(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) **World Intellectual