrarapid regimens of 1000 cGy in one fraction or 1200 cGy in two fractions produced improvement rates and median survivals that were approximately equal with the more protracted regimens. However, patients progressed neurologically more quickly, and some suffered severe acute neurotoxicities including herniation and death; such regimens are no longer used [60]. Additionally, initial data suggested that hyperfractionated regimens of up to 7040 cGy might improve survival further [61,62], but these results were not replicated with additional study [63]. Finally, studies with radiation sensitizers have been disappointing [64–66].

WBRT effectively palliates neurologic disease in most patients, but the median survival is only approximately four months because many succumb to uncontrolled systemic disease. Even brain metastases from relatively radioresistant tumors such as melanoma may respond [67,68]. There are occasional patients with prolonged survival following WBRT and even autopsied cases of pathologically documented brain metastases that were cured following WBRT in patients who later died from their systemic cancer [69].

Surgery

For close to a century, attempts have been made to improve survival by surgically removing metastatic lesions. Many uncontrolled studies demonstrate benefit from surgery, but patient selection bias has always clouded the interpretation [70]. There have been three randomized prospective trials comparing WBRT alone with resection followed by WBRT for single-brain metastasis (Table 3). Two showed unequivocal benefit from surgery [35,71,72]. The third showed no survival advantage or difference in quality of life [73]. In the third trial, however, almost 25% of the patients

Table 3
Outcome after treatment of single brain metastases (median in weeks)

	Surgery + Whole-brain Radiotherapy (WBRT)		WBRT alone	
	Survival	Functional independence	Survival	Functional independence
Patchell et al [35]	40	38	15	8
Vecht et al [71]	40	30	24	14
Mintz et al [73]	22	—	25	—

randomized to WBRT alone (10 of 43) had surgical resection of their brain metastasis. Furthermore, almost 20% (7 of 41) of patients in the surgery group also violated protocol for various reasons. These problems may account for the lack of difference between the treatment groups.

The authors strongly advocate surgery for single-brain metastasis in patients with controlled or controllable systemic disease. Many also advocate resection of a dominant single lesion even if a patient has multiple other brain metastases. This may be necessary if a single lesion is causing severe or impending neurologic compromise, such as a large cerebellar metastasis compressing the fourth ventricle. Extirpation of such a lesion may relieve acute neurologic symptoms and also facilitate the safe administration of subsequent WBRT.

There is a growing trend toward resecting multiple brain metastases when two to three surgically accessible lesions are present. Retrospective data suggest these patients also may do as well with surgery as those who have a single lesion removed. In a retrospective study, the records of 56 patients with multiple metastases and 26 matched patients with single metastasis were assessed. The median postoperative survival was six months among 30 of the 56 patients who had surgery for brain metastases but in whom all lesions were not resected (Group A). In contrast, the median survival was 14 months for the 26 of 56 patients who had resection of all multiple lesions (Group B) and for the 26 matched patients who underwent resection of a single metastasis (Group C). The differences in survival were statistically significant between Groups A and B ($P = 0.003$) and between Groups A and C ($P = 0.012$) [74]. When patients have a good performance status and limited systemic disease, the authors recommended surgery for two or even three brain metastases. In addition, select patients benefit from reoperation for recurrent brain metastasis [75,76]. These data give hope to patients with recurrent brain metastases, as studies for re-irradiation conflict [77,78]. With the advent of stereotactic radiosurgery (discussed later), however, the use of multiple craniotomies may diminish.

Postoperative radiotherapy

Brain metastases usually are more encapsulated and easily removed than primary glial tumors, but surgery may leave microscopic tumor cells behind

in the operative bed. In addition, there may be micrometastases elsewhere in the brain not visible on MRI. These considerations often have led to the empiric use of postoperative WBRT. In a well-designed prospective trial, patients who had a complete resection of a single-brain metastasis were randomized either to receive immediate WBRT or not. The study demonstrated that postoperative WBRT prolonged control of brain metastases and reduced neurologic death rates. The overall median mortality of 48 weeks was equivalent in the two groups, however. Moreover, there was no statistical difference between the two groups in the duration of functional independence (37 and 35 weeks, respectively) [79].

In addition, a significant problem with WBRT is the delayed neurotoxicity that can cognitively debilitate patients, especially the elderly. WBRT of brain metastases that led to long-term survival in 12 patients was associated with delayed dementia; these patients accounted for approximately 20% of those who survived a median of one year after treatment with WBRT. Many of these patients also developed gait ataxia and urinary incontinence suggestive of normal pressure hydrocephalus. Radiographic findings included brain atrophy, hydrocephalus, and leukoencephalopathy (changes in the white matter) that corresponded to chronic edema when examined pathologically. Treatment with CSF shunting and steroids provided some relief [80]. Even in younger patients, there are often detrimental effects on cognition. Therefore, the authors often forgo postoperative WBRT, especially in older patients and in those with relatively radioresistant tumors [81].

Stereotactic radiosurgery

Stereotactic radiosurgery (SRS) delivers an extremely high dose of focused radiation in one fraction to maximize the dose to the tumor and minimize the effect on surrounding normal tissue. Although Leksell first coined the term "radiosurgery" in the 1950s [82], the technique was first used to treat other brain lesions, such as vascular malformations. There are two commonly used methods of delivering SRS: the linear accelerator (LINAC), which delivers X-rays; and the gamma-knife, which delivers gamma rays from multiple Cobalt-60 sources. Many recent studies demonstrate the efficacy of SRS in treating brain metastases [83]. SRS especially is useful for patients unable to tolerate surgery and for patients with lesions that are surgically inaccessible, such as metastases to the brain stem [84]. Many investigators also are using it to treat multiple lesions instead of WBRT. Most agree that SRS is limited to lesions no larger than 3 cm in diameter. SRS has several advantages over surgery, including convenience (it is usually an outpatient procedure) and ability to treat multiple lesions simultaneously. Like surgery, however, it probably should be limited to treating no more than three lesions. Prior WBRT does not preclude salvage treatment with SRS [54,85,86].

Because SRS is a highly focused treatment, as is surgery, one major question is whether SRS combined with WBRT is superior to WBRT alone. A retrospective analysis of 502 patients treated with SRS at 10 different institutions shows that the combination of SRS with WBRT leads to superior survival when compared with WBRT alone [87]. The study reduces selection bias by stratifying patients into the three prognostic tiers defined by recursive partitioning analysis of the RTOG data [41]. For all three tiers, adding SRS to WBRT lengthens survival when compared to historical controls.

There is also a small prospective randomized trial of 27 patients with two to four metastases in which 13 patients received SRS plus WBRT and 14 patients received WBRT alone. [88] The patients who received SRS demonstrated better local control and time to recurrence of brain metastases. There was also a trend toward improved survival (11 months versus 7.5 months), but this was not statistically significant ($P = 0.22$). A larger randomized trial comparing SRS plus WBRT to WBRT alone is in progress, and the preliminary results suggest similarly improved performance status and local control with the addition of SRS, but without a survival advantage [87].

Retrospective reviews comparing surgery with SRS suggest the techniques may be equivalent in their efficacy. There are studies demonstrating the superiority of one approach over the other [89], but differences in outcome are small and the problem of selection bias obscures a definitive analysis. Encouraging data come from a retrospective analysis of 122 patients at four institutions who received SRS and WBRT as upfront therapy for newly diagnosed single-brain metastases [90]. This patient population studied was designed to parallel the surgical arm of the randomized studies of surgery with and without WBRT from Patchell et al [35] and Noordijk et al [72]. The results suggested that combining SRS and WBRT led to survival, functional independence, and recurrence rates that were equivalent to treatment with surgery and WBRT. There is no randomized clinical trial comparing SRS with surgery.

An important additional question is whether WBRT when combined with SRS is superior to SRS alone. In effect, this is the same question addressed by Patchell et al [79], who investigated whether WBRT following surgical resection improved survival and quality of life. SRS is effective as a sole treatment of brain metastases, yielding a median survival of approximately nine months in one study [91]. Retrospective analysis comparing SRS alone with SRS combined with WBRT yielded no difference in survival (11 months), although there was a trend toward improved local control but worsened overall risk of new brain metastases in those treated without WBRT, presumably by leaving micrometastases elsewhere untreated [92]. Other retrospective studies found similar results [88,93], although some found a trend toward increased survival among those patients without extracranial disease who received WBRT [94]. There is a prospective randomized study in progress to assess the value of WBRT

following SRS. Until those data become available, the authors approach SRS as surgery and usually hold WBRT until the time of tumor progression.

Complications of SRS may include seizures, worsening neurologic deficits, and nausea. These side effects occurred in less than 10% of patients in most series but may necessitate restarting or increasing the dose of corticosteroids [83]. Radiation necrosis developed in 17% of patients in one study [95], although this rate may rise with longer follow up. Hemorrhage or radiation necrosis may require surgical excision in some patients [88].

Chemotherapy

Currently, chemotherapy also has a limited role in treating most brain metastases. One difficulty is choosing drugs or doses that penetrate the blood–brain barrier (BBB) [96]. The intact BBB excludes agents that are hydrophilic or large, although this may be less of an issue in patients with brain metastases that have a significant amount of contrast enhancement on CT or MR scanning indicating disruption of the BBB. Nonetheless, an intact BBB creates a "sanctuary site" in the brain, and brain metastases may occur long after systemic chemotherapy has rendered extracranial disease quiescent [97]. Some investigators have attempted to improve efficacy with intra-arterial drug delivery, at times with simultaneous BBB disruption; however, these measures have only limited success at best and the authors do not favor them.

More important than the BBB is the intrinsic chemosensitivity of the metastasis, as certain tumors respond well to chemotherapy. For example, many studies demonstrate that brain metastases from small-cell lung cancer (SCLC) are particularly chemosensitive [98,99]. Initial treatment with chemotherapy of brain metastases from SCLC results in high response rates; it even may be useful to delay WBRT until recurrence or progression of brain metastases, especially in those who need on-going chemotherapy for their systemic disease. Agents used include cyclophosphamide, vincristine, etoposide, and doxorubicin, which led to an 82% response rate in one series [100]. More recently, teniposide demonstrated activity, but was most useful when combined with WBRT [101]. It also has shown some activity in non–small-cell lung cancer (NSCLC) [102]. Although a recent meta-analysis demonstrated a small but significant survival and quality of life advantage and lower rates of brain metastases after prophylactic WBRT in patients with SCLC in remission [13], this remains controversial because of the risk of WBRT induced neurotoxicity.

Although NSCLC is less chemosensitive than SCLC, a prospective study of up-front cisplatin and etoposide shows encouraging results in patients with brain metastases from breast cancer and NSCLC; patients with melanoma respond poorly [103]. Temozolomide, however, recently has shown activity against brain metastases from melanoma and NSCLC and other primary tumors (Fig. 2) [104,105]. A regimen of cisplatin ifosfamide

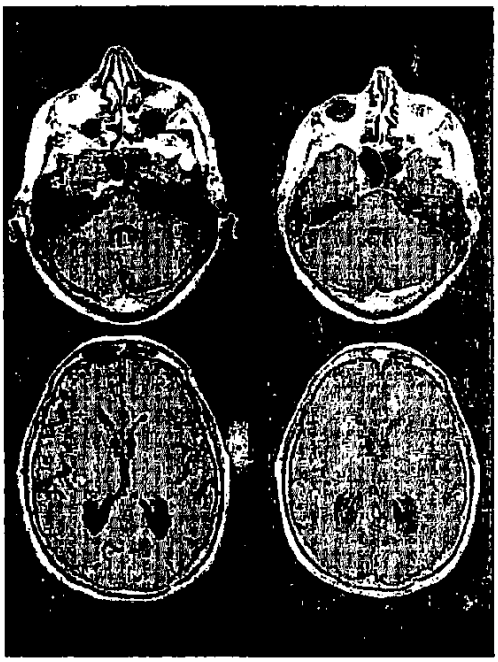

Fig. 2. Contrast-enhanced brain Magnetic Resonance Imaging (MRI) before (*left*) and after (*right*) two cycles of Temodar for brain metastases from Non-Small-Cell Lung Cancer (NSCLC). The lesions have disappeared.

and irinotecan produced responses in more than 90% of patients with SCLC [106] and in approximately half of patients with NSCLC [107].

Brain metastases from breast cancer also may be chemosensitive. Among 100 patients with brain metastases from breast cancer, partial or complete responses occurred in 50 patients, and stable disease resulted in nine patients after treatment with various regimens including cyclophosphamide, methotrexate, fluorouracil, prednisone, vincristine, and doxorubicin. Overall median survival was 5.5 months in all 100 patients, but this varied greatly within subgroups. Median survival was 39.5 months in the 10 complete responders, 10.5 months in the 40 partial responders, 6.5 months in the stable patients, and only 1.5 months in nonresponders [108]. In another series of 20 breast cancer patients treated with cyclophosphamide, fluorouracil, and either methotrexate or doxorubicin, up to 76% showed a response; median overall survival was approximately 6 months, again varying greatly within subgroups [109]. Among responders, it was approximately 17 months. Of note, these results were better than historic controls treated with WBRT. Others showed a similar response rate of 55% after treatment with platinum and etoposide [110]. Regimens with other agents, such as lomustine, carboplatin, vinorelbine, and fluorouracil, also have shown activity in breast cancer and NSCLC

[111]. There are sporadic reports of responses to hormonal therapy such as tamoxifen [112,113,114], especially in patients with estrogen receptor-positive tumors. Responses to megestrol acetate [115] and melatonin [116] also have been reported.

Brain metastases from choriocarcinoma also are extraordinarily chemosensitive [117,118]. Regimens of etoposide, methotrexate, actinomycin, vincristine, and cyclophosphamide (EMA/CO) produced prolonged survival in 72% of patients treated (13 of 18 patients) [118]. Radiotherapy after the chemotherapy is controversial. The authors recently studied an experience with high-dose intravenous methotrexate in patients with recurrent brain or leptomeningeal metastases. The high dose penetrates the BBB, but most tumors that metastasize to the brain are not sensitive to methotrexate. Among 21 patients with CNS metastases, more than half initially improved or remained stable clinically and radiographically, although median survival after initiating treatment was only three months [119]. No patient developed severe leukoencephalopathy, but survival may have been too short to observe this toxicity.

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EXHIBIT C

Cytopathologic Evaluation of Lung Carcinomas Presenting as Brain Metastasis

Cesar V. Reyes, M.D.,* Karen Sue Thompson, M.D., and JoAnne D. Jensen, S.C.T. (A.S.C.P.) (C.M.I.A.C.)

Brain metastasis is an uncommon initial presentation of lung carcinoma. One arm of this analysis is a retrospective review of 137 cases of surgically diagnosed solitary brain metastasis, which were eventually found to be of lung origin, encountered at Hines VA Hospital during the period 1958 to 1996. The second arm is composed of fine-needle aspiration biopsy specimens of primary lung tumor in 23 patients with an initial clinical diagnosis of brain metastasis and without the benefit of surgery, seen from 1981 through 1996. Our results in both analyses indicate that pulmonary adenocarcinoma is the predominant primary tumor that initially manifests as a brain metastasis, approaching 76% (107 and 17 cases, respectively), followed by small-cell carcinoma at 20% (24 and five cases, respectively) and large-cell undifferentiated carcinoma and squamous-cell carcinoma at 2% each. The predominance of adenocarcinoma as a source of brain metastasis in lung cancer patients probably reflects its rising incidence overall of late. Collateral findings also suggest that surgical resection of a solitary and small brain metastasis as well as of a discrete lung primary, whenever feasible, as the most effective procedure to improve survival and quality of life of patients. Diagn. Cytopathol. 1999;20:325–327.

Key Words: brain metastasis; lung carcinoma; adenocarcinoma; small-cell carcinoma

Lung cancer is the most common source of metastasis to the brain.[1–3] In large series, between 20% and 50% of lung cancer patients are noted to have brain involvement. As an initial presentation of lung cancer, however, brain metastasis is uncommon.[1] On microscopic examination, small-cell and large-cell undifferentiated carcinomas appear to be the usual types, although other studies have indicated that adenocarcinoma is the more predominant type.[4–6] While most published data focus on the clinical syndrome, treatment, and prognosis of brain metastasis, the histologic and cytologic aspects of the tumor have received little attention. The goal of this report is to address the cytologic and light microscopic features of lung carcinoma that initially manifests as brain metastasis.

Materials, Methods, and Results

Patients with Craniotomy

The surgical pathology files and tumor registry at Hines VA Hospital from 1958 to 1996 listed 137 patients who underwent craniotomy with frozen-section evaluation for metastatic carcinoma, which was their initial complaint. Subsequently all cases were confirmed to be primary in the lung. Histologic diagnosis of the brain metastases in the 137 patients was typed as follows: adenocarcinoma in 107 cases, small-cell carcinoma (SCC) in 24 cases, large-cell undifferentiated carcinoma (LCUC) in three cases, and squamous-cell carcinoma in three cases (Table 1). The examination was complemented by histochemical testing and immunostaining in almost all cases and by electron microscopy in 72 cases.

Special stains demonstrated secretory features in almost all of the 87 adenocarcinomas analyzed with mucicarmine and periodic acid–Schiff with diastase. Two cases initially interpreted as LCUC were found to be adenocarcinomas on histochemical testing. In addition, three LCUCs showed no evidence of secretion on special stains. Immunostaining displayed neuroendocrine differentiation in the 24 SCCs and one LCUC, but none in 17 adenocarcinomas studied. Keratin was confirmed in 28 adenocarcinomas and three LCUCs evaluated. Prostatic-specific antigen, trypsin, and chemotrypsin and alpha-fetoprotein evaluation showed negative results in 15 adenocarcinomas and two LCUCs tested.

Electron microscopic study essentially reaffirmed the light microscopic findings in 72 cases. Almost all the SCCs and one LCUC were neuroendocrine tumors. Among the adenocarcinomas (n = 48), 35 were of nonciliated bronchiolar cell origin, two each were of mucous and bronchioloal-

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Table I. Surgical Pathologic Analysis of Lung Cancer Presenting as Brain Metastasis at Hines VA Hospital (IL), 1958–1996

Histologic types	*Cases*
Adenocarcinoma	107
Small-cell carcinoma	24
Large-cell undifferentiated carcinoma	3
Squamous-cell carcinoma	3
Total	137

veolar cell types, and the remaining cases were poorly differentiated adenocarcinomas.

In a review of the clinical records, all 137 patients were evaluable with a complete history, physical examination, chest radiographs and other radiographic studies (fluoroscopy, tomogram, CT scans, and MRI), and laboratory tests (including blood cell counts, urinalysis, and blood chemistries). The cytologic and/or histologic diagnosis of the primary lung tumor was also available in all 137 cases. Sputa, bronchial brushing and washing, transthoracic fine-needle aspiration biopsy (n = 34), and transbronchial Wang fine-needle aspiration biopsy (n = 12) were used for cytologic studies. For the histologic evaluation, bronchial biopsy specimens (n = 91) and resection tumor tissue (n = 10) were evaluated. Essentially, the lung diagnosis was in agreement with the initial findings in the brain metastasis in all cases. Postmortem examination in 32 cases also confirmed the histologic diagnosis.

One hundred fifteen of the 137 patients were evaluable for survival (22 were lost to follow-up). The overall survival rate was 44% at 6 mo, 27% at 1 yr, and 9% at 2 yr. Four of the 10 patients who underwent complete resection of both the brain metastasis and lung primary survived for 2 yr; one survived more than 3 yr.

Patients Without the Benefit of Surgery

From 1981 through 1996, the cases of 23 patients who initially had massive or multifocal brain metastasis on CT scans (Fig. 1) were reviewed. Their ages ranged from 45 to 68 yr (mean, 63 yr). All were male and Caucasian. Chest radiographs and CT scans revealed clinically inoperable lung neoplasms with mediastinal lymphadenopathy and signs of metastasis at other sites. Fine-needle aspiration biopsy of the lung lesions showed adenocarcinoma (Fig. 2) in 17 cases, SCC in five cases, and LCUC in one case (Table 2). On electron microscopy, 16 adenocarcinomas were found to be of nonciliated bronchiolar cell origin; one was of bronchioloalveolar cell origin. The SCCs were of the neuroendocrine type.

All 23 patients received irradiation to the brain and lung lesions and/or systemic cis-platinum, taxol, or VP-16-based chemotherapy, along with symptomatic and supportive regimens. Twelve patients succumbed to the tumor at 6 mo, and none survived 10 mo. Autopsy findings in six cases reaffirmed the cytologic diagnosis.

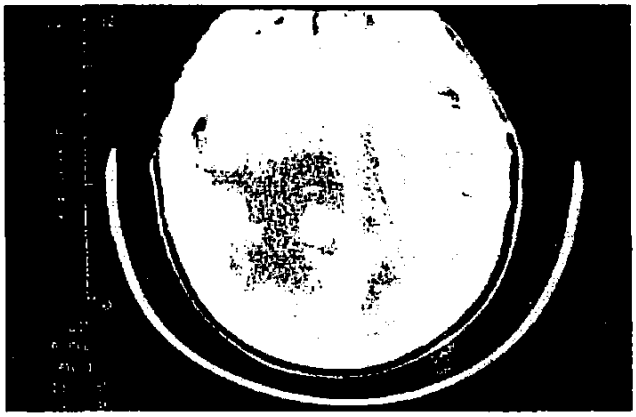

Fig. 1. A 54-yr-old man was completely well until 3 wk before admission, when he had episodes of seizure followed by unconsciousness of 4 days' duration. CT scan showed a massive mass in the right posterior cerebrum.

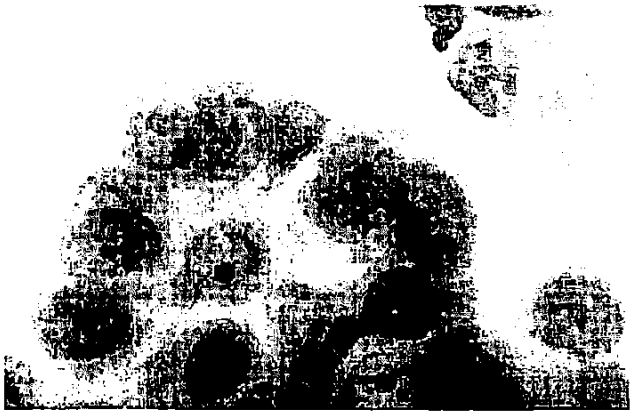

Fig. 2. Chest radiographs showed signs of mediastinal lymphadenopathy and a large left-sided, posteriorly located lung lesion, which was biopsied by transcutaneous fine-needle aspiration under CT guidance. On cytologic examination, the interpretation was adenocarcinoma (Papanicolaou stains, ×400). At autopsy, the diagnosis of pulmonary adenocarcinoma and right-sided posterior cerebral metastasis was confirmed.

Table II. Cytologic Analysis of Lung Cancer Presenting as Brain Metastasis at Hines VA Hospital (IL), 1981–1996

Cytologic types	*Cases*
Adenocarcinoma	17
Small-cell carcinoma	5
Large-cell undifferentiated carcinoma	1
Total	23

Comments

The brain is highly susceptible to metastasis from cancer of the lung. A solitary brain metastasis has been reported in one-third of cases. The syndrome of brain metastasis as the first presentation of lung cancer portends a grave prognosis and seems to be influenced by radiotherapy and/or chemotherapy only minimally. The average reported survival time of untreated patients with brain metastasis ranges from 1.5 to

6 mo.[1-10] Sequential brain and lung surgeries appear to have considerably better results than either radiotherapy or chemotherapy alone or in combination, in terms of prolonging survival and improving the quality of patients' life. The one-yr survival rate after combined resection increases to 30%, and has been reported up to as high as 55%.[1,6,7,10]

In this series, the 1-yr survival rate was 27%, and one patient survived for 3 yr after initial diagnosis, with both brain lesion and lung primary resected. These results are much improved in comparison with the group treated with radiation and chemotherapy regimen(s). Evidence of encouraging results and the possibility of a more accurate selection of patients with solitary brain metastasis by means of CT scan or MRI support combined surgical resection for lung cancer and brain metastasis as the better treatment regimen.

The predominance of adenocarcinoma as a source of brain metastasis in lung cancer patients is probably proportional to its rising incidence overall of late. This observation has been attributed to improved criteria for evaluating tumor pathologic characteristics; the increased incidence of lung cancer among women, who tend to have adenocarcinoma; and a wider exposure to new occupational/environmental carcinogens. Histochemical and electron microscopic evaluation in cancer diagnosis has also made possible the reclassification of many poorly differentiated carcinomas and LCUCs into the category of adenocarcinoma.[11]

Recent epidemiologic data likewise have shown that smoking plays a premier role in the development of lung adenocarcinoma.[11] Brain metastases are present more frequently when the primary tumor is located in the lung periphery; such a tumor is usually an adenocarcinoma.[2] Adenocarcinoma is also the most common type of lung carcinoma to metastasize to the brain in our study. Although sequential brain and lung resections appear to be the superior treatment regimen, only certain patients are good candidates for the procedure. Those patients most likely to benefit from surgical resections are those with a single, accessible brain lesion, systemic cancer limited to the primary site, and life expectancy of at least 2 mo.[7]

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EXHIBIT D

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Product Sheet

A549 (ATCC® CCL-185™)

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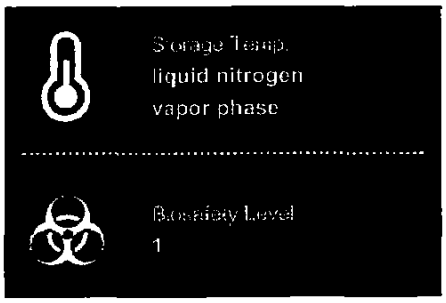

Intended Use

This product is intended for research use only. It is not intended for any animal or human therapeutic or diagnostic use.

Complete Growth Medium

The base medium for this cell line is ATCC-formulated F-12K Medium, Catalog No. 30-2004. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%.

Citation of Strain

If use of this culture results in a scientific publication, it should be cited in that manuscript in the following manner: A549 (ATCC® CCL-185™)

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Email: Tech@atcc.org

Or contact your local distributor

Description

Organism: *Homo sapiens*, human
Tissue:
lung
Disease: Carcinoma
Age: 58 years
Gender: male
Morphology: epithelial
Growth Properties: adherent
Isoenzymes:
G6PD, B
DNA Profile:
Amelogenin: X,Y
CSF1PO: 10,12
D13S317: 11
D16S539: 11,12
D5S818: 11
D7S820: 8,11
THO1: 8,9.3
TPOX: 8,11
vWA: 14
Cytogenetic Analysis: This is a hypotriploid human cell line with the modal chromosome number of 66, occurring in 24% of cells. Cells with 64 (22%), 65, and 67 chromosome counts also occurred at relatively high frequencies; the rate with higher ploidies was low at 0.4%. There were 6 markers present in single copies in all cells. They include der(6)t(1;6) (q11;q27); ?del(6) (p23); del(11) (q21), del(2) (q11), M4 and M5. Most cells had two X and two Y chromosomes. However, one or both Y chromosomes were lost in 40% of 50 cells analyzed. Chromosomes N2 and N6 had single copies per cell; and N12 and N17 usually had 4 copies.

SAFETY PRECAUTION

ATCC highly recommends that protective gloves and clothing always be used and a full face mask always be worn when handling frozen vials. It is important to note that some vials leak when submersed in liquid nitrogen and will slowly fill with liquid nitrogen. Upon thawing, the conversion of the liquid nitrogen back to its gas phase may result in the vessel exploding or blowing off its cap with dangerous force creating flying debris.

Unpacking & Storage Instructions

1. Check all containers for leakage or breakage.
2. Remove the frozen cells from the dry ice packaging and immediately place the cells at a temperature below -130°C, preferably in liquid nitrogen vapor, until ready for use.

Handling Procedure for Frozen Cells

Handling Procedure for Frozen Cells

To insure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If upon arrival, continued storage of the frozen culture is necessary, it should be stored in liquid nitrogen vapor phase and not at -70°C. Storage at -70°C will result in loss of viability.

SAFETY PRECAUTION: ATCC highly recommends that protective gloves and clothing always be used and a full face mask always be worn when handling frozen vials. It is important to note that some vials leak when submersed in liquid nitrogen and will slowly fill with liquid nitrogen. Upon thawing, the conversion of the liquid nitrogen back to its gas phase may result in the vessel exploding or blowing off its cap with dangerous force creating flying debris.

1. Thaw the vial by gentle agitation in a 37°C water bath. To reduce the possibility of contamination, keep the O-ring and cap out of the water. Thawing should be rapid (approximately 2 minutes).

2. Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by dipping in or spraying with 70% ethanol. All of the operations from this point on should be carried out under strict aseptic conditions.

3. Transfer the vial contents to a centrifuge tube containing 9.0 mL complete culture medium, and spin at approximately 125 xg for 5 to7 minutes.

4. Resuspend cell pellet with the recommended complete medium (see the specific batch information for the culture recommended dilution ratio). It is important to avoid excessive alkalinity of the medium during recovery of the cells. It is suggested that, prior to the addition of the vial contents, the culture vessel containing the complete growth medium be placed into the incubator for at least 15 minutes to allow the

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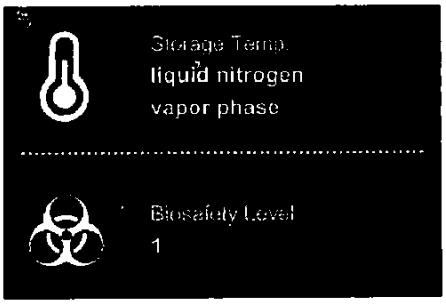

Intended Use

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Citation of Strain

If use of this culture results in a scientific publication, it should be cited in that manuscript in the following manner: A549 (ATCC® CCL-185™)

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medium to reach its normal pH (7.0 to 7.6). pH (7.0 to 7.6).

5. Incubate the culture at 37°C in a suitable incubator. A 5% CO_2 in air atmosphere is recommended if using the medium described on this product sheet.

Handling Procedure for Flask Cultures

Handling Procedure for Flask Cultures

The flask was seeded with cells (see specific batch information) grown and completely filled with medium at ATCC to prevent loss of cells during shipping.

1. Upon receipt visually examine the culture for macroscopic evidence of any microbial contamination. Using an inverted microscope (preferably equipped with phase-contrast optics), carefully check for any evidence of microbial contamination. Also check to determine if the majority of cells are still attached to the bottom of the flask: during shipping the cultures are sometimes handled roughly and many of the cells often detach and become suspended in the culture medium (but are still viable).
2. **If the cells are still attached,** aseptically remove all but 5 to 10 ml of the shipping medium. The shipping medium can be saved for reuse. Incubate the cells at 37°C in a 5% CO_2 in air atmosphere until they are ready to be subcultured.
3. **If the cells are not attached,** aseptically remove the entire contents of the flask and centrifuge at 125 xg for 5 to 10 minutes. Remove shipping medium and save. Resuspend the pelleted cells in 10 mL of this medium and add to 25 cm^2 flask. Incubate at 37°C in a 5% CO_2 in air atmosphere until cells are ready to be subcultured.

Subculturing Procedure

Protocol:

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin- 0.53 mM EDTA solution to remove all traces of serum that contains trypsin inhibitor.
3. Add 2.0 to 3.0 mL of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes).
   Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.
4. Add 6.0 to 8.0 mL of complete growth medium and aspirate cells by gently pipetting.
5. Add appropriate aliquots of the cell suspension to new culture vessels.
   Cultures can be established between 2 x 10^3 and 1 x 10^4 viable cells/cm^2. Do not exceed 7 x 10^4 cels/cm^2.
6. Incubate cultures at 37°C.

Interval: Maintain cultures at a cell concentration between 6 X 10^3 and 6 X 10^4 cell/cm^2.
Subcultivation Ratio: A subcultivation ratio of 1:3 to 1:8 is recommended
Medium Renewal: 2 to 3 times per week

Cryopreservation Medium

Cryoprotectant Medium

Complete growth medium described above supplemented with 5% (v/v) DMSO.
Cell culture tested DMSO is available as ATCC Catalog No. 4-X.

Comments

Studies by M. Lieber, et al. revealed that A549 cells could synthesize lecithin with a high percentage of desaturated fatty acids utilizing the cytidine diphosphocholine pathway.

References

References and other information relating to this product are available online at www.atcc.org.

Biosafety Level: 1

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the current publication of the *Biosafety in Microbiological and Biomedical Laboratories* from the U.S. Department of Health and Human Services Centers for Disease Control and Prevention and National Institutes for Health.

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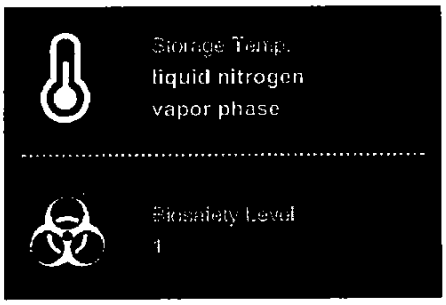

Intended Use

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Complete Growth Medium

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Review

Ehrlich ascites carcinoma

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Experimental tumors have great importance in modeling, and Ehrlich ascites carcinoma (EAC) is one of the commonest tumors. EAC is referred to as an undifferentiated carcinoma and is originally hyperdiploid, has high transplantable capability, no-regression, rapid proliferation, shorter life span, 100% malignancy and also does not have tumor-specific transplantation antigen (TSTA). Frequently, tumor virulence increases via repetitious passages, while the proliferating rate of such tumors increases gradually. However, the differentiation gradually disappears, while the cells get free growth control mechanisms, gain hetero-transplantability and in the end, they are converted to the ascites' form. EAC resembles human tumors which are the most sensitive to chemotherapy due to the fact that they are undifferentiated and that they have a rapid growth rate. The ideal drug being ineffective or minimally effective for normal cells have been focused on, and at this point, the usage of natural sources as an alternative cancer therapy is thought to have a great value for cancer control and programs' destruction.

Key words: carcinoma, transplantability

EHRLICH ASCITES CARCINOMA

The intensive studies on the transplantable tumors were taken into consideration in the last 2 to 3 decades. The planned goal of that research was to improve new techniques especially for experimental tumors in animals that have been underlain at the basis of recent achievements in cancer therapy. Experimental tumors have great importance for the purposes of modeling, and Ehrlich ascites carcinoma (EAC) is one of the commonest. It appeared firstly as a spontaneous breast cancer in a female mouse (Aktaş, 1996; Taşkin, 2002), and then Ehrlich and Apolant (1905) used it as an experimental tumor by transplanting tumor tissues subcutaneously from mouse to mouse. In 1932, Loewenthal and Jahn (1932) obtained the liquid form in the peritoneum of the mouse and named it as "Ehrlich ascites carcinoma" due to the ascites liquid, together with the carcinoma cells. Lettre et al. (1972) had provided not only the seizure of this tumor, but also the conversion of it to the test system which is suitable for qualitative and quantitative cancer researches by their studies during World War II. After 1948, EAC cells had spread rapidly around the research institutes all over the world.

EAC is referred to as an undifferentiated carcinoma, and is originally hyperdiploid, has high transplantable capability, no-regression, rapid proliferation, shorter life span, 100% malignancy and also does not have tumor-specific transplantation antigen (TSTA) (Kaleoğlu and İşli, 1977). In 1953, Haucscka (Lettre et al. 1972) obtained a sub-clone whose chromosome was tetraploid, while in the following years, such studies about diploid, hypertetraploid (Lennartz et al., 1968) and hypotetraploid (Burns, 1968) sub-clones were performed. However, Lettre et al. (1972) succeeded in obtaining colchicine resistant tumor clone and Sholz, with glycogen (+) and glycogen Ø Ehrlich clones as well (Aktaş, 1996).

The effusion, which contained neoplastic cells that are proliferated after injection of tumor cells into the peritoneal cavity, is referred to as the "ascites". Frequently, tumor virulence increases via repetitious passages, while the proliferating rate of such tumors increases gradually. However, differentiation gradually disappears, while the cells get free growth control mechanisms, gain hetero-transplantability and in the end, are converted to the ascites form (Kaleoğlu and İşli, 1977). Ascites liquid is gray-white, or sometimes has a light bloody viscose liquid

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and contains 10 million neoplastic cells in 0.1 cc (Aktaş, 1996; Kaleoğlu and İşli, 1977).

Following the obtained Ehrlich ascites form, this has been preferred frequently in researches. The reason for its load usage is that the suspension contained homogeneous free tumor cells of the Ehrlich ascites tumor, and in this way, it has a transplantable capacity for certain quantitative tumor cells to another mouse (Klein, 1951). Therefore, it is not only the tumor cell count that is transplanted, but also, the growing tumor size can be determined by common basic counter systems (Ekinci, 2000).

EAC is used as ascites or a solid form due to these purposes, that is, if ascites fluid contains the tumor cell that injects i.p., the ascites form is obtained, but if it contains s.c., a solid form is obtained (Okay, 1998; Zeybek, 1996).

EAC cells grow in suspension in the peritoneal cavity of mice and they do not adhere to the synthetic surface *in vitro* (Aktaş, 1996; Lazebnik et al., 1991; Song et al., 1993; Vinuela et al., 1991). In 4 or 6 days after passage, the ascites fluid is formed and a total of 5 or 12 cc ascites fluid is accumulated (Gümüşhan, 2002).

Following the inoculation into the peritoneal cavity of mice, EAC cells grow in two phases. These two phases are: a proliferating phase, in which the number of cells increases exponentially, and a plateau phase followed by a resting phase, in which a number of cells stay almost constant (Song et al., 1993; Siems et al., 1993; Grune et al., 1992; Skog et al., 1990; Tannock, 1969). Several studies reported that following the 3 x 10^6 EAC cells transplantation i.p., the number of cells increased exponentially in the 9th day and they were transmitted from the exponential phase to the plateau phase starting from the 9th and 10th day (Bulan, 1990; Altun, 1996; Öner, 1985). In another study, the proliferating rate of EAC cells was characterized in 4 phases. These phases are: a logarithmic phase for 4 or 5 days, following the 10^7 tumor cells transplantation i.p.; a plateau phase, in which the number of cells stayed practically constant on the 5th to 13th day; a transitory proliferating phase on the 13th to 15th day and a second plateau phase on the 15th to 18th day (Szikla et al., 1981).

During the EAC cells transition from the proliferating phase into the plateau phase, morphological and metabolic changes (except the changes in cell kinetics) occur (Aktaş, 1996), such as: structural deterioration (Aktaş, 1996; Siems et al., 1993; Schmidt et al., 1991; Schwendel et al., 1994; Senger et al., 1983; Siems, 1989; Segura et al., 2001; Latha et al., 2000; Haris et al., 1970), decreased number of mitochondria (Siems et al., 1993; Schmidt et al., 1991; Schwendel et al., 1994; Siems, 1989), decreased DNA and RNA biosynthesis (Aktaş, 1996; Siems et al., 1993; Bulan, 1990; Schmidt et al., 1991; Siems, 1989), loss of intracellular purine and pirimidine nucleotides, nucleosides and bases (Siems et al., 1993; Grune et al., 1992; Schmidt et al., 1991; Schwendel et al., 1994; Siems, 1989), a decline of the ATP concentration and turnover (Siems et al., 1993; Skog et al., 1990; Öner, 1985), decreased protein synthe-sis (Burns, 1968, Siems et al., 1993; Skog et al., 1990; Schmidt et al., 1991; Estrela et al., 1992), increased thymidine concentration with a decrease of thymidine kinase activity (Aktaş, 1996; Skog et al., 1990; Szıkla et al.,1981), decreased glutathione (GSH) concentration (Marquez et al., 1989; Lobo et al., 2000; Balint and Holczinger, 1984) and increased triglycerides, cholesterol esters and free fatty acids (Aktaş, 1996; Burns et al., 1983).

The inhibition of NK and T cell responses was dramatically reported to be parallel with an inclination of the repressed macrophages and down-regulator humoral factors (Haris et al., 1970).

EAC cells increased via rapid cell division during the proliferating phase and in the load peritoneal cavity. Ascites fluid accumulation occurred in parallelism with the proliferation of tumor cells. After a given time, the host animal died due to the pressure exerted by the tumor volume and/or the damage that resulted from the tumor (Aktaş, 1996; Altun, 1996; Öner, 1985). During the transition of the EAC cells from the proliferating phase to the plateau phase, the rates of cell viability did not decrease significantly (Schmidt et al., 1991).

For the accumulation of ascites fluid, whether or not the tumor cells secrete a vascular permeability factor that stimulated the accumulation of ascites fluid was investigated, and in conclusion, vessels in the peritoneal cavity of mice with EAC showed that the microvascular permeability increased significantly in comparison with those of the control group. This increased permeability was detected by an effective permeability factor in ascites fluid, but not in the normal plasma and serum (Senger et al., 1983).

Altun (1996) reported that the rate of cell proliferation in the bone marrow was inhibited, depending on the age of the tumor in mice. This showed that inhibitor factors in ascites fluid affected the normal cell population of the host animal.

Contrary to these studies, Altun (1996) in another study investigated the liver regeneration in mice with EAC and reported that tumor growth stimulated the regenerative growth. Gabrilovac et al. (1982) reported that peritoneal fluids, collected in the early phase of tumor growth on the 4th and 6th day after tumor transplantation, were ineffective on the proliferation of EAC cells *in vitro,* but those collected on the 15th day increased DNA synthesis (Gabrilovac et al., 1982). Burns et al. (1968) examined the mitogenic activity of Ehrlich ascites carcinoma factor (EACF) isolated from the cellular ascitic fluid in liver and in other tissues of adult mice, and reported that DNA synthesis was stimulated by this factor's mitogenic activity in liver, submandibular gland, exorbital lachrymal gland and the epithelium of the tongue of adult mice (Yeh et al., 1985).

Donenko et al. (1992) examined the effect of Ehrlich tumor cell's dialysate and ascites fluid on the *in vivo* progression of EAC and teratoma T-36 and concluded that, the ascites fluid, together with the tumor cell's dialysate, protected the tumor cells *in vivo*. In comparison with the control group, EAC dialysate and ascites fluid increased the rates of the tumor cell progression by 195 and 153%, respectively.

ALTERNATIVE APPROACHES IN CANCER THERAPY

In modern medicine, 3 methods are generally used for cancer therapy; chemotherapy, radiotherapy and surgery. Nowadays, chemotherapy has been thought to be the best effective therapy (Kayaalp, 1996).

The main principle of chemotherapy, which serves as a drug treatment in cancer, is to prevent the growth and progression of tumor cells or to destroy them by the effect it has on tumor cells more than the normal cells of the patient without side effect or with minimum side effect (Mycek et al., 1998). In consequence, the aim is to provide a lethal toxic effect of the used drugs to tumor progression. Generally, the prevention of metabolic pathways in cell replication is aimed. Furthermore, this effect is aimed to be specific for only malign cells. However, all the used drugs for cancer therapy are not specific on cancer cells, in that they do not only affect the proliferated cells, but also the normal cells. Therefore, all cancer therapeutics are toxic and their dosage-response curves are upright.

If tumor metastasis occurs and surgical treatment is impossible, chemotherapy is preferably used for the therapy. At the same time, it is applied after surgical and radiation therapies to prevent micro-metastasis.

Although the cancer chemotherapy has a half century clinical story, thousands of chemicals were investigated in this study. However, only a few of these chemicals classified due to different characteristics is used as a drug to treat cancer nowadays (Öner, 1985).

The most sensitive tumors to chemotherapy are poorly differentiated and they grow rapidly (Mycek et al., 1998). Nonetheless, lots of cancer chemotherapeutics affect the normal cells of patients seriously (Mascarenhas, 1994). For instance, cytostatics in cancer therapy focus on the intracellular targets and its effect mechanism, which is a natural cell damage. However, the resistance of some tumor types against this drug group and also hepatoxic, nephrotoxic, cardiotoxic, etc side effects on normal cells make new agents for cancer therapy necessary (Soini et al., 1998).

Scientists' studies about cancer therapy have focused on the ideal drug being ineffective or minimally effective for normal cells (Gümüşhan, 2002). At this point, the usage of natural sources is thought to have a great value for cancer control and programs' destruction (Suffiness and Pezzuto, 1991).

The usage of plant preparations in medicine has a great historical inheritance among people (Duke, 1985). Nature gives a great deal of effective anti-cancer agents such as dactinomycin and doxorubicin derived from microorganisms and vinblastine, irinotecan, topotecan, vincristine and taxanes from plants which are used frequently in recent years. Several plants were reported to stimulate the immune system in different pathways. In addition, they increased specific cellular and humoral immune responses (Bhakuni et al., 1969). Moreover, there is a growing trend for herbal drugs because of low toxicity and high medical effectiveness of the extracts from these plants.

CONCLUSION

EAC has a resemblance with human tumors which are the most sensitive to chemotherapy due to the fact that it is undifferentiated and that it has a rapid growth rate. Due to the resemblance, some researchers reported that some plant extracts were effective against EAC (Ozaslan et al., 2007, 2009a, 2009b; Cragg and Newmann, 1999).

Although there are a lot of floristic studies, approximately 10% of the 250,000 complex plant species only were investigated at their chemical and pharmacological sites. Nonetheless, the search of new toxic agents from natural sources has been conducted in collaboration with scientists, worldwide (Cragg and Newmann, 1999).

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First Named Inventor/Applicant Name:	Heidi Lane
Filer:	Ann R. Pokalsky/Maggi Leone
Attorney Docket Number:	031671-US-CNT03 167-62 C3

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First Named Inventor/Applicant Name:	Heidi Lane
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If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

<u>National Stage of an International Application under 35 U.S.C. 371</u>
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

<u>New International Application Filed with the USPTO as a Receiving Office</u>
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

PATENT
Attorney Docket: PAT031671-US-CNT03 (167-62 CON III)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s): Lane et al. Examiner: Klinkel, Kortney L.

Serial No.: 13/546,686 Confirmation No.: 8586

Filed: July 11, 2012 Dated: March 11, 2013

For: TREATMENT OF SOLID TUMORS WITH RAPAMYCIN DERIVATIVES

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

AMENDMENT TRANSMITTAL FORM

Sir:

Transmitted herewith is an amendment in the above-identified application.

[ ] Small entity status of this application under 37 C.F.R. §1.9 and §1.27 has been established by a verified statement previously submitted.

[ ] A verified statement to establish small entity under 37 C.F.R. §1.9 and §1.27 is enclosed.

[X] No additional fee is required.

For	Claims Remaining After Amendment	Highest No. Previously Paid For	Present Extra	Rate (Small Entity)	Addit. Fee	Rate (Large Entity)	Addit. Fee
TOTAL CLAIMS*	6	20	0	x **31** =	$ 0.00	x **62** =	$0.00
INDEPENDENT CLAIMS	1	3	0	x **125** =	$0.00	x **250** =	$0.00
[ ] First Presentation of Multiple Dep. Claim				**230**		**460**	$0.00

Total: 0.00

Certificate of EFS-Web Transmission

I hereby certify that this correspondence is being transmitted to the U.S. Patent and Trademark Office via the Office's electronic filing system.

Date: March 11, 2013 Name: Ann R. Pokalsky

Ann R. Pokalsky

[ ] Fees are to be charged to a credit card. A separate form PTO-2038 is attached with credit card information.

[ ] Please charge Deposit Account No. **04-1121** in the amount of $0.00.

[X] The Commissioner if hereby authorized to charge any additional fees, which may be required, or credit any overpayment to Deposit Account No. **04-1121**.

Respectfully submitted,

Ann R. Pokalsky
Reg. No. 34,697
Attorney for Applicant(s)

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PATENT APPLICATION FEE DETERMINATION RECORD

Substitute for Form PTO-875

Application or Docket Number	Filing Date	☐ To be Mailed
13/546,686	07/11/2012	

APPLICATION AS FILED – PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA	SMALL ENTITY ☐ RATE ($)	SMALL ENTITY ☐ FEE ($)	OR	OTHER THAN SMALL ENTITY RATE ($)	OTHER THAN SMALL ENTITY FEE ($)
☐ BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A			N/A	
☐ SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A			N/A	
☐ EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A			N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	minus 20 =	*	X $ =		OR	X $ =	
INDEPENDENT CLAIMS (37 CFR 1.16(h))	minus 3 =	*	X $ =			X $ =	
☐ APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $250 ($125 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).						
☐ MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))							
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL			TOTAL	

APPLICATION AS AMENDED – PART II

AMENDMENT 03/11/2013	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	SMALL ENTITY RATE ($)	SMALL ENTITY ADDITIONAL FEE ($)	OR	OTHER THAN SMALL ENTITY RATE ($)	OTHER THAN SMALL ENTITY ADDITIONAL FEE ($)
Total (37 CFR 1.16(i))	* 6	Minus	** 20	= 0	X $ =		OR	X $62=	0
Independent (37 CFR 1.16(h))	* 1	Minus	***3	= 0	X $ =		OR	X $250=	0
☐ Application Size Fee (37 CFR 1.16(s))									
☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
					TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	0

AMENDMENT	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	RATE ($)	ADDITIONAL FEE ($)		RATE ($)	ADDITIONAL FEE ($)
Total (37 CFR 1.16(i))	*	Minus	**	=	X $ =		OR	X $ =	
Independent (37 CFR 1.16(h))	*	Minus	***	=	X $ =		OR	X $ =	
☐ Application Size Fee (37 CFR 1.16(s))									
☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
					TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.

** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".

*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".

The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.

Legal Instrument Examiner:
/CATHERINE SMITH/

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PRIORITY DOCUMENT EXCHANGE

FAILURE STATUS REPORT

An attempt by the Office to electronically retrieve, under the Priority Document Exchange programs (PDX and DAS), 0104072.4 to which priority is claimed has FAILED on 06/10/2013.

For further questions or assistance, please contact our EBC Customer Support Center at

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
13/546,686	07/11/2012	Heidi Lane	031671-US-CNT03 167-62 C3	8586

28249 7590 06/14/2013

DILWORTH & BARRESE, LLP
1000 WOODBURY ROAD
SUITE 405
WOODBURY, NY 11797

EXAMINER	
KLINKEL, KORTNEY L	
ART UNIT	**PAPER NUMBER**
1611	

MAIL DATE	DELIVERY MODE
06/14/2013	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	13/546,686	LANE ET AL.	
	Examiner	Art Unit	
	Kortney L. Klinkel	1611	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *11 March 2013*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

4)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

5)☒ Claim(s) *1 and 3-7* is/are pending in the application.

5a) Of the above claim(s) _____ is/are withdrawn from consideration.

6)☐ Claim(s) _____ is/are allowed.

7)☒ Claim(s) *1 and 3-7* is/are rejected.

8)☐ Claim(s) _____ is/are objected to.

9)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

10)☐ The specification is objected to by the Examiner.

11)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

12)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

13)☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☒ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☒ Certified copies of the priority documents have been received in Application No. 10/468520.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Acknowledgement is made of the remarks/amendments dated 3/11/2013. Claim 1 was amended to incorporate the limitations of claim 2 and claim 2 was cancelled.

Rejections and/or objections not reiterated from previous office actions are hereby withdrawn. The following rejections and/or objections are either reiterated or newly applied and constitute the complete set presently being applied to the instant application.

Priority

Acknowledgement is made that the instant application is a CON of 10/468520 filed 1/27/2004 which is a 371 of PCT/EP02/01714 filed 2/18/2002. Acknowledgement is also made of applicant's foreign priority claim to UK patent applications 0104072.4 filed 2/19/2001 and 0124957.2 filed 10/17/2001. Receipt is acknowledged of papers submitted under 35 U.S.C. 119(a)-(d), which papers have been placed of record in the file.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

The factual inquiries set forth in *Graham* v. *John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966), that are applied for establishing a background for determining obviousness under 35 U.S.C. 103(a) are summarized as follows:

1. Determining the scope and contents of the prior art.
2. Ascertaining the differences between the prior art and the claims at issue.
3. Resolving the level of ordinary skill in the pertinent art.
4. Considering objective evidence present in the application indicating obviousness or nonobviousness.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

Claims 1 and 3-7 are rejected under 35 U.S.C. 103(a) as being unpatentable over Geoerger et al. ("Antitumor Activity of the Rapamycin Analog CCI-779 in Human Primitive Neuroectodermal Tumor/Medulloblastoma Models as Single Agent and in Combination Chemotherapy", Cancer Research, 61, 2/15/2001, 1527-1532, as per Applicant's IDS) in view of Cottens et al. (WO 94/09010, as per Applicant's IDS). This rejection is maintained.

Geoerger et al. teach that administration of rapamycin has antitumor activity (p. 1527, 1st column). Co-administration of rapamycin with cisplatin, or 5-fluouracil and

cyclophosphamide exhibited enhanced apoptosis in human cell lines and cytotoxicity in colon tumor models respectively (p. 1527, 1st column). Rapamycin and its 40-O substituted analog CCI-779 are effective brain tumor therapeutics both alone and in combination with chemotherapeutics such as cisplatin and camptothecin (p. 1527, abstract and 2nd column). Geoerger et al. teach that brain tumor cell lines are exquisitely sensitive to rapamycin (p. 1527, 2nd column, first full paragraph). Geoerger et al. teach that rapamycin in combination with cisplatin or camptothecin has an additive effect in cell lines resistant to rapamycin (p. 1528, 1st paragraph of Results section). The antitumor activity of rapamycin has been demonstrated in tumors. The antitumor activity of rapamycin has been demonstrated in human rhabdomyosarcoma and neuroblastoma tumor cell lines *in vitro* and in B16 melanocarcinoma, Colon 38 tumors, CD8F1 mammary tumors, EM ependymoblastoma, and U251 glioblastoma brain tumors *in vivo* (p. 1530, Discussion). Geoerger et al. also teach that tumor toxicity can be increased by using combination chemotherapy with a rapamycin without the risk of increased systemic cyctotoxicity (p. 1530, Discussion). Geoerger et al. teach that cisplatin, camptothecin, CPT 11 and topotecan are effective agents in the chemotherapeutic treatment of brain tumors but that dosages of these agents are limited due to their toxicity. Because rapamycin and the 40-O-substituted derivative CCI-779 show at least an additive effect when combined with chemotherapeutics and they have low toxicity, they are good adjuvants for these toxic chemotherapeutics (p. 1532, first column). Additionally CCI-779 exhibits an enhanced antitumor effect when combined with cisplatin in vivo (p. 1532, first column).

Geoerger et al. also teach that either 20 mg/kg/d in a single dose or 100 mg/kg/d in a divided dose of the rapamycin derivative CCI-779 is administered via intraperitoneal injection (p. 1528 1st col., p. 1532 1st col.). Dosages of 100, 200, 400 or 800 mg/kg/d of rapamycin are also taught to be effective (p. 1531, 1st col.).

The teachings of Geoerger et al. differ from the instant claims in that rapamycin or the 40-O substituted rapamycin derivative CCI-779 are administered either alone or in combination with other chemotherapeutics for the treatment of brain tumors *inter alia*, rather than the claimed rapamycin derivative 40-O-(2-hydroxyethyl) rapamycin (AKA everolimus). Geoerger et al. also fail to teach explicit dosages in terms of mg administered, but rather teaches dosages in terms of mg/kg. The dosages described by Geoerger et al. are all administered intraperiotoneally rather than orally as required by instant claim 7.

Cottens et al. teach compounds of formula I, including the instant claimed compound i.e. 40-O-(2-hydroxyethyl) rapamycin (pages 2-4, see particularly p. 3 compound 8, last line; 21-22; Example 8 p. 21-22; claim 2, compound 8) and that these derivatives of rapamycin have an improved pharmacologic profile over rapamycin, exhibit greater stability and bioavailability and allow for greater ease in producing gelenic formulations (p. 2, first full paragraph). Cottens et al. teach that the use of rapamycin as an antitumor agent is restricted by its low and variable bioavailability (p. 2, lines 1-4).

Cottens et al. teach that compounds of formula I have demonstrated antitumor activity and the ability to enhance performance of antitumor agents by alleviating

multidrug resistance e.g. by administration with anticancer agent e.g. colchicine or etoposide, to multidrug resistant cells and drug sensitive cells in vitro or to animals having multidrug resistant or drug sensitive tumors (page 12, first full para.). Cottens et al. teach that the compounds may be administered as the sole active ingredient or together with other drugs e.g. corticosteroids, azathioprine, immunosuppressive monoclonal antibodies (page 8, second full para.).

Cottens et al. teach a method of treating tumors or hyperproliferative disorders comprising administering a compound of formula I (page 6, items “d and e;” page 40, claim 8). Cottens et al. teach that generally the dose of the instant claimed compounds is from 0.05 to 10 mg/kg/d orally in individual dosages of 0.1 to 7.5 mg/kg/day for up to 4 divided doses per day. Typical dosages for intravenous injection range from 0.01 to 5 mg/kg/day (page 7, first para to page 8, first para.). In total, for an average human, dosages range from 5 to 100 mg p.o. up to 500 mg/d p.o. or on the order of 0.5 to 250 mg i.v. with individual dosages from 2.5 to 50 mg i.v. (p. 8 first para.). These absolute dosage amounts overlap with the dosage amounts required by claims 3-6.

It would have been prima facie obvious to one of ordinary skill in the art at the time of the instant invention to substitute rapamycin or CCI-779 of Geoerger et al. for the claimed rapamycin derivative 40-O-(2-hydroxyethyl)rapamycin of Cottens et al. with the reasonable expectation that solid tumors, including brain tumors or brain carcinoma would be treated when administered alone or in combination with other chemotherapeutics such as cisplatin, 5-fluoruracil, and topotecan. One would have been motivated to do so because it is well known in the art that 40-O-(2-

hydroxyethyl)rapamycin is useful for treating tumors and hyperproliferative disorders and that it exhibits an improved pharmacologic profile over rapamycin, exhibits greater stability and bioavailability and allows for greater ease in formulating. One of ordinary skill in the art would be imbued with the reasonable expectation that the combination of 40-O-(2-hydroxyethyl)rapamycin with the chemotherapeutics 5-fluorouracil and topotecan would exhibit at least an additive effect as this is what is observed for the combination of rapamycin or CCI-779 with these agents. One would be imbued with the reasonable expectation that the combination of 40-O-(2-hydroxyethyl)rapamycin with cisplatin would exhibit an enhanced antitumor effect, as this is what is observed for the 40-O-substituted rapamycin derivative CCI-779. Additionally, "[i]t is *prima facie* obvious to combine two compositions each of which is taught by the prior art to be useful for the same purpose, in order to form a third composition to be used for the very same purpose.... [T]he idea of combining them flows logically from their having been individually taught in the prior art." *In re Kerkhoven*, 626 F.2d 846, 850, 205 USPQ 1069, 1072 (CCPA 1980) (citations omitted).

Regarding the dosage amounts of about 0.1-25 mg as a single or divided dosage of claim 3, a unit dosage of about 0.05 to 12.5 mg of claim 4, a unit dosage from about 0.25 to 10 mg of claim 5 and a unit dosage form of 10 mg of claim 6, the Examiner notes that depending on the size of the subject, both the teachings of Geoerger et al. and Cottens et al. teach amounts which fall within or overlap with the claimed amounts. Generally, differences in concentration or temperature will not support the patentability of subject matter encompassed by the prior art unless there is evidence indicating such

concentration or temperature is critical. "[W]here the general conditions of a claim are disclosed in the prior art, it is not inventive to discover the optimum or workable ranges by routine experimentation." *In re Aller*, 220 F.2d 454, 456, 105 USPQ 233, 235 (CCPA 1955). Here, one of ordinary skill in the art would be motivated to adjust the relative amount of drug administered to suite the subject's mass and condition and to balance beneficial effects with negative side effects. It is well within the purview of one of ordinary skill in the art to determine the optimal dosage amount.

Response to Arguments

Applicant's arguments regarding the rejection of claims over Geoerger et al. in view of Cottens et al. have been fully considered, but are not persuasive. Applicant argues that carcinomas are a type of cancer arising from epithelial (outer layer, coverings) cells of lung, breast, skin, etc. and points to Wikipedia's page on carcinoma (response p. 6). Applicant argues that brain metastasis is a complex process by which cells of the primary carcinoma (most commonly, lung, breast and melanoma) travel through the blood stream and establish residence in the brain (Steeg, see Exhibit A, p. 5, left col.). Applicant notes that non-small cell lung carcinoma is the most common primary carcinoma causing carcinoma in the brain (see response p. 6 and Exhibits B and C). Applicant points to pp. 12-13 of the instant specification which is directed to an Example where fragments of A549 tumors were transplanted subcutaneously into the left flank of BALB/c nude mice. The A549 cell line was derived from a 58-year old patient suffering from lung adenocarcinoma (see pp. 6-7 of response and Exhibit D). Applicant argues that Geoeger et al. does not teach or suggest anything about

administration of rapamycin or a rapamycin derivative for the treatment of brain carcinomas. Applicant argues that as evidenced by the attached exhibits, brain carcinomas are distinct from primary brain tumors and that the human primitive neuroectodermal tumor and medulloblastoma which Geoerger et al. studied are considered primary brain tumors and not brain carcinomas. Applicant also argues that Cottens et al. does not teach or suggest anything about administration of everolimus for the treatment of brain carcinomas and that the Ehrlich ascites carcinoma used as a model on p. 12 of Cottens is derived from a mouse and there is no evidence that it metastasized to the brain even in mice (remarks p. 7). Applicant argues that it was not well known prior to the instant invention that everolimus is useful for treating tumors and hyperproliferative disorders. Applicant argues Cottens et al. teach 28 different preferred compounds one of which is everolimus and that Cottens also teach that everolimus is especially preferable for immunosuppressive use (see p. 7-8 of remarks). Applicant concludes that since neither Geoerger nor Cottens even mention treatment of brain carcinomas, there would have been no motivation to combine the two references, and even if there were reason to combine, one of ordinary skill in the art would not have had a reasonable expectation of success that everolimus would be useful in treating brain carcinoma as presently recited in the claims. Applicant argues that the rejection lacks motivation and a reasonable expectation of success which are both required for a proper rejection. Thus applicant requests withdrawal of the rejection. These arguments have been fully considered, but are not persuasive.

First, the examiner maintains that there is motivation to combine the prior art Geoerger and Cottens references. One would have been motivated to do so because it is well known in the art that 40-O-(2-hydroxyethyl)rapamycin (everolimus) is useful for treating tumors and hyperproliferative disorders and that it exhibits an improved pharmacologic profile over rapamycin, exhibits greater stability and bioavailability and allows for greater ease in formulating. The Federal Circuit has repeatedly held that:

> an implicit motivation to combine exists not only when a suggestion may be gleaned from the prior art as a whole, but when the 'improvement' is technology-independent and the combination of references results in a product or process that is more desirable, for example because it is stronger, cheaper, cleaner, faster, lighter, smaller, more durable, or more efficient. Because the desire to enhance commercial opportunities by improving a product or process is universal—and even common-sensical—we have held that there exists in these situations a motivation to combine prior art references even absent any hint of suggestion in the references themselves. See *Dystar Textilfarben GmbH & Co. Deutschland KG v. C.H. Patrick*, 464 F.3d 1356, 1368, 80 USPQ2d 1641, 1651 (Fed. Cir. 2006).

Thus, given the fact that everolimus is known to have an improved pharmacologic profile over rapamycin and exhibits greater stability and bioavailabliliyt and allows for greater ease in formulating, there is implicit motivation to combine the prior art teachings.

Regarding the issue of a reasonable expectation for success and again motivation, the examiner maintains that there would have been a reasonable expectation that solid tumors, including brain tumors and brain carcinoma would be treated when everolimus is administered alone or in combination with other chemotherapeutics such as cisplatin, 5-fluoruracil, and topotecan. Geoerger teach that administration of rapamycin has antitumor activity (p. 1527, 1st column). Geoerger

demonstrate that rapamycin and the 40-O substituted derivative CCI-779 alone and in combination with other known chemotherapeutics are effective against a range of different types of cancer including, human rhabdomyosarcoma and neuroblastoma tumor cell lines *in vitro* and in B16 melanocarcinoma, Colon 38 tumors, CD8F1 mammary tumors, EM ependymoblastoma, and U251 glioblastoma brain tumors *in vivo* (p. 1530, Discussion), several of which are carcinomas. Additionally, Geoerger teach that brain tumor cell lines are exquisitely sensitive to rapamycin (p. 1527, 2nd column, first full paragraph). Coupled with these teachings, Cottens teach a method of treating tumors or hyperproliferative disorders comprising administering a compound of formula I which includes everolimus (page 6, items "d and e;" page 40, claim 8). Note also that Cottens demonstrates that the rapamycin derivatives including everolimus are effective against Ehrlich ascites carcinoma (p. 12). The examiner concedes that Cottens does not specify whether or not the EA carcinoma metastasized to the brain or not. Likewise, the examiner notes that despite the fact that non-small cell lung carcinoma is the primary carcinoma causing carcinoma in the brain, applicant has not demonstrated that the example in the specification on pp. 12-13 where fragments of A549 (lung tumor cells) were implanted in mice necessarily metastasized in the brain. The examiner notes that Exhibit C suggests that 20-50% of patients with brain metastasis also presented with primary lung carcinoma. Lung carcinoma does not inherently result in brain carcinoma. The examiner also notes that the fact that Cottens also teaches that the rapamycin derivatives are immunosuppressive, does in no way detract from the fact

that Cottens also teaches that these compounds have anticancer properties. An active compound can exhibit more than one therapeutic property.

The fact that Geoerger teach that a wide variety of different cancers are responsive to rapamycin and the 40-O substituted derivative CCI-779 coupled with the fact that Cottens teach that everolimus has anticancer properties, exhibits an improved pharmacologic profile over rapamycin, exhibits greater stability and bioavailability and allows for greater ease in formulating provide clear motivation and a reasonable expectation of success for treating brain carcinoma. Obviousness does not require absolute predictability, however, at least some degree of predictability is required. Evidence showing there was no reasonable expectation of success may support a conclusion of nonobviousness. *In re Rinehart*, 531 F.2d 1048, 189 USPQ 143 (CCPA 1976). Applicant has not demonstrated that there would have been no reasonable expectation of success. Nor has applicant demonstrated that everolimus exhibits unexpected properties over those suggested by the prior art. As such, the claims remain properly rejected under 35 USC 103(a).

Conclusion

Claims 1 and 3-7 are rejected. No claim is allowed.

No new ground(s) of rejection were presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL.** Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Kortney Klinkel, whose telephone number is (571)270-5239. The examiner can normally be reached on Monday-Friday 10 am to 7 pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Daniel Sullivan can be reached at (571)272-0779. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a

USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Kortney L. Klinkel/
Primary Examiner, Art Unit 1611

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	13546686	LANE ET AL.
	Examiner	Art Unit
	KORTNEY L KLINKEL	1611

CPC- SEARCHED		
Symbol	Date	Examiner

CPC COMBINATION SETS - SEARCHED		
Symbol	Date	Examiner

US CLASSIFICATION SEARCHED			
Class	Subclass	Date	Examiner

SEARCH NOTES		
Search Notes	Date	Examiner
Searched inventor names in PALM	9/28/2012	KLK
Searched EAST, see history attached	9/28/2012	KLK
Searched Pubmed, see history attached	9/28/2012	KLK
searched EAST	6/10/2013	KLK
searched keywords (carcinoma, brain carcinoma, etc) in google	6/10/2013	KLK

INTERFERENCE SEARCH			
US Class/ CPC Symbol	US Subclass / CPC Group	Date	Examiner

/K.L.K./ Examiner.Art Unit 1611	

EAST Search History

EAST Search History (Prior Art)

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
S1	32	("20020022054" | "20020098278" | "20030100886" | "20030100887" | "4885171" | "5066493" | "5194447" | "5206018" | "5362718" | "5922730" | "5985890" | "6333348" | "6569463" | "6617333" | "6641822").PN.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/09/28 13:06
S2	33	heidi near2 lane	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/09/28 13:06
S3	35	terence near2 o'reilly	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/09/28 13:07
S4	68	jeanette near2 wood	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/09/28 13:07
S5	114	S2 or S3 or S4	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/09/28 13:07
S6	4	S2 and S3 and S4	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/09/28 13:07
S7	604202	cancer or carcinoma or tumor	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/09/28 13:09

S8	83	S5 and S7	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/09/28 13:10
S9	25275	rapamycin or everolimus	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/09/28 13:10
S10	19	S8 and S9	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/09/28 13:10
S11	5952358	brain or cns or central near2 nervous near2 system	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/09/28 13:23
S12	109650	S7 same S11	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/09/28 13:24
S13	460	S7 same S11 same S9	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/09/28 13:24
S14	31535727	@py<="2002"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/09/28 13:24
S15	20	S13 and S14	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/09/28 13:24
S16	583	brain near2 carcinoma near10 glioblastoma	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO;	OR	ON	2013/06/07 17:52

			DERWENT; IBM_TDB			
S17	532	brain near2 carcinoma near5 glioblastoma	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2013/06/07 17:52

6/10/2013 3:53:42 PM
C:\Users\kklinkel\Documents\EAST\Workspaces\13546686.wsp

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
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PRIORITY DOCUMENT EXCHANGE

FAILURE STATUS REPORT

An attempt by the Office to electronically retrieve, under the Priority Document Exchange programs (PDX and DAS), 0124957.2 to which priority is claimed has FAILED on 10/04/2013.

For further questions or assistance, please contact our EBC Customer Support Center at

1-866-217-9197 (toll-free)

571-272-4100 (local)

M-F 6AM - Midnight (Eastern Time)

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UNITED STATES DEPARTMENT OF COMMERCE
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Alexandria, Virginia 22313-1450
www.uspto.gov

PRIORITY DOCUMENT EXCHANGE

FAILURE STATUS REPORT

An attempt by the Office to electronically retrieve, under the Priority Document Exchange programs (PDX and DAS), 0104072.4 to which priority is claimed has FAILED on 10/04/2013.

For further questions or assistance, please contact our EBC Customer Support Center at

1-866-217-9197 (toll-free)

571-272-4100 (local)

M-F 6AM - Midnight (Eastern Time)

Doc code: RCEX
Doc description: Request for Continued Examination (RCE)

PTO/SB/30EFS (07-09)
Approved for use through 07/31/2012. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

REQUEST FOR CONTINUED EXAMINATION(RCE)TRANSMITTAL
(Submitted Only via EFS-Web)

Application Number	13546686	Filing Date	2012-07-11	Docket Number (if applicable)	167-62 CON III	Art Unit	1611
First Named Inventor	Heidi Lane			Examiner Name	KLINKEL, Kortney L.		

This is a Request for Continued Examination (RCE) under 37 CFR 1.114 of the above-identified application.
Request for Continued Examination (RCE) practice under 37 CFR 1.114 does not apply to any utility or plant application filed prior to June 8, 1995, or to any design application. The Instruction Sheet for this form is located at WWW.USPTO.GOV

SUBMISSION REQUIRED UNDER 37 CFR 1.114

Note: If the RCE is proper, any previously filed unentered amendments and amendments enclosed with the RCE will be entered in the order in which they were filed unless applicant instructs otherwise. If applicant does not wish to have any previously filed unentered amendment(s) entered, applicant must request non-entry of such amendment(s).

☐ Previously submitted. If a final Office action is outstanding, any amendments filed after the final Office action may be considered as a submission even if this box is not checked.

☐ Consider the arguments in the Appeal Brief or Reply Brief previously filed on _____

☐ Other _____

☒ Enclosed

☒ Amendment/Reply

☐ Information Disclosure Statement (IDS)

☐ Affidavit(s)/ Declaration(s)

☐ Other _____

MISCELLANEOUS

☐ Suspension of action on the above-identified application is requested under 37 CFR 1.103(c) for a period of months _____
(Period of suspension shall not exceed 3 months; Fee under 37 CFR 1.17(i) required)

☐ Other _____

FEES

The RCE fee under 37 CFR 1.17(e) is required by 37 CFR 1.114 when the RCE is filed.

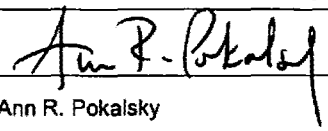
☒ The Director is hereby authorized to charge any underpayment of fees, or credit any overpayments, to Deposit Account No 041121

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT REQUIRED

☒ Patent Practitioner Signature

☐ Applicant Signature

PTO/SB/30EFS (07-09)
Approved for use through 07/31/2012. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Signature of Registered U.S. Patent Practitioner			
Signature		Date (YYYY-MM-DD)	2013-10-15
Name	Ann R. Pokalsky	Registration Number	34697

This collection of information is required by 37 CFR 1.114. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450.

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s): Lane et al.

Examiner: Klinkel, Kortney L.

U.S. Appl. No.: 13/546,686

Group Art Unit:

Filed: July 11, 2012

Docket: 031671-US-CNT03 (167-62 CON III)

For: **TREATMENT OF SOLID TUMORS WITH RAPAMYCIN DERIVATIVES**

Confirmation No.: 8586

MS:AF
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

AMENDMENT UNDER 37 C.F.R. §1.116

In response to the Office Action of June 14, 2013, please amend the above-identified application as follows:

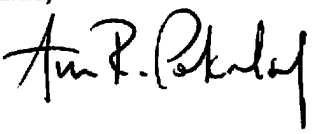
Amendments to the Claims are reflected in the listing of claims, which begins on page 2 of this paper.

Remarks / Arguments begin on page 4 of this paper.

Certificate of EFS-Web Transmission

I hereby certify that this correspondence is being transmitted to the U.S. Patent and Trademark Office via the Office's electronic filing system on October 15, 2013.

Ann R. Pokalsky
(Printed Name)

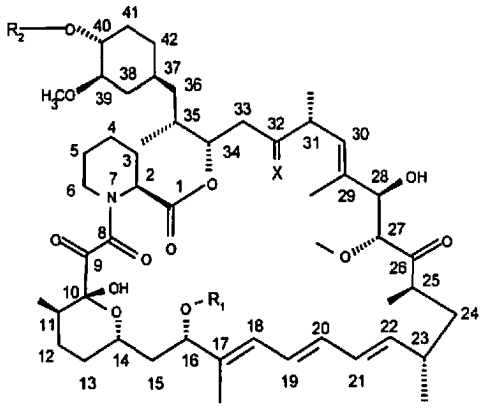
Signature: 

Amendments to the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application.

Listing of Claims:

Claim 1 (currently amended): A method for inhibiting growth of non-malignant solid tumors of the brain in a subject, ~~wherein the solid tumor of the brain is a carcinoma~~ [[,]]said method comprising administering to said subject a therapeutically effective amount of a compound of formula I



I

wherein

R_1 is CH_3,

R_2 is $-CH_2-CH_2-OH$, and

X is =O.

Claim 2 (canceled).

Claim 3 (previously presented): The method of claim 1 wherein the compound of formula I is administered at a daily dose range of from about 0.1 to 25 mg, as a single dose or in divided doses.

Claim 4 (previously presented): The method of claim 1 wherein the compound of formula I is administered in a unit dosage form of from about 0.05 to 12.5 mg.

Claim 5 (previously presented): The method of claim 1 wherein the compound of formula I is administered in a unit dosage form of from about 0.25 to 10 mg.

Claim 6 (previously presented): The method of claim 1 wherein the compound of formula I is administered in a unit dosage form of 10 mg.

Claim 7 (previously presented): The method of claim 1 wherein the compound of formula I is administered orally.

REMARKS / ARGUMENTS

In response to the Final Office Action of June 14, 2013, Applicants have amended claim 1, which when considered with the following remarks, is deemed to advance prosecution of this application. Favorable consideration of the claims is respectfully requested.

Claims 1 and 3-7 remain rejected under 35 U.S.C. §103(a) as allegedly unpatentable over Geoerger et al. ("Antitumor Activity of the Rapamycin Analog CCI-779 in Human Primitive Neuroectodermal Tumor/Medulloblastoma Models as Single Agent and in Combination Chemotherapy" *Cancer Research 61*:1527-1532, 2/15/2001) in view of Cottens et al. (WO 94/09010).

The alleged teachings of both Geoerger et al. and Cottens et al., are fully set forth in the previous office action.

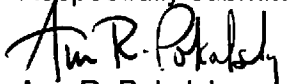
In response to the rejection, and in order to advance prosecution of this application, claim 1 has been amended to recite a non-malignant solid tumor of the brain. It is respectfully submitted that neoplastic diseases, such as solid tumors (as these terms are used in the middle of page 4 of the specification), are known in the art to be non-malignant, pre-malignant, or malignant.

Applicants respectfully submit that Geoerger et al. does not teach or suggest anything about administration of rapamycin or rapamycin derivatives for the treatment of non-malignant solid tumors of the brain. Geoerger et al. examined the cytotoxicity of rapamycin and the rapamycin analog CCI-779 in human malignant brain tumor cell lines *in vitro* and *in vivo* as single agents and in combination with standard chemotherapeutic drugs. The key finding of the study was that malignant tumor toxicity can be increased by using combination chemotherapy and that CCI-779 inhibited growth of xenografts derived from U251 malignant glioma cells, a human cell line resistant to rapamycin *in vitro*. The study reported no finding, conclusion or suggestion about using rapamycin or the rapamycin analog CCI-779 as a single agent to increase tumor toxicity in non-malignant brain tumors. In addition, the findings related to malignant brain tumors are limited to rapamycin and CCI-779.

Cottens et al. does not teach or suggest anything about administration of 40-O-(2-hydroxyethyl)rapamycin for the treatment of non-malignant brain tumors.

Accordingly, the presently claimed invention is not obvious, and withdrawal of the rejection of claims 1 and 3-7 under 35 U.S.C. §103(a) is warranted.

Respectfully submitted,



Ann R. Pokalsky
Registration No.: 34,697
Attorney for Applicants

DILWORTH & BARRESE, LLP
1000 Woodbury Road, Suite 405
Woodbury , New York 11797

Tel. No. (516) 228-8484
Fax No. (516) 228-8516
ARP/ml

Electronic Patent Application Fee Transmittal

Application Number:	13546686
Filing Date:	11-Jul-2012
Title of Invention:	TREATMENT OF SOLID TUMORS WITH RAPAMYCIN DERIVATIVES
First Named Inventor/Applicant Name:	Heidi Lane
Filer:	Ann R. Pokalsky/Suzanne Schmidt
Attorney Docket Number:	031671-US-CNT03 167-62 C3

Filed as Large Entity

Utility under 35 USC 111(a) Filing Fees

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Request for Prioritized Examination	1817	1	4000	4000
Pages:				
Claims:				
Miscellaneous-Filing:				
Publ. Fee- Early, Voluntary, or Normal	1504	1	300	300
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Extension-of-Time:				
Extension - 1 month with $0 paid	1251	1	200	200
Miscellaneous:				
Request for Continued Examination	1801	1	1200	1200
		Total in USD ($)		**5700**

Electronic Acknowledgement Receipt

EFS ID:	17132749
Application Number:	13546686
International Application Number:	
Confirmation Number:	8586
Title of Invention:	TREATMENT OF SOLID TUMORS WITH RAPAMYCIN DERIVATIVES
First Named Inventor/Applicant Name:	Heidi Lane
Customer Number:	28249
Filer:	Ann R. Pokalsky/Suzanne Schmidt
Filer Authorized By:	Ann R. Pokalsky
Attorney Docket Number:	031671-US-CNT03 167-62 C3
Receipt Date:	15-OCT-2013
Filing Date:	11-JUL-2012
Time Stamp:	16:37:50
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	Credit Card
Payment was successfully received in RAM	$5700
RAM confirmation Number	4253
Deposit Account	041121
Authorized User	HARRISON, HELENE

The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:

Charge any Additional Fees required under 37 C.F.R. Section 1.16 (National application filing, search, and examination fees)

Charge any Additional Fees required under 37 C.F.R. Section 1.17 (Patent application and reexamination processing fees)

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Fee Worksheet (SB06)	fee-info.pdf	35605 e9a4a27d5ae23b671e6973dc2e08268e270e82dc	no	2

Warnings:

Information:

Total Files Size (in bytes):	35605

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

Doc Code: TRACK1.REQ
Document Description: TrackOne Request

PTO/AIA/424 (03-13)

CERTIFICATION AND REQUEST FOR PRIORITIZED EXAMINATION UNDER 37 CFR 1.102(e) (Page 1 of 1)

First Named Inventor:	Heidi Lane	Nonprovisional Application Number (if known):	13/546,686
Title of Invention:	Treatment of Solid Tumors with Rapamycin Derivatives		

APPLICANT HEREBY CERTIFIES THE FOLLOWING AND REQUESTS PRIORITIZED EXAMINATION FOR THE ABOVE-IDENTIFIED APPLICATION.

1. The processing fee set forth in 37 CFR 1.17(i)(1), the prioritized examination fee set forth in 37 CFR 1.17(c), and if not already paid, the publication fee set forth in 37 CFR 1.18(d) have been filed with the request. The basic filing fee, search fee, examination fee, and any required excess claims and application size fees are filed with the request or have been already been paid.

2. The application contains or is amended to contain no more than four independent claims and no more than thirty total claims, and no multiple dependent claims.

3. The applicable box is checked below:

I. ☐ **Original Application (Track One) - Prioritized Examination under § 1.102(e)(1)**

i. (a) The application is an original nonprovisional utility application filed under 35 U.S.C. 111(a). This certification and request is being filed with the utility application via EFS-Web.

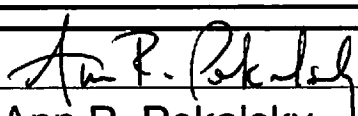
---OR---

(b) The application is an original nonprovisional plant application filed under 35 U.S.C. 111(a). This certification and request is being filed with the plant application in paper.

ii. The executed inventor's oath or declaration is filed with the application. (37 CFR 1.63 and 1.64)

II. ☑ **Request for Continued Examination - Prioritized Examination under § 1.102(e)(2)**

i. A request for continued examination has been filed with, or prior to, this form.
ii. If the application is a utility application, this certification and request is being filed via EFS-Web.
iii. The application is an original nonprovisional utility application filed under 35 U.S.C. 111(a), or is a national stage entry under 35 U.S.C. 371.
iv. This certification and request is being filed prior to the mailing of a first Office action responsive to the request for continued examination.
v. No prior request for continued examination has been granted prioritized examination status under 37 CFR 1.102(e)(2).

Signature 	Date October 15, 2013
Name (Print/Typed) Ann R. Pokalsky	Practitioner Registration Number 34,697

Note: *This form must be signed in accordance with 37 CFR 1.33. See 37 CFR 1.4(d) for signature requirements and certifications. Submit multiple forms if more than one signature is required.**

☐ *Total of _____ forms are submitted.

Electronic Patent Application Fee Transmittal

Application Number:	13546686
Filing Date:	11-Jul-2012
Title of Invention:	TREATMENT OF SOLID TUMORS WITH RAPAMYCIN DERIVATIVES
First Named Inventor/Applicant Name:	Heidi Lane
Filer:	Ann R. Pokalsky/Suzanne Schmidt
Attorney Docket Number:	031671-US-CNT03 167-62 C3

Filed as Large Entity

Utility under 35 USC 111(a) Filing Fees

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Request for Prioritized Examination	1817	1	4000	4000
Pages:				
Claims:				
Miscellaneous-Filing:				
Publ. Fee- Early, Voluntary, or Normal	1504	1	300	300
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Extension-of-Time:				
Extension - 1 month with $0 paid	1251	1	200	200
Miscellaneous:				
Request for Continued Examination	1801	1	1200	1200
		Total in USD ($)		**5700**

Electronic Acknowledgement Receipt

EFS ID:	17131705
Application Number:	13546686
International Application Number:	
Confirmation Number:	8586
Title of Invention:	TREATMENT OF SOLID TUMORS WITH RAPAMYCIN DERIVATIVES
First Named Inventor/Applicant Name:	Heidi Lane
Customer Number:	28249
Filer:	Ann R. Pokalsky/Suzanne Schmidt
Filer Authorized By:	Ann R. Pokalsky
Attorney Docket Number:	031671-US-CNT03 167-62 C3
Receipt Date:	15-OCT-2013
Filing Date:	11-JUL-2012
Time Stamp:	16:34:14
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Request for Continued Examination (RCE)	RCE.pdf	88888 0f9bbbe6d784dbc4f894c3095e2e77b1c587a152	no	2

Warnings:

This is not a USPTO supplied RCE SB30 form.

Information:					
2	Amendment Submitted/Entered with Filing of CPA/RCE	AMENDMENT.pdf	152178 f34e8a25e9a756cdcdcd572b9846bd02bc47579a	no	5
Warnings:					
Information:					
3	TrackOne Request	CERT_PRIORITIZED_EXAM.pdf	74489 ab346633a341df1e12166e194ac353a65cbfcb9d	no	1
Warnings:					
Information:					
4	Fee Worksheet (SB06)	fee-info.pdf	35605 388b53c0c8fa0aebd9877c9708be894a8cf90b2c	no	2
Warnings:					
Information:					
			Total Files Size (in bytes):	351160	

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New Applications Under 35 U.S.C. 111

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New International Application Filed with the USPTO as a Receiving Office

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PTO/SB/06 (09-11)
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PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875	Application or Docket Number 13/546,686	Filing Date 07/11/2012	☐ To be Mailed

ENTITY: ☒ LARGE ☐ SMALL ☐ MICRO

APPLICATION AS FILED – PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA	RATE ($)	FEE ($)
☐ BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A	
☐ SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A	
☐ EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	minus 20 =	*	X $ =	
INDEPENDENT CLAIMS (37 CFR 1.16(h))	minus 3 =	*	X $ =	
☐ APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $310 ($155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).			
☐ MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))				
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL	

APPLICATION AS AMENDED – PART II

AMENDMENT **10/15/2013**

	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	RATE ($)	ADDITIONAL FEE ($)
Total (37 CFR 1.16(i))	* 6	Minus	** 20	= 0	X $80 =	0
Independent (37 CFR 1.16(h))	* 1	Minus	***3	= 0	X $420 =	0
☐ Application Size Fee (37 CFR 1.16(s))						
☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))						
					TOTAL ADD'L FEE	0

AMENDMENT

	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	RATE ($)	ADDITIONAL FEE ($)
Total (37 CFR 1.16(i))	*	Minus	**	=	X $ =	
Independent (37 CFR 1.16(h))	*	Minus	***	=	X $ =	
☐ Application Size Fee (37 CFR 1.16(s))						
☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))						
					TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".
The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.

LIE
/PAUL STANBACK/

Electronic Acknowledgement Receipt

EFS ID:	17132749
Application Number:	13546686
International Application Number:	
Confirmation Number:	8586
Title of Invention:	TREATMENT OF SOLID TUMORS WITH RAPAMYCIN DERIVATIVES
First Named Inventor/Applicant Name:	Heidi Lane
Customer Number:	28249
Filer:	Ann R. Pokalsky/Suzanne Schmidt
Filer Authorized By:	Ann R. Pokalsky
Attorney Docket Number:	031671-US-CNT03 167-62 C3
Receipt Date:	15-OCT-2013
Filing Date:	11-JUL-2012
Time Stamp:	16:37:50
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	Credit Card
Payment was successfully received in RAM	$5700
RAM confirmation Number	4253
Deposit Account	041121
Authorized User	HARRISON, HELENE

10/21/2013 CKHLOK 00000002 041121 13546686
01 FC:1830 140.00 DA

The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:

Charge any Additional Fees required under 37 C.F.R. Section 1.16 (National application filing, search, and examination fees)

Charge any Additional Fees required under 37 C.F.R. Section 1.17 (Patent application and reexamination processing fees)

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Fee Worksheet (SB06)	fee-info.pdf	35605 e9a4a27d5ae23b671e6973dc2e08268e270e82dc	no	2

Warnings:	
Information:	
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New International Application Filed with the USPTO as a Receiving Office
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DIL WORTH & BARRESE, LLP
1000 WOODBURY ROAD
SUITE 405
WOODBURY NY 11797

Doc Code: TRACK1.GRANT

Decision Granting Request for Prioritized Examination (Track I or After RCE)	Application No.: 13/546,686

1. THE REQUEST FILED 10/15/13 IS **GRANTED**.

The above-identified application has met the requirements for prioritized examination

A. ☐ for an original nonprovisional application (Track I).
B. ☒ for an application undergoing continued examination (RCE).

2. **The above-identified application will undergo prioritized examination.** The application will be accorded special status throughout its entire course of prosecution until one of the following occurs:

A. filing a **petition for extension of time** to extend the time period for filing a reply;
B. filing an **amendment to amend the application to co`tain more than four independent claims, more than thirty total claims**, or a multiple dependent claim;
C. filing a **request for continued examination**;
D. filing a notice of appeal;
E. filing a request for suspension of action;
F. mailing of a notice of allowance;
G. mailing of a final Office action;
H. completion of examination as defined in 37 CFR 41.102; or
I. abandonment of the application.

Telephone inquiries with regard to this decision should be directed to Terri Johnson at 571-272-2991. In his/her absence, calls may be directed to Brian Brown at 571-272-5338.

/Terri Johnson/ — Paralegal Specialist

[*Signature*] — (Title)

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An attempt by the Office to electronically retrieve, under the Priority Document Exchange programs (PDX and DAS), 0124957.2 to which priority is claimed has FAILED on 12/25/2013.

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571-272-4100 (local)

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
13/546,686	07/11/2012	Heidi Lane	031671-US-CNT03 167-62 C3	8586

28249 7590 02/04/2014

DILWORTH & BARRESE, LLP
1000 WOODBURY ROAD
SUITE 405
WOODBURY, NY 11797

EXAMINER	
KLINKEL, KORTNEY L	
ART UNIT	**PAPER NUMBER**
1611	

MAIL DATE	DELIVERY MODE
02/04/2014	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No. 13/546,686	Applicant(s) LANE ET AL.	
	Examiner Kortney L. Klinkel	Art Unit 1611	AIA (First Inventor to File) Status No

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTHS FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *10/15/2013*.

☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

4)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims*

5)☒ Claim(s) *1 and 3-7* is/are pending in the application.

5a) Of the above claim(s) _____ is/are withdrawn from consideration.

6)☐ Claim(s) _____ is/are allowed.

7)☒ Claim(s) *1 and 3-7* is/are rejected.

8)☐ Claim(s) _____ is/are objected to.

9)☐ Claim(s) _____ are subject to restriction and/or election requirement.

* If any claims have been determined allowable, you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to PPHfeedback@uspto.gov.

Application Papers

10)☐ The specification is objected to by the Examiner.

11)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

Priority under 35 U.S.C. § 119

12)☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

Certified copies:

a)☒ All b)☐ Some** c)☐ None of the:

1.☐ Certified copies of the priority documents have been received.

2.☒ Certified copies of the priority documents have been received in Application No. 10/468520.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

** See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)

2) ☐ Information Disclosure Statement(s) (PTO/SB/08a and/or PTO/SB/08b) Paper No(s)/Mail Date _____.

3) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .

4) ☐ Other: _____.

DETAILED ACTION

The present application is being examined under the pre-AIA first to invent provisions.

Continued Examination Under 37 CFR 1.114

A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on 10/15/2013 has been entered.

Claim 1 was amended. Claim 2 stands cancelled. Claims 1 and 3-7 are pending and under consideration in the instant office action.

Rejections and/or objections not reiterated from previous office actions are hereby withdrawn. The following rejections and/or objections are either reiterated or newly applied and constitute the complete set presently being applied to the instant application.

Claim Rejections - 35 USC § 103

The following is a quotation of pre-AIA 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which

said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

The factual inquiries set forth in *Graham v. John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966), that are applied for establishing a background for determining obviousness under pre-AIA 35 U.S.C. 103(a) are summarized as follows:

1. Determining the scope and contents of the prior art.
2. Ascertaining the differences between the prior art and the claims at issue.
3. Resolving the level of ordinary skill in the pertinent art.
4. Considering objective evidence present in the application indicating obviousness or nonobviousness.

This application currently names joint inventors. In considering patentability of the claims under pre-AIA 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of pre-AIA 35 U.S.C. 103(c) and potential pre-AIA 35 U.S.C. 102(e), (f) or (g) prior art under pre-AIA 35 U.S.C. 103(a).

Claims 1 and 3-7 are rejected under pre-AIA 35 U.S.C. 103(a) as being unpatentable over Geoerger et al. (“Antitumor Activity of the Rapamycin Analog CCI-779 in Human Primitive Neuroectodermal Tumor/Medulloblastoma Models as Single Agent and in Combination Chemotherapy”, Cancer Research, 61, 2/15/2001, 1527-1532, as per Applicant’s IDS) in view of Cottens et al. (WO 94/09010, as per Applicant’s IDS).

Geoerger et al. teach that administration of rapamycin has antitumor activity in general (p. 1527, 1st column). Co-administration of rapamycin with cisplatin, or 5-fluouracil and cyclophosphamide exhibited enhanced apoptosis in human cell lines and cytotoxicity in colon tumor models respectively (p. 1527, 1st column). Rapamycin and its 40-O substituted analog CCI-779 are effective brain tumor therapeutics both alone and in combination with chemotherapeutics such as cisplatin and camptothecin (p. 1527, abstract and 2nd column). Geoerger et al. teach that brain tumor cell lines are exquisitely sensitive to rapamycin (p. 1527, 2nd column, first full paragraph). Geoerger et al. teach that rapamycin in combination with cisplatin or camptothecin has an additive effect in cell lines resistant to rapamycin (p. 1528, 1st paragraph of Results section). The antitumor activity of rapamycin has been demonstrated in tumors. The antitumor activity of rapamycin has been demonstrated in human rhabdomyosarcoma and neuroblastoma tumor cell lines *in vitro* and in B16 melanocarcinoma, Colon 38 tumors, CD8F1 mammary tumors, EM ependymoblastoma, and U251 glioblastoma brain tumors *in vivo* (p. 1530, Discussion). Geoerger et al. also teach that tumor toxicity can be increased by using combination chemotherapy with a rapamycin without the risk of increased systemic cyctotoxicity (p. 1530, Discussion). Geoerger et al. teach that cisplatin, camptothecin, CPT 11 and topotecan are effective agents in the chemotherapeutic treatment of brain tumors but that dosages of these agents are limited due to their toxicity. Because rapamycin and the 40-O-substituted derivative CCI-779 show at least an additive effect when combined with chemotherapeutics and they have low toxicity, they are good adjuvants for these toxic chemotherapeutics (p.

1532, first column). Additionally CCI-779 exhibits an enhanced antitumor effect when combined with cisplatin in vivo (p. 1532, first column).

Geoerger et al. also teach that either 20 mg/kg/d in a single dose or 100 mg/kg/d in a divided dose of the rapamycin derivative CCI-779 is administered via intraperitoneal injection (p. 1528 1st col., p. 1532 1st col.). Dosages of 100, 200, 400 or 800 mg/kg/d of rapamycin are also taught to be effective (p. 1531, 1st col.).

The teachings of Geoerger et al. differ from the instant claims in that rapamycin or the 40-O substituted rapamycin derivative CCI-779 are administered either alone or in combination with other chemotherapeutics for the treatment of brain tumors *inter alia*, rather than the claimed rapamycin derivative 40-O-(2-hydroxyethyl) rapamycin (AKA everolimus). Geoerger et al. also fail to teach explicit dosages in terms of mg administered, but rather teaches dosages in terms of mg/kg. The dosages described by Geoerger et al. are all administered intraperiotoneally rather than orally as required by instant claim 7. The examiner also notes that Geoerger et al. generally mentions that rapamycin and the rapamycin derivative CCI-779 are effective against brain tumors (i.e. no mention of whether they are benign (non-malignant) or malignant), but the working examples therein are directed to malignant forms of brain tumor.

Cottens et al. teach compounds of formula I, including the instant claimed compound i.e. 40-O-(2-hydroxyethyl) rapamycin (pages 2-4, see particularly p. 3 compound 8, last line; 21-22; Example 8 p. 21-22; claim 2, compound 8) and that these derivatives of rapamycin have an improved pharmacologic profile over rapamycin, exhibit greater stability and bioavailability and allow for greater ease in producing

gelenic formulations (p. 2, first full paragraph). Cottens et al. teach that the use of rapamycin as an antitumor agent is restricted by its low and variable bioavailability (p. 2, lines 1-4).

Cottens et al. teach that compounds of formula I have demonstrated antitumor activity and the ability to enhance performance of antitumor agents by alleviating multidrug resistance e.g. by administration with anticancer agent e.g. colchicine or etoposide, to multidrug resistant cells and drug sensitive cells in vitro or to animals having multidrug resistant or drug sensitive tumors (page 12, first full para.). Note that Cottens et al. teaches these compounds have general antitumor activity and does not state that these compounds only work on malignant tumors. Cottens et al. teach that the compounds may be administered as the sole active ingredient or together with other drugs e.g. corticosteroids, azathioprine, immunosuppressive monoclonal antibodies (page 8, second full para.).

Cottens et al. teach a method of treating tumors (in general) or hyperproliferative disorders comprising administering a compound of formula I (page 6, items “d and e;” page 40, claim 8). Cottens et al. teach that generally the dose of the instant claimed compounds is from 0.05 to 10 mg/kg/d orally in individual dosages of 0.1 to 7.5 mg/kg/day for up to 4 divided doses per day. Typical dosages for intravenous injection range from 0.01 to 5 mg/kg/day (page 7, first para to page 8, first para.). In total, for an average human, dosages range from 5 to 100 mg p.o. up to 500 mg/d p.o. or on the order of 0.5 to 250 mg i.v. with individual dosages from 2.5 to 50 mg i.v. (p. 8

first para.). These absolute dosage amounts overlap with the dosage amounts required by claims 3-6.

Newton et al. review the clinical presentation, diagnosis and pharmacotherapy of patients with primary brain tumors (title). Chemotherapy is a common treatment for patients with both malignant and selected recurrent and progressive benign (or non-malignant) neoplasms (abstract, Data Synthesis). Newton et al. note that cisplatin, cyclophosphamide and etoposide *inter alia* are particularly useful in treating such neoplasms (abstract, Data Synthesis)

It would have been *prima facie* obvious to one of ordinary skill in the art at the time of the instant invention to substitute rapamycin or CCI-779 of Geoerger et al. for the claimed rapamycin derivative 40-O-(2-hydroxyethyl)rapamycin of Cottens et al. with the reasonable expectation that non-malignant (or benign) solid tumors of the brain would be treated when administered alone or in combination with other chemotherapeutics such as cisplatin, 5-fluoruracil, topotecan, cyclosphsphamide and etoposide. One would have been motivated to do so because it is well known in the art that 40-O-(2-hydroxyethyl)rapamycin is useful for treating tumors in general and hyperproliferative disorders and that it exhibits an improved pharmacologic profile over rapamycin, exhibits greater stability and bioavailability and allows for greater ease in formulating. One of ordinary skill in the art would be imbued with the reasonable expectation that the combination of 40-O-(2-hydroxyethyl)rapamycin with the chemotherapeutics 5-fluorouracil, topotecan, cyclophosphamide and etoposide inter alia, would exhibit at least an additive effect as this is what is observed for the

combination of rapamycin or CCI-779 with these agents. One would be imbued with the reasonable expectation that the combination of 40-O-(2-hydroxyethyl)rapamycin with cisplatin would exhibit an enhanced antitumor effect, as this is what is observed for the 40-O-substituted rapamycin derivative CCI-779. Additionally, "[i]t is *prima facie* obvious to combine two compositions each of which is taught by the prior art to be useful for the same purpose, in order to form a third composition to be used for the very same purpose.... [T]he idea of combining them flows logically from their having been individually taught in the prior art." *In re Kerkhoven*, 626 F.2d 846, 850, 205 USPQ 1069, 1072 (CCPA 1980) (citations omitted).

Further, one of ordinary skill in the art would be motivated to administer 40-O-(2-hydroxyethyl)rapamycin alone or in combination with a second known chemotherapeutic to a subject having a non-malignant solid brain tumor as the state of the art is such that chemotherapy in general, and including therapy with cisplatin, cyclophosphamide and etoposide is a known means of treating benign solid brain tumors. Additionally, as cisplatin and rapamycin are known to exhibit a synergistic effect and cisplatin is known to be an effective benign solid brain tumor treatment, one of ordinary skill in the art would be imbued with a reasonable expectation of success for this combination in particular. The Examiner also notes that the fact that Geoerger et al. and Cottens et al. both teach that rapamycin and 40-O-(2-hydroxyethyl)rapamycin have demonstrated antitumor activity in general is sufficient to imbue one of ordinary skill in the art that there would be a reasonable expectation of success that non-malignant solid brain tumors would be treated with the monotherapy as well.

Regarding the dosage amounts of about 0.1-25 mg as a single or divided dosage of claim 3, a unit dosage of about 0.05 to 12.5 mg of claim 4, a unit dosage from about 0.25 to 10 mg of claim 5 and a unit dosage form of 10 mg of claim 6, the Examiner notes that depending on the size of the subject, both the teachings of Geoerger et al. and Cottens et al. teach amounts which fall within or overlap with the claimed amounts. Generally, differences in concentration or temperature will not support the patentability of subject matter encompassed by the prior art unless there is evidence indicating such concentration or temperature is critical. "[W]here the general conditions of a claim are disclosed in the prior art, it is not inventive to discover the optimum or workable ranges by routine experimentation." *In re Aller*, 220 F.2d 454, 456, 105 USPQ 233, 235 (CCPA 1955). Here, one of ordinary skill in the art would be motivated to adjust the relative amount of drug administered to suite the subject's mass and condition and to balance beneficial effects with negative side effects. It is well within the purview of one of ordinary skill in the art to determine the optimal dosage amount.

Response to Arguments

Applicant's arguments regarding the rejection of claims have been fully considered, but are moot in light of the new grounds of rejection presented above. However, as Geoerger et al. and Cottens et al. were used in the previous rejection, the examiner will address any arguments still relevant.

Applicant argues that Geoerger et al. does not teach or suggest anything about administration of rapamycin or rapamycin derivatives for the treatment of non-malignant solid tumors of the brain. Applicant also argues that Geoerger et al. reported no finding,

conclusion or suggestion about using rapamycin or the rapamycin analog as a single agent to increase tumor toxicity in non-malignant brain tumors. Applicant also argues that Cottens et al. fails to teach or suggest anything about administration of 40-O-(2-hydroxyethyl)rapamycin for the treatment of non-malignant brain tumors. These arguments have been fully considered, but are not persuasive. Both Geoerger et al. and Cottens et al. teach that generally rapamycin, the rapamycin derivative CCI-779 and 40-O-(2-hydroxyethyl)rapamycin have antitumor effects in general. Note that a tumor is either benign, pre-malignant or malignant (note Applicant's arguments p. 4 as well). It is true that Geoerger et al. demonstrates examples with rapamycin and CCI-779 against malignant tumor lines, however, this cannot detract from the broader teachings. Additionally, the examiner importantly notes that, one cannot show nonobviousness by attacking references individually where the rejections are based on combinations of references. See *In re Keller*, 642 F.2d 413, 208 USPQ 871 (CCPA 1981); *In re Merck & Co.,* 800 F.2d 1091, 231 USPQ 375 (Fed. Cir. 1986). Here the rejection is over the combined teachings of Geoerger et al., Cottens et al. and Newton et al. together, as detailed in the above rejection, these references render obvious the instantly claimed method and provide one of ordinary skill in the art with a reasonable expectation of success.

Regarding the argument that Geoerger et al. fails to report a finding or suggestion about using rapamycin or the rapamycin analog CCI-779 as a single agent to increase tumor toxicity in non-malignant brain tumors, the Examiner disagrees. On p. 1527 second column Geoerger et al. states that "Rapamycin and its analogue, CCI-779,

are attractive candidates for brain tumor therapy.". Furthermore, Cottens et al. also teaches that the rapamycin derivatives therein, including the claimed 40-O-(2-hydroxyethyl)rapamycin have antitumor activity alone or in combination. Finally, the examiner notes that applicant's claims are limited to monotherapy with 40-O-(2-hydroxyethyl)rapamycin. The comprising or open claim language allows for the inclusion of additional therapeutics to be administered with the claimed 40-O-(2-hydroxyethyl)rapamycin.

Conclusion

Claims 1 and 3-7 are rejected. No claim is allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Kortney Klinkel whose telephone number is (571)270-5239. The examiner can normally be reached on Monday-Friday 10 am to 7 pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Daniel Sullivan can be reached at (571)272-0779. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Kortney L. Klinkel/
Primary Examiner, Art Unit 1611

Notice of References Cited	Application/Control No. 13/546,686	Applicant(s)/Patent Under Reexamination LANE ET AL.	
	Examiner Kortney L. Klinkel	Art Unit 1611	Page 1 of 1

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	Classification
	A	US-			
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NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	Newton et al. "Clinical presentation, diagnosis, and pharmacotherapy of patients with primary brain tumors." Ann Pharmacother. 1999, July-Aug, 33(7-8); 816-32, abstract only.
	V	
	W	
	X	

*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	13546686	LANE ET AL.
	Examiner	Art Unit
	KORTNEY L KLINKEL	1611

CPC- SEARCHED		
Symbol	Date	Examiner
A61K 31/436, 45/06, 2300/00	1/31/2014	KLK

CPC COMBINATION SETS - SEARCHED		
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Class	Subclass	Date	Examiner

SEARCH NOTES		
Search Notes	Date	Examiner
Searched inventor names in PALM	9/28/2012	KLK
Searched EAST, see history attached	9/28/2012	KLK
Searched Pubmed, see history attached	9/28/2012	KLK
searched EAST	6/10/2013	KLK
searched keywords (carcinoma, brain carcinoma, etc) in google	6/10/2013	KLK
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searched Pubmed, see history 2x attached	1/31/2014	KLK

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#4	Add	Search **((benign or nonmalignant)) AND brain tumor** Filters: **Review; Free full text available**	86	17:35:52
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EAST Search History

EAST Search History (Prior Art)

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S3	35	terence near2 o'reilly	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/09/28 13:07
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S5	114	S2 or S3 or S4	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/09/28 13:07
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S7	604202	cancer or carcinoma or tumor	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/09/28 13:09
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S10	19	S8 and S9	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/09/28 13:10
S11	5952358	brain or cns or central near2 nervous near2 system	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/09/28 13:23
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S19	2	("20060165635").PN.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2013/06/11 13:21
S20	10	"505378".ap.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2013/06/11 14:54
S21	108304	(nonmalignant or non-malignant or benign)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2014/01/29 15:23
S22	147721	malignant	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2014/01/29 15:24
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S29	4984	a61k31/436.cpc.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2014/01/29 15:28
S30	8	S28 and S29	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2014/01/29 15:28
S31	36745	rapamycin or everolimus or RAD-001 or RAD001 or sirolimus or zortress or certican or afinitor	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2014/01/29 15:32
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s): Lane et al.

Examiner: Klinkel, Kortney L.

U.S. Appl. No.: 13/546,686

Group Art Unit: 1611

Filed: July 11, 2012

Docket: 031671-US-CNT03 (167-62 CON III)

For: **TREATMENT OF SOLID TUMORS WITH RAPAMYCIN DERIVATIVES**

Confirmation No.: 8586

Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

AMENDMENT

In response to the Office Action of February 4, 2014, please amend the above-identified application as follows:

Amendments to the Claims are reflected in the listing of claims, which begins on page 2 of this paper.

Remarks / Arguments begin on page 4 of this paper.

Certificate of EFS-Web Transmission

I hereby certify that this correspondence is being transmitted to the U.S. Patent and Trademark Office via the Office's electronic filing system on May 5, 2014.

Ann R. Pokalsky
(Printed Name)

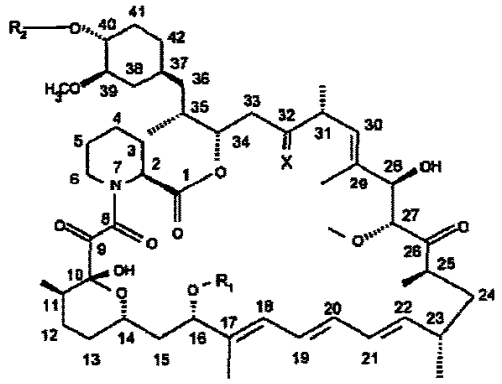
Signature: Ann R. Pokalsky

Amendments to the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application.

Listing of Claims:

Claim 1 (currently amended): A method for inhibiting growth of non-malignant solid tumors of the brain in a subject, said method ~~comprising~~ consisting of administering to said subject a therapeutically effective amount of a compound of formula I



I

wherein

R_1 is CH_3,

R_2 is $-CH_2-CH_2-OH$, and

X is =O.

Claim 2 (canceled).

Claim 3 (previously presented): The method of claim 1 wherein the compound of formula I is administered at a daily dose range of from about 0.1 to 25 mg, as a single dose or in divided doses.

Claim 4 (previously presented): The method of claim 1 wherein the compound of formula I is administered in a unit dosage form of from about 0.05 to 12.5 mg.

Claim 5 (previously presented): The method of claim 1 wherein the compound of formula I is administered in a unit dosage form of from about 0.25 to 10 mg.

Claim 6 (previously presented): The method of claim 1 wherein the compound of formula I is administered in a unit dosage form of 10 mg.

Claim 7 (previously presented): The method of claim 1 wherein the compound of formula I is administered orally.

REMARKS / ARGUMENTS

In response to the Office Action of February 4, 2014, Applicants have amended claim 1, which when considered with the following remarks, is deemed to advance prosecution of this application. Favorable consideration of the claims is respectfully requested.

In the February 4, 2014, Office Action, the Examiner has indicated on page 9 that *new grounds of rejection have been applied to the claims.* On page 3, final paragraph, of the Office Action, however, the Examiner has indicated that claims 1 and 3-7 have been rejected under 35 U.S.C. §103(a) as allegedly unpatentable over Geoerger et al. ("Antitumor Activity of the Rapamycin Analog CCI-779 in Human Primitive Neuroectodermal Tumor/Medulloblastoma Models as Single Agent and in Combination Chemotherapy" *Cancer Research 61*:1527-1532, 2/15/2001) in view of Cottens et al. (WO 94/09010). Newton et al. ("Clinical presentation, diagnosis, and pharmacotherapy of patients with primary brain tumors" *Ann Pharmacother.* 1999, July-Aug, 33(7-8); 816-32, abstract only) has been cited as a reference as indicated on the Form PTO-892, and as discussed on page 7, first full paragraph, of the Office Action. Thus, it appears that the claims have been rejected over Geoerger et al. in view of Cottens et al. and Newton et al. *See also* page 10 of the Office Action: "here the rejection is over the combined teachings of Geoerger et al., Cottens et al., and Newton et al., together."

The alleged teachings of both Geoerger et al. and Cottens et al. are fully set forth on pages 3-5 of the February 4, 2014, Office Action.

Newton et al. (abstract) has been cited for allegedly reviewing the clinical presentation, diagnosis and pharmacotherapy of patients with primary brain tumors. The reference has also been cited for allegedly teaching that chemotherapy is a common treatment for patients with both malignant and selected recurrent and progressive benign (or nonmalignant) neoplasms.

In response to the rejection, and in order to advance prosecution of this application, claim 1 has been amended to recite in relevant part: "A method for inhibiting growth of non-malignant solid tumors of the brain in a subject, said method *consisting of* administering to said subject a therapeutically effective amount of a compound of formula I..."

Since the claims have been amended to recite the transitional phrase "consisting of," the following discussion focuses on the teachings provided by Geoerger et al. as they pertain to monotherapy using rapamycin or the rapamycin analog CCI-779.

Applicants respectfully submit that Geoerger et al. does not teach or suggest anything about administration of rapamycin or rapamycin derivatives for the treatment of non-malignant solid tumors of the brain. Geoerger et al. examined the cytotoxicity of rapamycin and the rapamycin analog CCI-779 in human malignant brain tumor cell lines *in vitro* and *in vivo* as single agents and in combination with standard chemotherapeutic drugs. It is respectfully submitted that the key finding of the study was that malignant tumor toxicity can be increased by using combination chemotherapy and that CCI-779 inhibited growth of xenografts derived from U251 malignant glioma cells, a human cell line resistant to rapamycin *in vitro*. The study reported no finding, conclusion or suggestion about using rapamycin or the rapamycin analog CCI-779 as a single agent to increase tumor toxicity in non-malignant brain tumors. In addition, the findings related to malignant brain tumors are limited to rapamycin and CCI-779.

At page 4 of the office action, the Examiner has cited Geoerger et al. for teaching that brain tumor cell lines are exquisitely sensitive to rapamycin. Applicants respectfully submit that Geoerger et al. teach "*in vitro* studies in our laboratory find that brain tumor cell lines *can* be exquisitely sensitive to rapamycin." Geoerger et al., page 1527, second column, first full paragraph (emphasis added). Applicants also respectfully submit however, that the fact that the rapamycin analog CCI-779 produced growth inhibition of xenografts derived from U251 malignant glioma cells, a human cell line *resistant* to rapamycin *in vitro*, actually shows the unpredictability of the effectiveness of rapamycin and rapamycin analogs on brain tumor cells. That is, Geoerger et al. found that brain tumor cell lines (e.g., U251 malignant glioma) can also be insensitive (resistant) to rapamycin.

Applicants acknowledge Geoerger et al. teach that since CCI-779 produced growth inhibition of xenographs derived from U251 malignant glioma cell lines, a human cell line resistant to rapamycin *in vitro*, such results suggest that the rapamycin analog CCI-779 is an important new agent to investigate in the treatment of human brain tumors, particularly PNET/MB. *See* abstract, final two sentences. PNET/MB is indicated as the most common

malignant brain tumors in children. *See* Geoerger et al., page 1527, left column, third paragraph under "INTRODUCTION."

Regarding the teaching provided by Cottens et al., Applicants respectfully submit that the reference does not teach or suggest anything about administration of 40-O-(2-hydroxyethyl)rapamycin for the treatment of non-malignant brain tumors. Rather, the reference teaches that compounds for *immunosuppressive* use preferably include the presently claimed 40-O-(2-hydroxy)ethyl-rapamycin. *See* Cottens et al., page 4, last full paragraph, reproduced below:

> The Novel Compounds for immunosuppressive use are preferably the 40-O-substituted rapamycins where X and Y are both O, R^2 is H, R^4 is methyl and R^1 is other than H; most preferably where R^1 is selected from hydroxyalkyl, hydroxyalkoxyalkyl, acylaminoalkyl, and aminoalkyl; especially **40-O-(2-hydroxy)ethyl-rapamycin**, 40-O-(3-hydroxy)propyl-rapamycin, 40-O-[2-(2-hydroxy)ethoxy]ethyl-rapamycin, 40-O-(3-hydroxy)propyl-rapamycin, 40-O-[2-(2-hydroxy)ethoxy]ethyl-rapamycin, and 40-O-(2-acetaminoethyl)-rapamycin.
>
> (Emphasis added.)

Although "treatment of proliferative disorders, e.g., tumors" is disclosed on page 6 of Cottens et al., as conditions where any of the disclosed compounds might be used, the presently claimed compound, 40-O-(2-hydroxy)ethyl-rapamycin is clearly indicated as a compound for immunosuppressive use.

Cottens et al. teach at pages 3-4, twenty eight different "Preferred Novel Compounds", one of which is 40-O-(2-hydroxy)ethyl-rapamycin, presently recited in Applicants' claims. Page 4 of Cottens et al. also teaches that 40-O-(2-hydroxy)ethyl-rapamycin is especially preferable for *immunosuppressive use* and page 7 of Cottens et al. teaches that 27 of the 28 compounds taught at pages 3-4 (i.e., those which are O-substituted at C40, which would include 40-O-(2-hydroxy)ethyl-rapamycin as recited in Applicants' claims) are particularly useful in indications (a) and (b) as set forth on pages 5-6 therein. The conditions set forth in (a) on pages 5 of Cottens et al. include organ or tissue transplant rejection, and graft-versus-host disease. The conditions set forth in (b) on pages 5-6 of Cottens et al. comprise at least 40 different inflammatory diseases with an etiology including an autoimmune component.

The Examiner's position on page 7 of the office action is:

> It would have been *prima facie* obvious to one of ordinary skill in the art at the time of the instant invention to substitute rapamycin or CCI-779 of Geoerger et al. for the claimed rapamycin derivative 40-O-(2-hydroxy)ethyl-rapamycin of Cottens et al. with the reasonable expectation that non-malignant (or benign) sold tumors of the brain would be treated when administered alone...one would have been motivated to do so because it is well known in the art that 40-O-(2-hydroxy)ethyl-rapamycin is useful for treating tumors in general and hyperproliferative disorders and that it exhibits an improved pharmacologic profile over rapamycin, exhibits greater stability and bioavailability and allows for greater ease in formulating.

Applicants respectfully disagree with the Examiner's basis for the obviousness rejection, quoted above, for the following reasons.

The prior art of record would not have motivated a person of skill in the art to substitute 40-O-(2-hydroxy)ethyl-rapamycin taught by Cottens et al. for the CCI-779 taught by Geoerger et al. in the first instance. Applicants predicate this assertion on the following. A person of skill in the art would have known that CCI-779 is an ester prodrug of rapamycin having the chemical name 40-[3-hydroxy-2-(hydroxymethyl)-2-methylpropanoate]-rapamycin. *See e.g.*, specification, page 4. At the time the present invention was made, a person of skill in the art would have known that in the body, the 3-hydroxy-2-(hydroxymethyl)-2-methylpropanoate portion of CCI-779 gets cleaved from the macrolide. In contrast, the presently claimed 40-O-(2-hydroxy)ethyl-rapamycin is alkylated (not esterified) and upon administration, the CH_2CH_2-OH group at position 40 does *not* get cleaved from the macrolide. The twenty eight (28) compounds taught by Cottens et al. are all O-alkylated derivatives of rapamycin that do *not* get cleaved in the body. See Houghton, Peter J., March 2010 "Everolimus" *Clin Cancer Res.* 16(5):1368-1372, submitted herewith in an IDS. *See especially,* page 2, end of second full paragraph: "Unlike temsirolimus, everolimus is not converted to rapamycin *in* vivo." *Thus a person of skill in the art would not have combined the teachings of Geoerger et al. and Cottens et al. in the first instance.*

Newton et al. cannot cure the deficiency of teachings left by Geoerger et al. and Cottens et al. The teaching provided by Newton et al., which is relevant to the present case is that chemotherapy has been used to treat both malignant and benign tumors. Newton lists the most effective chemotherapeutic drugs as nitrosoureas, procarbazine, cisplatin and carboplatin. Other agents which Newton considers include cyclophosphamide, methotrexate, vincristine, and etoposide. Newton et al. conclude that the efficacy of chemotherapy for primary brain tumors remains modest. The reference does provide any motivation for combining the teachings of Geoerger et al. and Cottens et al., each of which as discussed *supra*, is directed to a completely different class of rapamycin derivatives.

Summarizing, a person of skill in the art would not have substituted the rapamycin derivative of Cottens et al. for the rapamycin derivative of Geoerger et al. because a person of skill in the art would not have combined the teachings of the two references in the first instance. The references would not have been combined because each of them is directed to a completely different class of rapamycin derivatives; esterified (Geoerger et al.) and alkylated (Cottens et al.). Newton et al. does not help in providing any motivation for combining Geoerger et al. with Cottens et al. Applicants further submit that since there would have been no motivation to combine Geoerger et al. with Cottens et al. in the first instance, there couldn't have been any reasonable expectation of success.

Even if there *was* motivation to combine the respective teachings of Geoerger et al. and Cottens et al., (a point on which Applicants do not agree with the Examiner, and on which Applicants do not acquiesce), there would not have been any reasonable expectation of success for arriving at the presently claimed invention. Cottens et al. teaches use of a group of 27 different O-alkylated rapamycin derivatives, 40-O-(2-hydroxy)ethyl-rapamycin being one of them, preferably for the treatment of organ or tissue transplant rejection, graft-versus-host disease and for immunosuppressive use in more than 40 different inflammatory conditions including: arthritis (for example rheumatoid arthritis, arthritis chronic progrediente and arthritis deformans) and rheumatic diseases, autoimmune hematological disorders (including e.g. hemolytic anaemia, aplastic anaemia, pure red cell anaemia and idiopathic thrombocytopenia), systemic lupus erythermatosus, polychondritis, sclerodoma, Wegener granulamoatosis, dermatomyositis, chronic active hepatitis, myasthenia gravis, psoriasis, Steven-Johnson syndrome, idiopathic sprue, autoimmune inflammatory bowel disease (including e.g. ulcerative colitis and Crohn's disease) endocrine opthalmopathy, Graves disease, sarcoidosis, multiple

sclerosis, primary billiary cirrhosis, juvenile diabetes (diabetes mellitus type I), uveitis (anterior and posterior), keratoconjunctivitis sicca and vernal keratoconjunctivitis, interstitial lung fibrosis, psoriatic arthritis, glomerulonephritis (with and without nephrotic syndrome, e.g. including idiopathic nephrotic syndrome or minimal change nephropathy) and juvenile dermatomyositis. *See* Cottens, page 6.

After disclosing more than 40 different types of inflammatory conditions with an etiology including an autoimmune component, which may be treated preferably using the subset of 27 "preferred novel compounds" disclosed therein, Cottens et al. does not disclose *any particular types of* cancer for which the compounds disclosed therein may be used. Cottens et al. simply discloses "treatment of proliferative disorders, e.g. tumors, hyperproliferative skin disorder and the like." *See* Cottens, page 6, "e". As such, a person of skill in the art would not have had any reasonable expectation of success that besides treating 40 different types of inflammatory conditions, one of the compounds, 40-O-(2-hydroxy)ethyl-rapamycin, would also work to inhibit growth of non-malignant brain tumors. A reasonable expectation of success would have been lacking because Cottens et al. does not provide any guidance as to which compound would work in which cancer. According to the National Cancer Institute's web site however, www.cancer.gov, there are more than **two hundred** types of cancer.

A proper obviousness determination requires two distinct elements: (1) motivation and (2) reasonable expectation of success. *Takeda Chem. Indus., Ltd. v. Alphapharm Pty., Ltd.*, 492 F3d 1350, 83 USPQ2d 1169 (Fed. Cir. 2007). Since neither element is present in the present obviousness finding, withdrawal of the rejection of claims 1 and 3-7 under 35 U.S.C.§ 103 is warranted.

Applicants provide herewith as Exhibit A, a copy of Franz et al. "Efficacy and safety of everolimus for subependymal giant cell astrocytomas associated with tuberous sclerosis complex (EXIST-1): a multicentere, randomized, placebo-controlled phase 3 trial" *The Lancet* January 12, 2013, Vol. 381, pages 125-132. Franz et al. provide data which further supports the teachings provided in the specification. Benign tumors in the brain, associated with tuberous sclerosis complex (TSC), were significantly reduced in size in patients treated with everolimus (40-O-(2-hydroxy)ethyl-rapamycin) relative to the placebo group. Since large astrocytomas are associated with increased morbidity and risk of hydrocephalus and potential death, stabilization or even slight reductions in tumor volume translate into clinical benefit.

In view of the foregoing remarks and amendments, it is firmly believed that the present claims are in condition for allowance, which action is earnestly solicited.

Respectfully submitted,

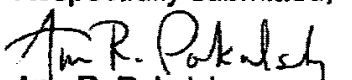


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EXHIBIT A

Efficacy and safety of everolimus for subependymal giant cell astrocytomas associated with tuberous sclerosis complex (EXIST-1): a multicentre, randomised, placebo-controlled phase 3 trial

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Summary

Background Tuberous sclerosis complex is a genetic disorder leading to constitutive activation of mammalian target of rapamycin (mTOR) and growth of benign tumours in several organs. In the brain, growth of subependymal giant cell astrocytomas can cause life-threatening symptoms—eg, hydrocephalus, requiring surgery. In an open-label, phase 1/2 study, the mTOR inhibitor everolimus substantially and significantly reduced the volume of subependymal giant cell astrocytomas. We assessed the efficacy and safety of everolimus in patients with subependymal giant cell astrocytomas associated with tuberous sclerosis complex.

Methods In this double-blind, placebo-controlled, phase 3 trial, patients (aged 0–65 years) in 24 centres in Australia, Belgium, Canada, Germany, the UK, Italy, the Netherlands, Poland, Russian Federation, and the USA were randomly assigned, with an interactive internet-response system, in a 2:1 ratio to oral everolimus 4·5 mg/m² per day (titrated to achieve blood trough concentrations of 5–15 ng/mL) or placebo. Eligible patients had a definite diagnosis of tuberous sclerosis complex and at least one lesion with a diameter of 1 cm or greater, and either serial growth of a subependymal giant cell astrocytoma, a new lesion of 1 cm or greater, or new or worsening hydrocephalus. The primary endpoint was the proportion of patients with confirmed response—ie, reduction in target volume of 50% or greater relative to baseline in subependymal giant cell astrocytomas. Analysis was by intention to treat. This study is registered with ClinicalTrials.gov, number NCT00789828.

Findings 117 patients were randomly assigned to everolimus (n=78) or placebo (n=39). 27 (35%) patients in the everolimus group had at least 50% reduction in the volume of subependymal giant cell astrocytomas versus none in the placebo group (difference 35%, 95% CI 15–52; one-sided exact Cochran-Mantel-Haenszel test, p<0·0001). Adverse events were mostly grade 1 or 2; no patients discontinued treatment because of adverse events. The most common adverse events were mouth ulceration (25 [32%] in the everolimus group *vs* two [5%] in the placebo group), stomatitis (24 [31%] *vs* eight [21%]), convulsion (18 [23%] *vs* ten [26%]), and pyrexia (17 [22%] *vs* six [15%]).

Interpretation These results support the use of everolimus for subependymal giant cell astrocytomas associated with tuberous sclerosis. Additionally, everolimus might represent a disease-modifying treatment for other aspects of tuberous sclerosis.

Funding Novartis Pharmaceuticals.

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See Comment page 95

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Introduction

Tuberous sclerosis complex is estimated to affect more than 1 million people worldwide.[1] It is an autosomal dominant genetic disorder characterised by benign tumours (hamartomas) that arise in many organs, including the brain, kidneys, skin, eyes, lungs, heart, and liver.[2] The most common manifestations of tuberous sclerosis are neurological (eg, epilepsy, intellectual disability, and neurobehavioural and psychiatric problems, including autism spectrum disorder) followed by renal and pulmonary symptoms.[1,3] Subependymal giant cell astrocytomas are slow-growing tumours, usually located near the foramen of Monro,[3] that develop in up to 20% of individuals with tuberous sclerosis.[4–6] They are typically asymptomatic until they reach a size sufficient to cause ventricular obstruction and hydrocephalus. Postoperative morbidity is substantial, although reports vary—about 20% of patients[7] and up to 50%.[6,8] Incomplete resection of subependymal giant cell astrocytomas leads to recurrence;[1,9] in a retrospective analysis, recurrence or contralateral occurrence was reported in 34% of patients, with 13% requiring repeat operations.[8]

The tuberous sclerosis genes *TSC1* (hamartin) and *TSC2* (tuberin) encode proteins that form the hamartin-tuberin tumour suppressor complex, which restricts the activation of the mammalian target of rapamycin complex 1 (mTORC1), a protein kinase that regulates protein synthesis, and cell growth and proliferation, through Rheb

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(Ras homologue enriched in brain).[10] Most patients with tuberous sclerosis have a mutation in either *TSC1* or *TSC2*,[11–13] resulting in activation of mTORC1. This finding has led to the investigation of mTORC1 blockade as a treatment approach in tuberous sclerosis. The results of case reports and preliminary studies have shown that mTOR inhibition is associated with improvements in the manifestation of tuberous sclerosis including subependymal giant cell astrocytomas, angiomyolipomas (benign renal tumours), and facial angiofibromas.[14–20] In an open-label study of 28 patients with evidence of serial growth of subependymal giant cell astrocytomas, the mTOR inhibitor everolimus (Afinitor, Novartis Pharma Stein AG, Stein, Switzerland) reduced the volume of subependymal giant cell astrocytomas, seizure frequency, and number of facial angiofibromas.[21] We assessed the efficacy and safety of everolimus against placebo in patients with subependymal giant cell astrocytomas associated with tuberous sclerosis complex in the phase 3 EXamining everolimus In a Study of Tuberous sclerosis complex (EXIST-1) trial.

Methods

Patients

Eligible patients (aged 0–65 years) had a definite diagnosis of tuberous sclerosis complex according to consensus criteria,[22,23] at least one target subependymal giant cell astrocytoma with the longest diameter 1 cm or greater as assessed with multiphase MRI, and one or more of the following when the results of an MRI done within 4 weeks of randomisation were compared with an earlier MRI: serial worsening (defined as an increase of at least 25% in volume of subependymal giant cell astrocytomas) based on the results of local imaging and radiographic assessment; presence of a new lesion 1 cm or greater in diameter; or new or worsening hydrocephalus (according to central radiological assessment of changes in ventricular configuration, periventricular oedema, and qualitative assessment of the dynamics of cerebrospinal fluid flow). Patients had to be medically stable and unlikely to require surgery for subependymal giant cell astrocytomas, with no critical hydrocephalus or imminent cerebral herniation.

The protocol was approved by an ethics committee at each centre, before the first patient was enrolled. The study was done in accordance with the principles of Good Clinical Practice, Declaration of Helsinki, and all local regulations. An independent data monitoring committee reviewed the safety every 6 months. All patients (or their legal representatives) provided written informed consent before enrolment.

Study design and treatment

The EXIST-1 double-blind, phase 3 trial was undertaken in ten countries (Australia, Belgium, Canada, Germany, UK, Italy, Netherlands, Poland, Russian Federation, and USA), in 24 centres. Patients were randomly assigned in a 2:1 ratio to everolimus or matching placebo, stratified according to the use of enzyme-inducing antiepileptic drugs. Everolimus was administered orally at a starting dose of 4·5 mg/m² body surface area per day and subsequently adjusted to attain blood trough concentrations of 5–15 ng/mL. In the event of treatment-related toxic effects, protocol-specified dose modifications were permitted. The starting dose was chosen to be just less than the maximum tolerated dose (5 mg/m² per day) in children with malignancies.[24] Patients were prohibited from using strong and moderate inhibitors of cytochrome P450 3A4 (CYP3A4) and P-glycoprotein (except antiepileptic drugs), strong inducers of CYP3A4 (except antiepileptic drugs), and concomitant use of antiproliferative drugs (those who had previously used antiproliferative agents were excluded from the study).

The trial consisted of a core phase from the start of the trial to the time when the last patient had been treated with everolimus or placebo for 6 months, and a planned extension phase in which all patients would be given the option of starting open-label everolimus if the results of the core phase favoured everolimus. The extension phase would continue until 4 years after the last patient was randomly assigned to treatment, ensuring follow-up of 4–5 years.

Randomisation and masking

An interactive internet-response system was used for random assignment of patients in a 2:1 ratio to everolimus and placebo and for management of their treatment to maintain allocation concealment. Patients were given masked study treatment (identical everolimus and placebo) unless discontinued as a result of unacceptable toxicity, withdrawal of consent, loss to follow-up, or progression of subependymal giant cell astrocytomas according to the results of independent, central radiological review. All study personnel were masked to treatment assignment. Dose adjustments for patients in the placebo group were recommended through the interactive internet-response system, in a randomised fashion, to maintain masking. Progression of subependymal giant cell astrocytomas was defined as an increase of 25% or more from the nadir volume at baseline; unequivocal worsening of non-target lesions of subependymal giant cell astrocytomas; the appearance of new lesions of 1 cm or more in diameter; or new or worsening hydrocephalus. Patients with progression of subependymal giant cell astrocytomas were unmasked to treatment, and those in the placebo group were offered open-label everolimus.

Efficacy and safety

The primary endpoint was the proportion of patients with confirmed tumour response, defined as a reduction in the total volume of all target subependymal giant cell astrocytomas of 50% or more relative to baseline, in the absence of worsening of non-target subependymal giant cell astrocytomas, new lesions of 1 cm or greater in

diameter, and new or worsening hydrocephalus. The initial tumour response required confirmation with an MRI scan 8–12 weeks later. Key secondary endpoints were absolute change from baseline to 24 weeks in seizure frequency per 24 h by use of a video electroencephalogram, time to progression of subependymal giant cell astrocytomas, and skin lesion response rate in patients with at least one skin lesion at baseline. Other secondary endpoints were angiomyolipoma response rate (defined as a reduction in the total volume of all target angiomyolipomas identified at baseline of 50% or more relative to baseline, with no new angiomyolipoma 1·0 cm or more in longest diameter, no increases in volume of kidney by more than 20% from nadir, and no angiomyolipoma-related bleeding of grade 2 or worse) in patients with one or more target angiomyolipomas, and time to, duration of, and correlation of response of subependymal giant cell astrocytomas with *TSC1* and *TSC2* gene mutation status.

Brain MRI was done at months 3, 6, and 12 after initiation of the treatment and yearly thereafter until discontinuation of the patient from study. For patients with one or more angiomyolipoma of at least 1 cm in diameter at screening or baseline, MRI or CT of the kidney was done on the same schedule as the brain MRI. All scans were assessed by central radiological review. All patients completed a 24 h video electroencephalogram at baseline and 24 weeks (or end of treatment for those who discontinued) that was sent for independent central review. Skin lesions were assessed with the Physician's Global Assessment of Clinical Condition scale[15,16] (a 7-point grading scale for evaluation of the overall extent of improvement or worsening of the patient's skin lesions compared with baseline) every 3 months. Blood was drawn every visit starting at week 2 for pharmacokinetic analysis. Laboratory assessments, including haematology and blood chemistry, were done every 2 weeks for the first 8 weeks, then at months 3, 4·5, and 6, and then every 3 months thereafter. DNA was isolated from whole blood at baseline for the purpose of sequencing *TSC1* and *TSC2* genes.

Adverse events were monitored continuously throughout the study with the Common Terminology Criteria for Adverse Events (version 3.0).[17] At each visit, patients or their carers were assessed for pulmonary symptoms consistent with interstitial pneumonitis, a known adverse effect of mTORC1 inhibition.

Statistical analysis

The planned sample size (n=99) was estimated with a simulation approach, giving the study 93% power to detect a 20% difference in response rates (assuming ≥20% with everolimus and 0 with placebo) of subependymal giant cell astrocytomas between treatment groups. The type 1 error was 2·5%.

Efficacy analyses included all patients (full analysis set) who were randomly assigned. Safety analyses included all patients who were given at least one dose of study drug and had at least one post-baseline assessment. The per-protocol set was used for supportive analysis of the primary endpoint and consisted of all patients from the full-analysis set without any major protocol deviations who could be assessed for efficacy and had been treated for at least 50% of the days in the first 12 weeks since the first day of treatment. The everolimus and placebo groups were compared with a

	Everolimus group (n=78)	Placebo group (n=39)
Age (years, median, range)	9·5 (1·0–23·5)	7·1 (0·8–26·6)
Age (years)		
<3	13 (17%)	7 (18%)
3 to <18	55 (71%)	26 (67%)
≥18	10 (13%)	6 (15%)
Sex		
Male	49 (63%)	18 (46%)
Female	29 (37%)	21 (54%)
Ethnic origin		
White	73 (94%)	36 (92%)
Black	3 (4%)	1 (3%)
Other	2 (3%)	2 (5%)
Body surface area (m², median, range)	1·07 (0·42–2·16)	0·96 (0·40–2·14)
Two or more main features of tuberous sclerosis complex	78 (100%)	39 (100%)
Use of enzyme-inducing antiepileptic drug	15 (19%)	7 (18%)
Presence of seizure on baseline electroencephalogram	27 (35%)	13 (33%)
One or more skin lesion	72 (92%)	38 (97%)
One or more angiomyolipoma	30 (38%)	14 (36%)
Hydrocephalus	8 (10%)	0
Previous treatment for subependymal giant cell astrocytomas	6 (8%)	2 (5%)
Drug	0	0
Surgery	6 (8%)	2 (5%)
Worsening subependymal giant cell astrocytomas confirmed by central review*	66 (85%)	34 (87%)
Serial growth	63 (81%)	32 (82%)
New lesion 1 cm or more in longest diameter	7 (9%)	5 (13%)
New or worsening hydrocephalus	5 (6%)	0
Number of target lesions of subependymal giant cell astrocytomas		
0	2 (3%)	0
1	40 (51%)	25 (64%)
2	34 (44%)	14 (36%)
3	1 (1%)	0
≥4	1 (1%)	0
Volume of subependymal giant cell astrocytomas (cm³, median, range)	2·63 (0·18–25·15)	1·30 (0·12–9·75)
TSC mutation status†		
TSC1 and *TSC2*	1 (1%)	0
TSC1	10 (13%)	3 (8%)
TSC2	55 (71%)	29 (74%)
None	11 (14%)	7 (18%)

Data are number (%) unless otherwise indicated. *At least one criterion for worsening of subependymal giant cell astrocytomas compared with previous visit. †One patient in the everolimus group did not have mutation analysis.

Table 2: Baseline demographic and clinical characteristics of patients

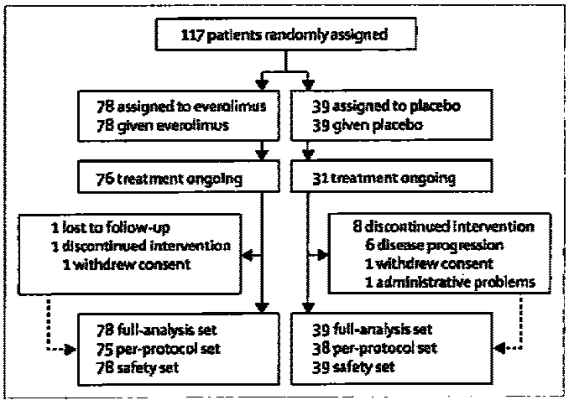

Figure 1: Trial profile

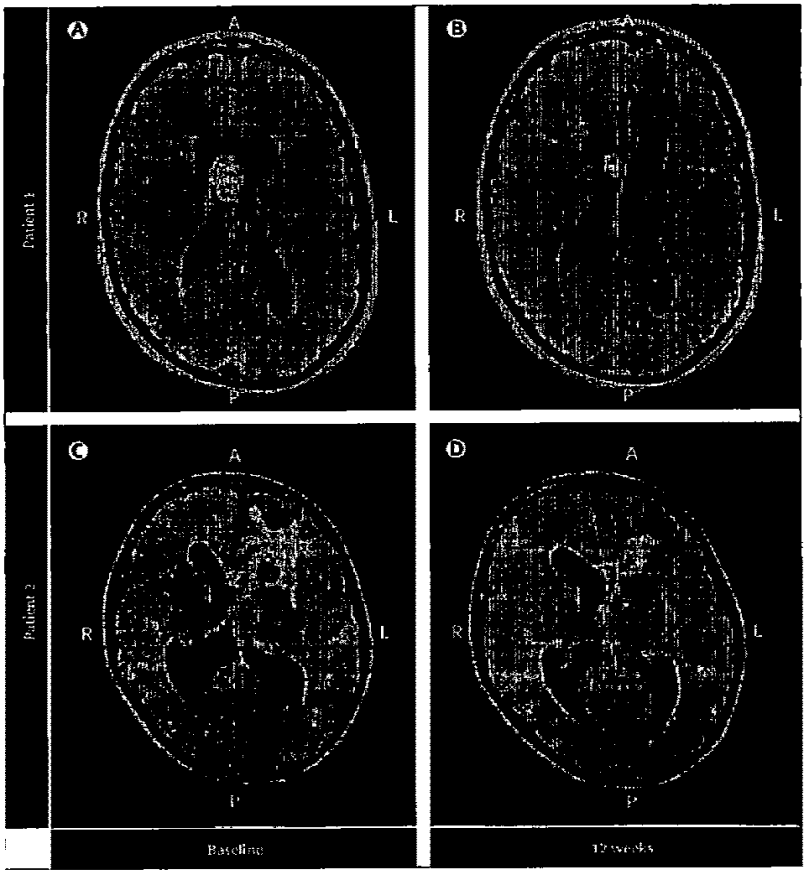

Figure 2: Contrast-enhanced axial fluid attenuated inversion recovery MRI of subependymal giant cell astrocytomas in two patients before (A, C) and after 12 weeks of everolimus (B, D)
Volume of subependymal giant cell astrocytomas was reduced in the two patients after 12 weeks of everolimus. Perituмoral oedema and white matter dysplasia were also reduced in patient 2, who had an unsuccessful resection for subependymal giant cell astrocytomas. Ventricular size was reduced overall in patient 2 despite an apparent increase in the right frontal horn due to resolution of the mass effect from the oedema. A=anterior. R=right. L=left. P=posterior.

one-sided exact Cochran-Mantel-Haenszel test for response rate of subependymal giant cell astrocytomas and skin lesions, a one-sided stratified log-rank test for the time to progression of the astrocytomas, and a one-sided test from rank ANOVA with baseline as covariate for seizure frequency.[28] All these tests were stratified according to the protocol (antiepileptic drug use *vs* no antiepileptic drug use) and done at the 2·5% level. Patients with unknown response of subependymal giant cell astrocytomas were judged non-responders for the analysis. For the key secondary endpoints, multiplicity was controlled through a predefined fixed-sequence testing procedure with a hierarchy of seizure frequency, time to progression of subependymal giant cell astrocytomas, and skin lesion response rate. Statistical analyses were done with SAS software (version 9.2). The data cutoff date for all analyses was 6 months after the last patient was randomly assigned to treatment.

The trial is registered with ClinicalTrials.gov, number NCT00789828.

Role of the funding source

The study was designed by academic investigators and representatives of the sponsor Novartis Pharmaceuticals. The data were analysed by the sponsor (monitored and stored by PAREXEL, Waltham, MA, USA). All authors contributed to data interpretation and amendment of the report, and attest to the accuracy and completeness of the reported data, and that the study conformed to the protocol and statistical analysis plan. The corresponding author made the final decision about where to submit the paper for publication.

Results

Between Aug 20, 2009, and Sept 2, 2010, 117 patients who had subependymal giant cell astrocytomas associated with tuberous sclerosis were randomly assigned to the everolimus (n=78) or placebo group (n=39). Baseline demographics and clinical characteristics were well balanced between the treatment groups, but the everolimus group had a higher proportion of men than did the placebo group and had hydrocephalus (table 1). The median age of patients was 9·5 years (range 0·8–26·6). Skin lesions were present at baseline in 110 patients (94%) and eight (7%) had a history of surgery related to their subependymal giant cell astrocytomas (table 1). Worsening of tumours at baseline, as ascertained by the local investigator, was confirmed by central review in 100 (85%) patients; the frequencies of 17 individuals whose worsening subependymal giant cell astrocytomas were not confirmed by central review were balanced between the treatment groups (12 [15%] in the everolimus group and five in [13%] the placebo group). 84 (72%) patients had *TSC2* mutations (table 1).

The per-protocol set comprised 75 patients in the everolimus group and 38 in the placebo group. Two patients in the everolimus group could not be assessed

because they did not have any target subependymal giant cell astrocytomas identified at baseline central review, one patient in the everolimus group was excluded because of insufficient treatment exposure, and one placebo-treated patient was excluded for protocol deviation.

After a median follow-up of 9·7 months, 76 (97%) patients in the everolimus group and 31 (79%) in the placebo group were still undergoing double-blind treatment (figure 1). The most common reason for discontinuation was disease progression, which was reported exclusively in the placebo group (six [15%] patients); these patients had their treatment changed to open-label everolimus and their data for the double-blind analysis were censored at that point for the analysis of the double-blind period. The median duration of study treatment was 41·9 weeks (range 24·0–78·9) for individuals in the everolimus group and 36·1 weeks (13·9–79·7) for those in the placebo group. The median dose intensity of everolimus was 5·9 mg/m² per day (range 2·3–11·8).

In the full-analysis set, 27 (35%) of 78 patients in the everolimus group and none of 39 in the placebo group had a response in terms of a reduction in the total volume of all target subependymal giant cell astrocytomas of 50% or more relative to baseline (difference 35%; 95% CI 15–52; one-sided exact Cochran-Mantel-Haenszel test, p<0·0001). The result obtained with the per-protocol analysis was similar—27 (36%) of 75 patients in the everolimus group versus none of 38 in the placebo group (36%, 17–53; p<0·0001) had a tumour response. Reductions in volume of subependymal giant cell astrocytomas were detectable with MRI by 12 weeks (figure 2). By week 24, 31 (42%) of 74 patients in the everolimus group had a reduction in total tumour volume of at least 50% versus one (3%) of 34 in the placebo group, and 58 (78%) and five (15%), respectively, had a reduction of 30% or more.

Figure 3 shows that the treatment effect estimates of the tumour response (≥50% reduction relative to baseline) were in favour of everolimus, irrespective of the subgroup—antiepileptic drug use, sex, or age. Some subgroups had few patients, as shown by the wide 95% CIs for the estimates. At data cutoff, 107 patients (76 in everolimus group and 31 in placebo group) were still undergoing treatment; nine had discontinued and one in the everolimus group was lost to follow-up. No cases of progression of subependymal giant cell astrocytomas were seen in the everolimus group; as a result, the duration of tumour response was censored for all everolimus-treated responders. All responses of subependymal giant cell astrocytomas were ongoing at the data cutoff date, and the duration of response was from more than 63 days to more than 255 days. No responses were seen in the placebo group. Response to everolimus was noted irrespective of whether the *TSC* mutation was *TSC1* or *TSC2*, but the rate was lower in patients with a *TSC2* mutation—five (50%) of ten individuals with a mutation in *TSC1* compared with 16 (29%) of 55 with a *TSC2* mutation. None of the patients in the placebo group, irrespective of the mutation status (*TSC1* and *TSC2*, *TSC1*, or *TSC2*), had a tumour response. In everolimus-treated patients with no mutation identified, five (45%) of 11 had a response; none of the seven placebo-treated patients with no mutation identified had a response.

At week 24, the median change from baseline in seizure frequency in 24 h with video electroencephalogram monitoring was 0 in the everolimus and placebo groups (p=0·2004). Because a large proportion of patients did not have any seizures at baseline 24 h electroencephalogram (table 1), we did a sensitivity analysis on the subset of individuals who had at least one seizure at baseline. Treatment groups were imbalanced—the placebo group

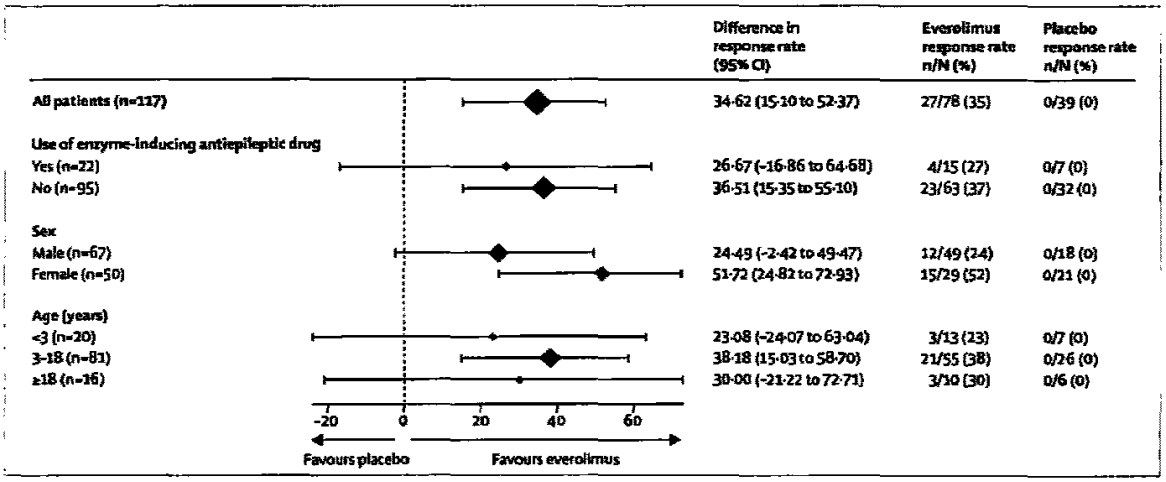

Figure 3: **Forest plot of subependymal giant cell astrocytomas response rates in subgroups of patients**
The area of each diamond is proportional to the number of patients in the subgroup. 95% CI were obtained from the exact unconditional confidence limits.

had a higher median baseline seizure frequency of 11·0 per 24 h (range 1·0 to 78·9) versus 5·9 per 24 h (1·0 to 42·6) in the everolimus group—the median change from baseline to week 24 was −2·9 per 24 h (95% CI −4·0 to −1·0) for the everolimus group and −4·1 per 24 h (−10·89 to 5·78) for the placebo group (p=0·2988).

As judged by central review, six patients, all in the placebo group, had progression of subependymal giant cell astrocytomas at the time of analysis. Median time to tumour progression was not reached in either treatment group, but the estimated progression-free rates at 6 months were 100% for everolimus and 86% for placebo (p=0·0002; figure 4).

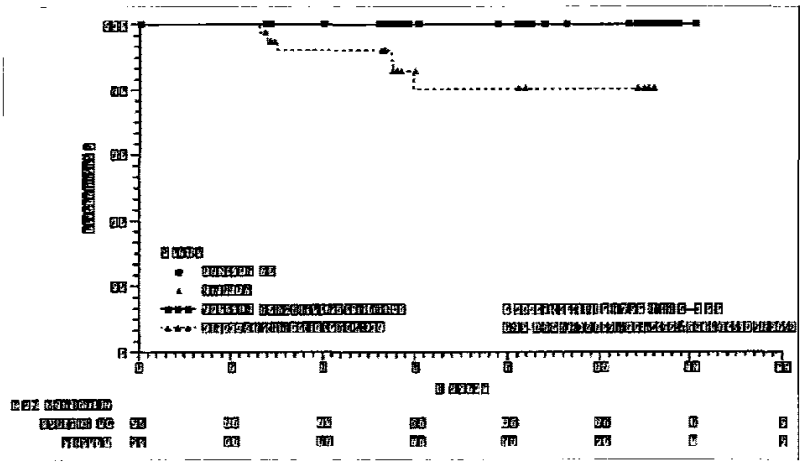

Figure 4: Kaplan-Meier plot of the estimated time to progression of subependymal giant cell astrocytomas
The hazard ratio could not be estimated because progression of the tumours occurred in the placebo group.
NA=not applicable.

	Everolimus group (n=78)		Placebo group (n=39)	
	All grades	Grade 3 or 4	All grades	Grade 3 or 4
Any adverse event	75 (96%)	26 (33%)*	35 (90%)	9 (23%)†
Mouth ulceration	25 (32%)	1 (1%)‡	2 (5%)	0
Stomatitis	24 (31%)	6 (8%)‡	8 (21%)	1 (3%)‡
Convulsion	18 (23%)	4 (5%)‡	10 (26%)	2 (5%)‡
Pyrexia	17 (22%)	5 (6%)‡	6 (15%)	0
Nasopharyngitis	14 (18%)	0	9 (23%)	0
Vomiting	13 (17%)	1 (1%)‡	5 (13%)	0
Upper respiratory tract infection	12 (15%)	1 (1%)‡	7 (18%)	0
Fatigue	11 (14%)	0	1 (3%)	0
Cough	10 (13%)	0	4 (10%)	0
Diarrhoea	10 (13%)	0	2 (5%)	0
Rash	9 (12%)	0	2 (5%)	0
Bronchitis	8 (10%)	3 (4%)‡	4 (10%)	1 (3%)‡
Otitis media	8 (10%)	1 (1%)‡	2 (5%)	1 (3%)‡
Pharyngitis	8 (10%)	0	1 (3%)	0

*Data are number (%). †All grade 3 except one grade 4 gastroenteritis. ‡All grade 3 except one grade 4 hyperglycaemia. §All grade 3.

Table 2: Adverse events of any cause in more than 10% of patients in the everolimus group

110 patients had at least one baseline skin lesion—30 (42%) of 72 patients in the everolimus group and four (11%) of 38 in the placebo group had a skin lesion response (p=0·0004). All skin lesion responses were incomplete.

44 patients had at least one renal angiomyolipoma at baseline (30 in everolimus group and 14 in placebo group); 16 (53%) of 30 patients in the everolimus group versus none of the 14 in the placebo group had an angiomyolipoma response.

The adverse event profile was consistent with the known safety profile of everolimus. Most adverse events were grade 1 or 2. The most common events were mouth ulceration, stomatitis, convulsion, and pyrexia (table 2). The most common grade 3 adverse events were stomatitis, pyrexia, and convulsion; grade 4 events were rare (table 2). Infections, mostly of the upper respiratory tract, were reported by 56 (72%) patients in the everolimus group and 26 (67%) in the placebo group. Other than one (1%) case of grade 1 herpes zoster in the everolimus group, no opportunistic infections were reported; one (1%) infection (gastroenteritis in the everolimus group) was classified as grade 4. One (1%) patient in the everolimus group had grade 2 interstitial pneumonitis after 197 days of treatment that resolved fully 8 weeks after reduction by one dose level.

38 (49%) patients in the everolimus group and four (10%) in the placebo group had adverse events requiring dose reduction or temporary interruption of treatment; most common were stomatitis (13 [17%] patients in everolimus group *vs* one [3%] patient in placebo group), mouth ulceration (six [8%] *vs* 0), pyrexia (five [6%] *vs* one [3%]), and pneumonia (four [5%] *vs* 0). No adverse events led to discontinuation from the study, and no patients died during the study.

In girls aged 13 years or older, three of eight in the everolimus group (aged 17 years, 19 years, and 19 years) and none of the five in the placebo group had secondary amenorrhoea lasting from 8 weeks to 14 months. Two cases resolved without intervention, and one resolved with progesterone.

Discussion

We noted a significant reduction in volume of subependymal giant cell astrocytomas associated with tuberous sclerosis complex in the everolimus group relative to the placebo group. Large astrocytomas are associated with increased morbidity and risk of hydocephalus and potential death,[29] so stabilisation or even slight reductions in tumour volume translate into clinical benefit, and the reductions noted in this trial are judged clinically significant. This result in a placebo-controlled, double-blind trial, provides confirmation of the findings of previous small studies and case reports[7,21,30,31] in which everolimus significantly reduced the tumour volume. The inclusion of a placebo group allowed the prospective comparison of efficacy and safety for the first time in this population. A placebo

group was judged necessary because no pharmacological treatments have been approved for subependymal giant cell astrocytomas associated with tuberous sclerosis complex (panel).

Important to assess long term is whether continuous everolimus is necessary to maintain the reduction in the total volume of subependymal giant cell astrocytomas. Regrowth of subependymal giant cell astrocytomas after discontinuation of everolimus was reported in the earlier open-label phase 1/2 trial.[21] The extension phase of our trial will provide data for long-term efficacy and safety that will help answer questions about the long-term effects of everolimus.

Analysis of change in seizure frequency was inconclusive because most patients had no seizures at baseline or at follow-up. Seizure frequency was evaluated as a secondary endpoint only and patients were selected for the trial on the basis of their need for intervention for progression of subependymal giant cell astrocytomas rather than presence of seizures.

Everolimus was associated with clinically meaningful increases in the time to progression of subependymal giant cell astrocytomas and skin lesion response rate compared with placebo. On the prespecified statistical analysis plan, formal significance could not be ascertained. However, if a Bonferroni approach, a more traditional means of controlling for multiplicity, had been used, the p values of 0·0002 for time to progression of subependymal giant cell astrocytomas and 0·0004 for best overall skin lesion response would have fallen to less than 0·025 and 0·0083, respectively, one-sided critical boundary. The benefit in time to progression of subependymal giant cell astrocytomas and skin lesion response rate is clinically relevant evidence of the efficacy of everolimus. Likewise, reduction or stabilisation of angiomyolipoma volume by everolimus is likely to have real clinical benefit by reducing the number of angiomyolipoma-related morbidities, such as risk of haemorrhage and chronic renal failure.

The safety profile of everolimus was consistent with that in the phase 1/2 study of everolimus in patients with tuberous sclerosis complex[21] and the overall safety profile in the paediatric setting[14] with the exception of secondary amenorrhoea in three of eight girls aged 13 years and older. This adverse event might have been a consequence of mTOR inhibition because data suggest that mTOR might play a part in energy sensing and the control of puberty onset.[22] A clear pattern was not noted in the three cases of amenorrhoea in our study in terms of risk factors, relation to study drug, or resolution patterns; however, continued vigilance is required. The EXIST-1 protocol was amended to add long-term assessment of potential effects of everolimus on growth, development, and sexual maturation in the paediatric population.

Although presence of a growing subependymal giant cell astrocytoma was the primary criterion for enrolment, everolimus resulted in clinically significant benefits not only with respect to reductions in volume of subependymal giant cell astrocytomas and delays in growth, but also reductions in comorbid skin lesions and kidney tumours. Other trials are in progress to investigate the effect of everolimus on intractable epilepsy and cognitive impairments associated with tuberous sclerosis complex. Our trial is now in the extension phase to assess whether the results can be safely maintained over a longer period. Our results support the use of everolimus for patients with subependymal giant cell astrocytomas associated with tuberous sclerosis. Furthermore, everolimus might represent a disease-modifying treatment for aspects of tuberous sclerosis other than subependymal giant cell astrocytomas.

Panel: Research in context

Systematic review

We searched PubMed for reports about clinical trials and case studies of patients with subependymal giant cell astrocytomas associated with tuberous sclerosis complex using the primary search terms "tuberous sclerosis complex," "subependymal giant cell astrocytoma," and "everolimus." Our search, which was not limited by date, did not identify any other placebo-controlled, randomised studies of everolimus in this patient population.

Interpretation

The results of this study confirm the effectiveness of inhibition of mammalian target of rapamycin complex 1 (mTORC1) with everolimus for tuberous sclerosis complex. mTORC1 inhibition could benefit other disorders with overactivation of this pathway, such as PTEN (phosphatase and tensin homologue) mutations, Cowden's syndrome, and Birt-Hogg-Dubé syndrome. Activation of autophagy by mTORC1 inhibitors is a potential treatment for other neurological disorders characterised by accumulation of pathogenic proteins such as Huntington's disease, Parkinson's disease, and collagen VI myopathies.

Contributors

PJdV was a member of the study steering committee and reviewed the literature. DNF, PJdV, VHW, JPF, GS, DL, and TS designed the study. TS was the team leader for scientists and physicians to undertake the trial in accordance with good clinical practice. DNF did the research and oversaw the data gathering. SS, EMB, MF, RK, MHK, JRF, JYW, EAT, JPF, GS, and SJ gathered the data. DNF, SS, EMB, MHK, JYW, GS, HC, DL, and SJ did the data analysis. SS, EMB, RK, OW, JYW, PC, PJdV, VHW, GS, DL, and SJ did the data interpretation. JRF and HC reviewed the data. JPF managed the study. DNF, OW, PJdV, VHW, GS, DL, and SJ wrote the report. SS edited and contributed to the rewriting of the report and was the primary investigator at the study site. EB, MF, RK, MHK, JRF, PC, and EAT were involved in patient recruitment or enrolment.

Conflicts of interest

JPF, GS, HC, DL, and TS are employees of Novartis. DNF, SS, EMB, MF, MHK, JYW, PJdV, and SJ are consultants for Novartis (including advisory boards), and have received travel payments, research funding, or speaker honoraria from Novartis. DNF has received compensation from various attorneys for legal work reviewing medical malpractice cases and occasionally gives expert testimony. SS and JYW have received honoraria from Lundbeck Pharmaceuticals. JYW serves on a professional advisory board for and receives research support from the Tuberous Sclerosis Alliance. PJdV has been a coprincipal investigator on research studies partly funded by Novartis Oncology. The other authors declare that they have no conflicts of interest.

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First Named Inventor/Applicant Name:	Heidi Lane
Filer:	Ann R. Pokalsky/Maggi Leone
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Pursuant to Applicant(s) duty of disclosure, the information listed on the attached form PTO/SB/08a is brought to the attention of the Examiner. A copy of the listed items is attached.

The citation of the listed items is not a representation that they constitute a complete or exhaustive listing of the relevant art or that the references are prior art. The items listed are submitted in good faith, but are not intended to a substitute for the Examiner's search. It is hoped, however, that in addition to apprising the Examiner of these particular items, they will assist in identifying fields of search and in making as full and complete a search as possible.

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[ ] This information disclosure statement is being filed within three (3) months of the filing date of this application.

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[ ] Please charge the **$130.00** petition fee to Deposit Account No. **04-1121**.

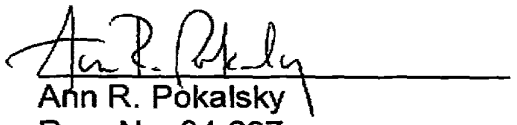
[X] Enclosed is the **$180.00** fee required by 37 C.F.R. § 1.17(p).

[ ] Please charge the **$180.00** fee required by 37 C.F.R. § 1.17(p) to Deposit Account No. **04-1121**.

[X] Please charge any deficiency as well as any other fee(s) which may become due under 37 C.F.R. § 1.16 and/or 1.17 at any time during the pendency of this application, or credit any overpayment of such fee(s) to Deposit Account **04-1121**. Also, in the event any extensions of time for responding are required for the pending application(s), please treat this paper as a petition to extend the time as required and charge Deposit Account No. **04-1121**.

Early and favorable consideration of the case is respectfully requested.

Respectfully submitted,



Ann R. Pokalsky
Reg. No. 34,697
Attorney for Applicant(s)

DILWORTH & BARRESE, LLP
1000 Woodbury Road, Suite 405
Woodbury, NY 11797
Phone: 516-228-8484
Facsimile: 516-228-8516

PTO/SB/08a (01-10)
Approved for use through 07/31/2012. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Not for submission under 37 CFR 1.99)

Application Number	13546686
Filing Date	2012-07-11
First Named Inventor	Lane et al.
Art Unit	1611
Examiner Name	KLINKEL, Kortney L.
Attorney Docket Number	167-62 CON III

U.S.PATENTS Remove

Examiner Initial*	Cite No	Patent Number	Kind Code[1]	Issue Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
	1					

If you wish to add additional U.S. Patent citation information please click the Add button. Add

U.S.PATENT APPLICATION PUBLICATIONS Remove

Examiner Initial*	Cite No	Publication Number	Kind Code[1]	Publication Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
	1					

If you wish to add additional U.S. Published Application citation information please click the Add button. Add

FOREIGN PATENT DOCUMENTS Remove

Examiner Initial*	Cite No	Foreign Document Number[3]	Country Code[2] i	Kind Code[4]	Publication Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear	T[5]
	1							☐

If you wish to add additional Foreign Patent Document citation information please click the Add button Add

NON-PATENT LITERATURE DOCUMENTS Remove

Examiner Initials*	Cite No	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc), date, pages(s), volume-issue number(s), publisher, city and/or country where published.	T[5]

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	13546686
	Filing Date	2012-07-11
	First Named Inventor	Lane et al.
	Art Unit	1611
	Examiner Name	KLINKEL, Kortney L.
	Attorney Docket Number	167-62 CON III

	1	HOUGHTON, Peter J., "Everolimus", Clin Cancer Res. 16(5) (2010) p 1-7.	☐

If you wish to add additional non-patent literature document citation information please click the Add button Add

EXAMINER SIGNATURE

Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through a citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

[1] See Kind Codes of USPTO Patent Documents at www.USPTO.GOV or MPEP 901.04. [2] Enter office that issued the document, by the two-letter code (WIPO Standard ST.3). [3] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. [4] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. [5] Applicant is to place a check mark here if English language translation is attached.

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	13546686
	Filing Date	2012-07-11
	First Named Inventor	Lane et al.
	Art Unit	1611
	Examiner Name	KLINKEL, Kortney L.
	Attorney Docket Number	167-62 CON III

CERTIFICATION STATEMENT

Please see 37 CFR 1.97 and 1.98 to make the appropriate selection(s):

☐ That each item of information contained in the information disclosure statement was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(1).

OR

☐ That no item of information contained in the information disclosure statement was cited in a communication from a foreign patent office in a counterpart foreign application, and, to the knowledge of the person signing the certification after making reasonable inquiry, no item of information contained in the information disclosure statement was known to any individual designated in 37 CFR 1.56(c) more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(2).

☐ See attached certification statement.

☐ The fee set forth in 37 CFR 1.17 (p) has been submitted herewith.

☒ A certification statement is not submitted herewith.

SIGNATURE

A signature of the applicant or representative is required in accordance with CFR 1.33, 10.18. Please see CFR 1.4(d) for the form of the signature.

Signature	/Ann R. Pokalsky/	Date (YYYY-MM-DD)	2014-05-05
Name/Print	Ann R. Pokalsky	Registration Number	34697

This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 1 hour to complete, including gathering, preparing and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether the Freedom of Information Act requires disclosure of these record s.

2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.

3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.

4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).

5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.

6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).

7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.

8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspections or an issued patent.

9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

PATENT
Attorney Docket: PAT031671-US-CNT03 (167-62 CON III)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s):	Lane et a.	Examiner:	Klinkel, Kortney L.
Serial No.:	13/546,686	Group Art Unit:	1611
Filed:	July 11, 2012	Dated:	May 5, 2014
For:	TREATMENT OF SOLID TUMORS WITH RAPAMYCIN DERIVATIVES	Confirmation No.:	8586

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

AMENDMENT TRANSMITTAL FORM

Sir:

Transmitted herewith is an amendment inthe above-identified application.

[ ] Small entity status of this application under 37 C.F.R. §1.9 and §1.27 has been established by a verified statement previously submitted.

[ ] A verified statement to establish small entity under 37 C.F.R. §1.9 and §.27 is enclosed.

[X] No additional fee is required.

For	Claims Remaining After Amendment	Highest No. Previously Paid For	Present Extra	Rate (Small Entity)	Addit. Fee	Rate (Large Entity)	Addit. Fee
TOTAL CLAIMS*	6	20	0	x **40.00**	$ 0.00	x **80.00**	$0.00
INDEPENDENT CLAIMS	1	3	0	x **210.00**	$0.00	x **420.00**	$0.00
[ ] First Presentation of Multiple Dep. Claim				**390.00**		**780.00**	$0.00

Total: 0.00

Certificate of EFS-Web Transmission

I hereby certify that this correspondence is being transmitted to the U.S. Patent and Trademark Office via the Office's electronic filing system.

Date: May 5, 2014 Name: Ann R. Pokalsky

[ ] Fees are to be charged to a credit card. A separate form PTO2038 is attached with credit card information.

[ ] Please charge Deposit Account No **04-1121** in the amount of $0.00.

[X] The Commissioner if hereby authorized to charge any additional fees, which may be required, or credit any overpayment to Deposit Account No.**04-1121**.

Respectfully submitted,

Ann R. Pokalsky
Reg. No. 34,697
Attorney for Applicant(s)

DILWORTH & BARRESE, LLP
1000 Woodbury Road, Suite 405
Woodbury, NY 11797
(516) 228-8484

Customer No. 28249

PTO/SB/06 (09-11)
Approved for use through 1/31/2014. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875	Application or Docket Number 13/546,686	Filing Date 07/11/2012	☐ To be Mailed

ENTITY: ☒ LARGE ☐ SMALL ☐ MICRO

APPLICATION AS FILED – PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA	RATE ($)	FEE ($)
☐ BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A	
☐ SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A	
☐ EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	minus 20 =	*	X $ =	
INDEPENDENT CLAIMS (37 CFR 1.16(h))	minus 3 =	*	X $ =	
☐ APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $310 ($155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).			
☐ MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))				
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL	

APPLICATION AS AMENDED – PART II

AMENDMENT

05/05/2014	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	RATE ($)	ADDITIONAL FEE ($)
Total (37 CFR 1.16(i))	* 6	Minus	** 20	= 0	X $80 =	0
Independent (37 CFR 1.16(h))	* 1	Minus	***3	= 0	X $420 =	0
☐ Application Size Fee (37 CFR 1.16(s))						
☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))						
					TOTAL ADD'L FEE	0

AMENDMENT

	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	RATE ($)	ADDITIONAL FEE ($)
Total (37 CFR 1.16(i))	*	Minus	**	=	X $ =	
Independent (37 CFR 1.16(h))	*	Minus	***	=	X $ =	
☐ Application Size Fee (37 CFR 1.16(s))						
☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))						
					TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".
The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.

LIE
/DANTE SMITH/

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

NOTICE OF ALLOWANCE AND FEE(S) DUE

28249 7590 05/19/2014
DILWORTH & BARRESE, LLP
1000 WOODBURY ROAD
SUITE 405
WOODBURY, NY 11797

EXAMINER
KLINKEL, KORTNEY L

ART UNIT	PAPER NUMBER
1611	

DATE MAILED: 05/19/2014

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
13/546,686	07/11/2012	Heidi Lane	031671-US-CNT03 167-62 C3	8586

TITLE OF INVENTION: TREATMENT OF SOLID TUMORS WITH RAPAMYCIN DERIVATIVES

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	UNDISCOUNTED	$960	$0	$0	$960	08/19/2014

THE APPLICATION IDENTIFIED ABOVE HAS BEEN EXAMINED AND IS ALLOWED FOR ISSUANCE AS A PATENT. PROSECUTION ON THE MERITS IS CLOSED. THIS NOTICE OF ALLOWANCE IS NOT A GRANT OF PATENT RIGHTS. THIS APPLICATION IS SUBJECT TO WITHDRAWAL FROM ISSUE AT THE INITIATIVE OF THE OFFICE OR UPON PETITION BY THE APPLICANT. SEE 37 CFR 1.313 AND MPEP 1308.

THE ISSUE FEE AND PUBLICATION FEE (IF REQUIRED) MUST BE PAID WITHIN THREE MONTHS FROM THE MAILING DATE OF THIS NOTICE OR THIS APPLICATION SHALL BE REGARDED AS ABANDONED. THIS STATUTORY PERIOD CANNOT BE EXTENDED. SEE 35 U.S.C. 151. THE ISSUE FEE DUE INDICATED ABOVE DOES NOT REFLECT A CREDIT FOR ANY PREVIOUSLY PAID ISSUE FEE IN THIS APPLICATION. IF AN ISSUE FEE HAS PREVIOUSLY BEEN PAID IN THIS APPLICATION (AS SHOWN ABOVE), THE RETURN OF PART B OF THIS FORM WILL BE CONSIDERED A REQUEST TO REAPPLY THE PREVIOUSLY PAID ISSUE FEE TOWARD THE ISSUE FEE NOW DUE.

HOW TO REPLY TO THIS NOTICE:

I. Review the ENTITY STATUS shown above. If the ENTITY STATUS is shown as SMALL or MICRO, verify whether entitlement to that entity status still applies.

If the ENTITY STATUS is the same as shown above, pay the TOTAL FEE(S) DUE shown above.

If the ENTITY STATUS is changed from that shown above, on PART B - FEE(S) TRANSMITTAL, complete section number 5 titled "Change in Entity Status (from status indicated above)".

For purposes of this notice, small entity fees are 1/2 the amount of undiscounted fees, and micro entity fees are 1/2 the amount of small entity fees.

II. PART B - FEE(S) TRANSMITTAL, or its equivalent, must be completed and returned to the United States Patent and Trademark Office (USPTO) with your ISSUE FEE and PUBLICATION FEE (if required). If you are charging the fee(s) to your deposit account, section "4b" of Part B - Fee(s) Transmittal should be completed and an extra copy of the form should be submitted. If an equivalent of Part B is filed, a request to reapply a previously paid issue fee must be clearly made, and delays in processing may occur due to the difficulty in recognizing the paper as an equivalent of Part B.

III. All communications regarding this application must give the application number. Please direct all communications prior to issuance to Mail Stop ISSUE FEE unless advised to the contrary.

IMPORTANT REMINDER: Utility patents issuing on applications filed on or after Dec. 12, 1980 may require payment of maintenance fees. It is patentee's responsibility to ensure timely payment of maintenance fees when due.

PART B - FEE(S) TRANSMITTAL

Complete and send this form, together with applicable fee(s), to: Mail **Mail Stop ISSUE FEE**
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450
or Fax (571)-273-2885

INSTRUCTIONS: This form should be used for transmitting the ISSUE FEE and PUBLICATION FEE (if required). Blocks 1 through 5 should be completed where appropriate. All further correspondence including the Patent, advance orders and notification of maintenance fees will be mailed to the current correspondence address as indicated unless corrected below or directed otherwise in Block 1, by (a) specifying a new correspondence address; and/or (b) indicating a separate "FEE ADDRESS" for maintenance fee notifications.

CURRENT CORRESPONDENCE ADDRESS (Note: Use Block 1 for any change of address)

28249 7590 05/19/2014
DILWORTH & BARRESE, LLP
1000 WOODBURY ROAD
SUITE 405
WOODBURY, NY 11797

Note: A certificate of mailing can only be used for domestic mailings of the Fee(s) Transmittal. This certificate cannot be used for any other accompanying papers. Each additional paper, such as an assignment or formal drawing, must have its own certificate of mailing or transmission.

Certificate of Mailing or Transmission
I hereby certify that this Fee(s) Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Mail Stop ISSUE FEE address above, or being facsimile transmitted to the USPTO (571) 273-2885, on the date indicated below.

(Depositor's name)
(Signature)
(Date)

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
13/546,686	07/11/2012	Heidi Lane	031671-US-CNT03 167-62 C3	8586

TITLE OF INVENTION: TREATMENT OF SOLID TUMORS WITH RAPAMYCIN DERIVATIVES

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	UNDISCOUNTED	$960	$0	$0	$960	08/19/2014

EXAMINER	ART UNIT	CLASS-SUBCLASS
KLINKEL, KORTNEY L	1611	514-291000

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363).
- ❑ Change of correspondence address (or Change of Correspondence Address form PTO/SB/122) attached.
- ❑ "Fee Address" indication (or "Fee Address" Indication form PTO/SB/47; Rev 03-02 or more recent) attached. **Use of a Customer Number is required.**

2. For printing on the patent front page, list
(1) The names of up to 3 registered patent attorneys or agents OR, alternatively,
(2) The name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed.
1 _____
2 _____
3 _____

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. If an assignee is identified below, the document has been filed for recordation as set forth in 37 CFR 3.11. Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE (B) RESIDENCE: (CITY and STATE OR COUNTRY)

Please check the appropriate assignee category or categories (will not be printed on the patent) : ❑ Individual ❑ Corporation or other private group entity ❑ Government

4a. The following fee(s) are submitted:
- ❑ Issue Fee
- ❑ Publication Fee (No small entity discount permitted)
- ❑ Advance Order - # of Copies _____

4b. Payment of Fee(s): **(Please first reapply any previously paid issue fee shown above)**
- ❑ A check is enclosed.
- ❑ Payment by credit card. Form PTO-2038 is attached.
- ❑ The Director is hereby authorized to charge the required fee(s), any deficiency, or credits any overpayment, to Deposit Account Number _____ (enclose an extra copy of this form).

5. **Change in Entity Status** (from status indicated above)
- ❑ Applicant certifying micro entity status. See 37 CFR 1.29 — NOTE: Absent a valid certification of Micro Entity Status (see forms PTO/SB/15A and 15B), issue fee payment in the micro entity amount will not be accepted at the risk of application abandonment.
- ❑ Applicant asserting small entity status. See 37 CFR 1.27 — NOTE: If the application was previously under micro entity status, checking this box will be taken to be a notification of loss of entitlement to micro entity status.
- ❑ Applicant changing to regular undiscounted fee status. — NOTE: Checking this box will be taken to be a notification of loss of entitlement to small or micro entity status, as applicable.

NOTE: This form must be signed in accordance with 37 CFR 1.31 and 1.33. See 37 CFR 1.4 for signature requirements and certifications.

Authorized Signature _____ Date _____

Typed or printed name _____ Registration No. _____

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
13/546,686	07/11/2012	Heidi Lane	031671-US-CNT03 167-62 C3	8586

28249 7590 05/19/2014

DILWORTH & BARRESE, LLP
1000 WOODBURY ROAD
SUITE 405
WOODBURY, NY 11797

EXAMINER	
KLINKEL, KORTNEY L	

ART UNIT	PAPER NUMBER
1611	

DATE MAILED: 05/19/2014

Determination of Patent Term Adjustment under 35 U.S.C. 154 (b)
(Applications filed on or after May 29, 2000)

The Office has discontinued providing a Patent Term Adjustment (PTA) calculation with the Notice of Allowance.

Section 1(h)(2) of the AIA Technical Corrections Act amended 35 U.S.C. 154(b)(3)(B)(i) to eliminate the requirement that the Office provide a patent term adjustment determination with the notice of allowance. See Revisions to Patent Term Adjustment, 78 Fed. Reg. 19416, 19417 (Apr. 1, 2013). Therefore, the Office is no longer providing an initial patent term adjustment determination with the notice of allowance. The Office will continue to provide a patent term adjustment determination with the Issue Notification Letter that is mailed to applicant approximately three weeks prior to the issue date of the patent, and will include the patent term adjustment on the patent. Any request for reconsideration of the patent term adjustment determination (or reinstatement of patent term adjustment) should follow the process outlined in 37 CFR 1.705.

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (571)-272-7702. Questions relating to issue and publication fee payments should be directed to the Customer Service Center of the Office of Patent Publication at 1-(888)-786-0101 or (571)-272-4200.

OMB Clearance and PRA Burden Statement for PTOL-85 Part B

The Paperwork Reduction Act (PRA) of 1995 requires Federal agencies to obtain Office of Management and Budget approval before requesting most types of information from the public. When OMB approves an agency request to collect information from the public, OMB (i) provides a valid OMB Control Number and expiration date for the agency to display on the instrument that will be used to collect the information and (ii) requires the agency to inform the public about the OMB Control Number's legal significance in accordance with 5 CFR 1320.5(b).

The information collected by PTOL-85 Part B is required by 37 CFR 1.311. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, Virginia 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, Virginia 22313-1450. Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

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5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
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7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Notice of Allowability	Application No. 13/546,686	Applicant(s) LANE ET AL.	
	Examiner Kortney L. Klinkel	Art Unit 1611	AIA (First Inventor to File) Status No

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address--

All claims being allowable, PROSECUTION ON THE MERITS IS (OR REMAINS) CLOSED in this application. If not included herewith (or previously mailed), a Notice of Allowance (PTOL-85) or other appropriate communication will be mailed in due course. **THIS NOTICE OF ALLOWABILITY IS NOT A GRANT OF PATENT RIGHTS.** This application is subject to withdrawal from issue at the initiative of the Office or upon petition by the applicant. See 37 CFR 1.313 and MPEP 1308.

1. ☒ This communication is responsive to *response and amendments 5/5/2014*.
   ☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on_____.
2. ☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.
3. ☒ The allowed claim(s) is/are *1 and 3-7*. As a result of the allowed claim(s), you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to PPHfeedback@uspto.gov .
4. ☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

   Certified copies:

   a) ☒ All b) ☐ Some *c) ☒ None of the:
   1. ☐ Certified copies of the priority documents have been received.
   2. ☒ Certified copies of the priority documents have been received in Application No. *10/468,520* .
   3. ☐ Copies of the certified copies of the priority documents have been received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

   * Certified copies not received: _____.

Applicant has THREE MONTHS FROM THE "MAILING DATE" of this communication to file a reply complying with the requirements noted below. Failure to timely comply will result in ABANDONMENT of this application.
THIS THREE-MONTH PERIOD IS NOT EXTENDABLE.

5. ☐ CORRECTED DRAWINGS (as "replacement sheets") must be submitted.
   ☐ including changes required by the attached Examiner's Amendment / Comment or in the Office action of Paper No./Mail Date _____.

   Identifying indicia such as the application number (see 37 CFR 1.84(c)) should be written on the drawings in the front (not the back) of each sheet. Replacement sheet(s) should be labeled as such in the header according to 37 CFR 1.121(d).
6. ☐ DEPOSIT OF and/or INFORMATION about the deposit of BIOLOGICAL MATERIAL must be submitted. Note the attached Examiner's comment regarding REQUIREMENT FOR THE DEPOSIT OF BIOLOGICAL MATERIAL.

Attachment(s)

1. ☐ Notice of References Cited (PTO-892)
2. ☒ Information Disclosure Statements (PTO/SB/08), Paper No./Mail Date _____
3. ☐ Examiner's Comment Regarding Requirement for Deposit of Biological Material
4. ☐ Interview Summary (PTO-413), Paper No./Mail Date _____ .
5. ☐ Examiner's Amendment/Comment
6. ☒ Examiner's Statement of Reasons for Allowance
7. ☐ Other _____.

/Kortney L. Klinkel/
Primary Examiner, Art Unit 1611

DETAILED ACTION

The present application is being examined under the pre-AIA first to invent provisions. Acknowledgement is made of the remarks/amendments dated 5/5/2014. Claim 1 was amended. Claim 2 stands cancelled. Claims 1 and 3-7 are pending.

REASONS FOR ALLOWANCE

The following is an examiner's statement of reasons for allowance: The amended claims directed to a method for inhibiting growth of non-malignant solid tumors of the brain in a subject consisting of administering everolimus of formula I are novel and non-obvious over the teachings of the prior art. The closest prior art, Geoeger et al. (of record) is directed to the study of rapamycin and the rapamycin derivative CCI-779 (which is a different derivative of rapamycin than instantly claimed and in a different class of derivatives than those in Cottens et al., see arguments pp. 7-8) in combination with additional chemotherapeutic agents in vitro on various malignant brain tumor cell lines. Geoeger et al. also notes that brain tumors *can* be exquisitely sensitive to rapamycin (p. 1527, second column, first full paragraph), but demonstrates that its activity is hit or miss. Rapamycin is effective against PNET/MB but not U251 cell lines suggesting unpredictability for monotherapy. The claimed monotherapy is enabled as evidenced by Franz et al. "Efficacy and safety of everolimus for subependymal giant cell astrocytomas associated with tuberous sclerosis complex (EXIST-I): a multicentere, randomized, placebo-controlled phase 3 trial" The Lancet January 12, 2013, Vol. 381, pages 125-132, work funded by Novartis Pharmaceuticals, copy submitted as Exhibit A

in the response dated 5/5/2014. Franz et al. show that everolimus stabilizes and reduces the size of non-malignant brain tumors relative to placebo.

Any comments considered necessary by applicant must be submitted no later than the payment of the issue fee and, to avoid processing delays, should preferably accompany the issue fee. Such submissions should be clearly labeled "Comments on Statement of Reasons for Allowance."

Conclusion

Claims 1 and 2-7 are allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Kortney L. Klinkel whose telephone number is (571)270-5239. The examiner can normally be reached on Monday-Friday 10 am to 7 pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Daniel Sullivan can be reached on (571)272-0779. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Kortney L. Klinkel/
Primary Examiner, Art Unit 1611

EAST Search History

EAST Search History (Prior Art)

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	45	("20020022054" | "20020098278" | "20030100886" | "20030100887" | "4885171" | "5066493" | "5194447" | "5206018" | "5362718" | "5922730" | "5985890" | "6333348" | "6569463" | "6617333" | "6641822").PN.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2014/05/09 12:03
L2	210	heidi near2 lane	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2014/05/09 12:03
L3	179	terence near2 o'reilly	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2014/05/09 12:03
L4	348	jeanette near2 wood	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2014/05/09 12:03
L5	611	L2 or L3 or L4	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2014/05/09 12:03
L6	6	"13546686".rlan. or ("13".src. and "546686".ap.)	US-PGPUB; USPAT; USOCR; DERWENT	OR	ON	2014/05/09 12:04
L7	6	"13546686".rlan. or ("13".src. and "546686".ap.)	US-PGPUB; USPAT; USOCR; DERWENT	OR	ON	2014/05/09 12:04
L8	6	"13546686".rlan. or ("13".src. and "546686".ap.)	US-PGPUB; USPAT; USOCR; DERWENT	OR	ON	2014/05/09 12:05
L10	5288	((A61K31/436).CPC.)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2014/05/09 12:41

C:\ Users\ kklinkel\ Documents\ EAST\ Workspaces\ 13546686.wsp

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	13546686	LANE ET AL.
	Examiner	Art Unit
	KORTNEY L KLINKEL	1611

CPC- SEARCHED		
Symbol	Date	Examiner
A61K 31/436, 45/06, 2300/00	1/31/2014	KLK
A61K31/436	5/9/2014	KLK

CPC COMBINATION SETS - SEARCHED		
Symbol	Date	Examiner

US CLASSIFICATION SEARCHED			
Class	Subclass	Date	Examiner

SEARCH NOTES		
Search Notes	Date	Examiner
Searched inventor names in PALM	9/28/2012	KLK
Searched EAST, see history attached	9/28/2012	KLK
Searched Pubmed, see history attached	9/28/2012	KLK
searched EAST	6/10/2013	KLK
searched keywords (carcinoma, brain carcinoma, etc) in google	6/10/2013	KLK
searched EAST, see history attached	1/31/2014	KLK
searched Pubmed, see history 2x attached	1/31/2014	KLK
searched EAST, see history attached	5/9/2014	KLK

INTERFERENCE SEARCH			
US Class/ CPC Symbol	US Subclass / CPC Group	Date	Examiner
A61K31/436		5/9/2014	KLK

	/K.L.K./ Primary Examiner.Art Unit 1611

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	13546686	LANE ET AL.
	Examiner	**Art Unit**
	KORTNEY L KLINKEL	1611

CPC						
Symbol					**Type**	**Version**
A61K		31	/	4196	I	2013-01-01
A61K		45	/	06	I	2013-01-01
A61K		31	/	475	I	2013-01-01
A61K		31	/	436	I	2013-01-01
A61K		31	/	439	I	2013-01-01
A61K		39	/	39558	F	2013-01-01
A61K		39	/	3955	I	2013-01-01
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CPC Combination Sets								
Symbol					**Type**	**Set**	**Ranking**	**Version**
A61K		2300	/	00	A	1	2	2013-01-01
A61K		31	/	436	I	1	1	2013-01-01

NONE		**Total Claims Allowed:**	
(Assistant Examiner)	(Date)	6	
/KORTNEY L KLINKEL/ Primary Examiner.Art Unit 1611	05/09/2014	O.G. Print Claim(s)	O.G. Print Figure
(Primary Examiner)	(Date)	1	----------

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	13546686	LANE ET AL.
	Examiner	**Art Unit**
	KORTNEY L KLINKEL	1611

US ORIGINAL CLASSIFICATION		INTERNATIONAL CLASSIFICATION												
CLASS	SUBCLASS	CLAIMED					NON-CLAIMED							
514	291	A	6	1	K	31 / 436 (2006.01.01)								
CROSS REFERENCE(S)		A	6	1	K	31 / 475 (2006.01.01)								
		A	6	1	P	35 / 00 (2006.01.01)								
CLASS	SUBCLASS (ONE SUBCLASS PER BLOCK)													

NONE		Total Claims Allowed:	
(Assistant Examiner)	(Date)	6	
/KORTNEY L KLINKEL/ Primary Examiner.Art Unit 1611	05/09/2014	O.G. Print Claim(s)	O.G. Print Figure
(Primary Examiner)	(Date)	1	----------

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	13546686	LANE ET AL.
	Examiner	**Art Unit**
	KORTNEY L KLINKEL	1611

☒ Claims renumbered in the same order as presented by applicant ☐ CPA ☐ T.D. ☐ R.1.47

Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original
1	1														
-	2														
2	3														
3	4														
4	5														
5	6														
6	7														

NONE (Assistant Examiner)	(Date)	**Total Claims Allowed:** 6	
/KORTNEY L KLINKEL/ Primary Examiner.Art Unit 1611 (Primary Examiner)	05/09/2014 (Date)	O.G. Print Claim(s) 1	O.G. Print Figure ---

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BIB DATA SHEET

CONFIRMATION NO. 8586

SERIAL NUMBER	FILING or 371(c) DATE RULE	CLASS	GROUP ART UNIT	ATTORNEY DOCKET NO.
13/546,686	07/11/2012	514	1611	031671-US-CNT03 167-62 C3

APPLICANTS

INVENTORS

Heidi Lane, Basel, SWITZERLAND;
Terence O'Reilly, Basel, SWITZERLAND;
Jeanette Marjorie Wood, Biel-Benken, SWITZERLAND;

**** CONTINUING DATA ***************************

This application is a CON of 10/468,520 01/27/2004 PAT 8410131
which is a 371 of PCT/EP02/01714 02/18/2002

**** FOREIGN APPLICATIONS ***************************

UNITED KINGDOM 0104072.4 02/19/2001
UNITED KINGDOM 0124957.2 10/17/2001

**** IF REQUIRED, FOREIGN FILING LICENSE GRANTED ****

07/24/2012

Foreign Priority claimed	☑ Yes ☐ No	☑ Met after Allowance	STATE OR COUNTRY	SHEETS DRAWINGS	TOTAL CLAIMS	INDEPENDENT CLAIMS
35 USC 119(a-d) conditions met	☑ Yes ☐ No	KLK	SWITZERLAND	0	7	1
Verified and Acknowledged	/KORTNEY L KLINKEL/ Examiner's Signature	Initials				

ADDRESS

DILWORTH & BARRESE, LLP
1000 WOODBURY ROAD
SUITE 405
WOODBURY, NY 11797
UNITED STATES

TITLE

TREATMENT OF SOLID TUMORS WITH RAPAMYCIN DERIVATIVES

FILING FEE RECEIVED		
1550	FEES: Authority has been given in Paper No._____ to charge/credit DEPOSIT ACCOUNT No._____ for following:	☐ All Fees ☐ 1.16 Fees (Filing) ☐ 1.17 Fees (Processing Ext. of time) ☐ 1.18 Fees (Issue) ☐ Other _____ ☐ Credit

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Not for submission under 37 CFR 1.99)

Application Number	13546686
Filing Date	2012-07-11
First Named Inventor	Lane et al.
Art Unit	1611
Examiner Name	KLINKEL, Kortney L.
Attorney Docket Number	167-62 CON III

U.S.PATENTS Remove

Examiner Initial*	Cite No	Patent Number	Kind Code[1]	Issue Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
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ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /KLK/

Receipt date: 05/05/2014	Application Number	13546686	13546686 - GAU: 1611
INFORMATION DISCLOSURE STATEMENT BY APPLICANT (**Not for submission under 37 CFR 1.99)**	Filing Date	2012-07-11	
	First Named Inventor	Lane et al.	
	Art Unit	1611	
	Examiner Name	KLINKEL, Kortney L.	
	Attorney Docket Number	167-62 CON III	

	1	HOUGHTON, Peter J., "Everolimus", Clin Cancer Res. 16(5) (2010) p 1-7.	☐

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EXAMINER SIGNATURE

Examiner Signature	/Kortney Klinkel/	Date Considered	05/09/2014

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[1] See Kind Codes of USPTO Patent Documents at www.USPTO.GOV or MPEP 901.04. [2] Enter office that issued the document, by the two-letter code (WIPO Standard ST.3). [3] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. [4] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. [5] Applicant is to place a check mark here if English language translation is attached.

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INSTRUCTIONS: This form should be used for transmitting the ISSUE FEE and PUBLICATION FEE (if required). Blocks 1 through 5 should be completed where appropriate. All further correspondence including the Patent, advance orders and notification of maintenance fees will be mailed to the current correspondence address as indicated unless corrected below or directed otherwise in Block 1, by (a) specifying a new correspondence address; and/or (b) indicating a separate "FEE ADDRESS" for maintenance fee notifications.

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28249 7590 05/19/2014
DILWORTH & BARRESE, LLP
1000 WOODBURY ROAD
SUITE 405
WOODBURY, NY 11797

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I hereby certify that this correspondence is being transmitted to the U.S. Patent and Trademark Office via the Office's electronic filing system

Ann R. Pokalsky	(Depositor's Name)
Ann R. Pokalsky	(Signature)
June 5, 2014	(Date)

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
13/546,686	07/11/2012	Heidi Lane	031671-US-CNT03 167-62 C3	8586

TITLE OF INVENTION: TREATMENT OF SOLID TUMORS WITH RAPAMYCIN DERIVATIVES

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	UNDISCOUNTED	$960	$0	$0	$960	08/19/2014

EXAMINER	ART UNIT	CLASS-SUBCLASS
KLINKEL, KORTNEY L	1611	514-291000

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363).
☐ Change of correspondence address (or Change of Correspondence Address form PTO/SB/122) attached.
☐ "Fee Address" indication (or "Fee Address" Indication form PTO/SB/47; Rev 03-02 or more recent) attached. **Use of a Customer Number is required.**

2. For printing on the patent front page, list
(1) The names of up to 3 registered patent attorneys or agents OR, alternatively,
(2) The name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed.
1 Ann R. Pokalsky
2 Dilworth & Barrese, LLP
3

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. If an assignee is identified below, the document has been filed for recordation as set forth in 37 CFR 3.11. Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE: Novartis Pharmaceuticals Corporation

(B) RESIDENCE: (CITY and STATE OR COUNTRY): East Hanover, New Jersey

Please check the appropriate assignee category or categories (will not be printed on the patent): ☐ Individual ☒ Corporation or other private group entity ☐ Government

4a. The following fee(s) are submitted:
☒ Issue Fee
☐ Publication Fee (No small entity discount permitted)
☐ Advance Order - # of Copies

4b. Payment of Fee(s): **(Please first reapply any previously paid issue fee shown above)**
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☒ Payment by credit card. Form PTO-2038 is attached.
☒ The Director is hereby authorized to charge the required fee(s), any deficiency, or credits any overpayment, to Deposit Account Number 041121 (enclose an extra copy of this form).

5. **Change in Entity Status** (from status indicated above)
☐ Applicant certifying micro entity status. See 37 CFR 1.29
NOTE: Absent a valid certification of Micro Entity Status (see forms PTO/SB/15A and 15B), issue fee payment in the micro entity amount will not be accepted at the risk of application abandonment.
☐ Applicant asserting small entity status. See 37 CFR 1.27
NOTE: If the application was previously under micro entity status, checking this box will be taken to be a notification of loss of entitlement to micro entity status.
☐ Applicant changing to regular undiscounted fee status.
NOTE: Checking this box will be taken to be a notification of loss of entitlement to small or micro entity status, as applicable.

NOTE: This form must be signed in accordance with 37 CFR 1.31 and 1.33. See 37 CFR 1.4 for signature requirements and certifications.

Authorized Signature Ann R. Pokalsky Date June 5, 2014
Typed or printed name Ann R. Pokalsky Registration No. 34,697

Electronic Patent Application Fee Transmittal

Application Number:	13546686
Filing Date:	11-Jul-2012
Title of Invention:	TREATMENT OF SOLID TUMORS WITH RAPAMYCIN DERIVATIVES
First Named Inventor/Applicant Name:	Heidi Lane
Filer:	Ann R. Pokalsky
Attorney Docket Number:	031671-US-CNT03 167-62 C3

Filed as Large Entity

Utility under 35 USC 111(a) Filing Fees

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Utility Appl Issue Fee	1501	1	960	960
Extension-of-Time:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Miscellaneous:				
			Total in USD ($)	**960**

Electronic Acknowledgement Receipt

EFS ID:	19217828
Application Number:	13546686
International Application Number:	
Confirmation Number:	8586
Title of Invention:	TREATMENT OF SOLID TUMORS WITH RAPAMYCIN DERIVATIVES
First Named Inventor/Applicant Name:	Heidi Lane
Customer Number:	28249
Filer:	Ann R. Pokalsky
Filer Authorized By:	
Attorney Docket Number:	031671-US-CNT03 167-62 C3
Receipt Date:	05-JUN-2014
Filing Date:	11-JUL-2012
Time Stamp:	11:06:06
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	Credit Card
Payment was successfully received in RAM	$960
RAM confirmation Number	7790
Deposit Account	041121
Authorized User	HARRISON, HELENE

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Charge any Additional Fees required under 37 C.F.R. Section 1.17 (Patent application and reexamination processing fees)

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Issue Fee Payment (PTO-85B)	Issue_Fee_Transmittal.pdf	568619 27d96e34f4ea1147c20eb3a6df1d4a8338cc7498	no	1
Warnings:					
Information:					
2	Fee Worksheet (SB06)	fee-info.pdf	30274 d8776e23e4846a490f0f87b91d397260a85f71e7	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	598893		

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New Applications Under 35 U.S.C. 111
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

Receipt date: 07/11/2012	Application Number	13546686 - GAU: 1611
INFORMATION DISCLOSURE STATEMENT BY APPLICANT (**Not for submission under 37 CFR 1.99)**	Filing Date	2012-07-11
	First Named Inventor	Lane et al.
	Art Unit	
	Examiner Name	
	Attorney Docket Number	031671-US-CNT03 (62 C 3)

	9	6569463		2003-05-27	PATEL et al.	
	10	6617333		2003-09-09	RABINDRAN et al.	
Change(s) applied to document, /C.C.B./ 6/13/2014	11	~~6641822~~ 6,641,811		2003-11-04	SUTHANTHIRAN et al.	

If you wish to add additional U.S. Patent citation information please click the Add button. Add

U.S.PATENT APPLICATION PUBLICATIONS Remove

Examiner Initial*	Cite No	Publication Number	Kind Code[1]	Publication Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
	1	20020022054		2002-02-21	SAWADA et al.	
	2	20020098278		2002-07-25	BATES et al.	
	3	20030100886		2003-05-29	SEGAL et al.	
	4	20030100887		2003-05-29	SCOTT et al.	

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ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /KLK/

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APPLICATION NO.	ISSUE DATE	PATENT NO.	ATTORNEY DOCKET NO.	CONFIRMATION NO.
13/546,686	07/15/2014	8778962	031671-US-CNT03 167-62 C3	8586

28249 7590 06/25/2014

DILWORTH & BARRESE, LLP
1000 WOODBURY ROAD
SUITE 405
WOODBURY, NY 11797

ISSUE NOTIFICATION

The projected patent number and issue date are specified above.

Determination of Patent Term Adjustment under 35 U.S.C. 154 (b)
(application filed on or after May 29, 2000)

The Patent Term Adjustment is 0 day(s). Any patent to issue from the above-identified application will include an indication of the adjustment on the front page.

If a Continued Prosecution Application (CPA) was filed in the above-identified application, the filing date that determines Patent Term Adjustment is the filing date of the most recent CPA.

Applicant will be able to obtain more detailed information by accessing the Patent Application Information Retrieval (PAIR) WEB site (http://pair.uspto.gov).

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (571)-272-7702. Questions relating to issue and publication fee payments should be directed to the Application Assistance Unit (AAU) of the Office of Data Management (ODM) at (571)-272-4200.

APPLICANT(s) (Please see PAIR WEB site http://pair.uspto.gov for additional applicants):

Heidi Lane, Basel, SWITZERLAND;
Terence O'Reilly, Basel, SWITZERLAND;
Jeanette Marjorie Wood, Biel-Benken, SWITZERLAND;

AO 120 (Rev. 08/10)

TO:	REPORT ON THE FILING OR DETERMINATION OF AN ACTION REGARDING A PATENT OR TRADEMARK
Mail Stop 8 Director of the U.S. Patent and Trademark Office P.O. Box 1450 Alexandria, VA 22313-1450	

In Compliance with 35 U.S.C. § 290 and/or 15 U.S.C. § 1116 you are hereby advised that a court action has been filed in the U.S. District Court for the District of Delaware on the following

☐ Trademarks or ☑ Patents. (☐ the patent action involves 35 U.S.C. § 292.):

DOCKET NO.	DATE FILED	U.S. DISTRICT COURT
	6/10/2016	for the District of Delaware

PLAINTIFF	DEFENDANT
NOVARTIS PHARMACEUTICALS CORPORATION and NOVARTIS AG	BRECKENRIDGE PHARMACEUTICAL, INC.

	PATENT OR TRADEMARK NO.	DATE OF PATENT OR TRADEMARK	HOLDER OF PATENT OR TRADEMARK
1	5,665,772	9/9/1997	Novartis AG
2	8,410,131	4/2/2013	Novartis Pharmaceuticals Corporation
3	8,778,962	7/15/2014	Novartis PhArmaceuticals Corporation
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In the above—entitled case, the following patent(s)/ trademark(s) have been included:

DATE INCLUDED	INCLUDED BY ☐ Amendment ☐ Answer ☐ Cross Bill ☐ Other Pleading	
PATENT OR TRADEMARK NO.	DATE OF PATENT OR TRADEMARK	HOLDER OF PATENT OR TRADEMARK
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In the above—entitled case, the following decision has been rendered or judgement issued:

DECISION/JUDGEMENT

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1:17-CV-54

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Mail Stop 8 Director of the U.S. Patent and Trademark Office P.O. Box 1450 Alexandria, VA 22313-1450	

In Compliance with 35 U.S.C. § 290 and/or 15 U.S.C. § 1116 you are hereby advised that a court action has been filed in the U.S. District Court for the Northern District of West Virginia on the following

☐ Trademarks or ☑ Patents. (☐ the patent action involves 35 U.S.C. § 292.):

DOCKET NO.	DATE FILED	U.S. DISTRICT COURT
	4/10/2017	for the Northern District of West Virginia

PLAINTIFF	DEFENDANT
Novartis Pharmaceuticals Corporation and Novartis AG	Mylan Pharmaceuticals Inc.

	PATENT OR TRADEMARK NO.	DATE OF PATENT OR TRADEMARK	HOLDER OF PATENT OR TRADEMARK
1	5,665,772	9/9/1997	Novartis AG
2	8,617,598	12/31/2013	Novartis AG
3	8,778,962	7/15/2014	Novartis Pharmaceuticals Corporation
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In the above—entitled case, the following patent(s)/ trademark(s) have been included:

DATE INCLUDED	INCLUDED BY ☐ Amendment ☐ Answer ☐ Cross Bill ☐ Other Pleading	
PATENT OR TRADEMARK NO.	DATE OF PATENT OR TRADEMARK	HOLDER OF PATENT OR TRADEMARK
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Mail Stop 8 Director of the U.S. Patent and Trademark Office P.O. Box 1450 Alexandria, VA 22313-1450	

In Compliance with 35 U.S.C. § 290 and/or 15 U.S.C. § 1116 you are hereby advised that a court action has been filed in the U.S. District Court for the District of Delaware on the following

☐ Trademarks or ☑ Patents. (☐ the patent action involves 35 U.S.C. § 292.):

DOCKET NO.	DATE FILED	U.S. DISTRICT COURT
	4/7/2017	for the District of Delaware

PLAINTIFF	DEFENDANT
Novartis Pharmaceuticals Corporation and Novartis AG	Mylan Pharmaceuticals Inc.

	PATENT OR TRADEMARK NO.	DATE OF PATENT OR TRADEMARK	HOLDER OF PATENT OR TRADEMARK
1	5,665,772	9/9/1997	Novartis AG
2	8,778,962	7/15/2014	Novartis Pharmaceuticals Corporation
3	8,617,598	12/31/2013	Novartis AG
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☐ Trademarks or ☑ Patents. (☐ the patent action involves 35 U.S.C. § 292.):

DOCKET NO.	DATE FILED	U.S. DISTRICT COURT
	4/7/2017	for the District of Delaware

PLAINTIFF	DEFENDANT
Novartis Pharmaceuticals Corporation and Novartis AG	Teva Pharmaceuticals USA, Inc.

	PATENT OR TRADEMARK NO.	DATE OF PATENT OR TRADEMARK	HOLDER OF PATENT OR TRADEMARK
1	9,006,224	4/14/2015	Novartis AG
2	8,410,131	4/2/2013	Novartis Pharmaceuticals Corporation
3	8,778,962	7/15/2014	Novartis Pharmaceuticals Corporation
4	8,436,010	5/7/2013	Novartis Pharmaceuticals Corporation
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In the above—entitled case, the following patent(s)/ trademark(s) have been included:

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☐ Trademarks or ☑ Patents. (☐ the patent action involves 35 U.S.C. § 292.):

DOCKET NO.	DATE FILED	U.S. DISTRICT COURT
	4/13/2017	for the District of Delaware

PLAINTIFF	DEFENDANT
Novartis Pharmaceuticals Corporation and Novartis AG	Breckenridge Pharmaceutical, Inc.

	PATENT OR TRADEMARK NO.	DATE OF PATENT OR TRADEMARK	HOLDER OF PATENT OR TRADEMARK
1	5,665,772	9/9/1997	Novartis AG
2	8,410,131	4/2/2013	Novartis Pharmaceuticals Corporation
3	8,778,962	7/15/2014	Novartis Pharmaceuticals Corporation
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☐ Trademarks or ☑ Patents. (☐ the patent action involves 35 U.S.C. § 292.):

DOCKET NO.	DATE FILED	U.S. DISTRICT COURT
17-389-RGA	4/7/2017	for the District of Delaware

PLAINTIFF	DEFENDANT
NOVARTIS PHARMACEUTICALS CORPORATION and NOVARTIS AG	MYLAN PHARMACEUTICALS INC.

	PATENT OR TRADEMARK NO.	DATE OF PATENT OR TRADEMARK	HOLDER OF PATENT OR TRADEMARK
1	5,665,772	9/9/1997	Novartis AG
2	US 8,778,962 B2	7/15/2014	Novartis Pharmaceuticals Corporation
3	US 8,617,598 B2	12/31/2013	Novartis AG
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In the above—entitled case, the following patent(s)/ trademark(s) have been included:

DATE INCLUDED	INCLUDED BY
4/28/2017	☐ Amendment ☑ Answer ☐ Cross Bill ☐ Other Pleading

	PATENT OR TRADEMARK NO.	DATE OF PATENT OR TRADEMARK	HOLDER OF PATENT OR TRADEMARK
1	US 7,297,703 B2	11/20/2007	Novartis AG
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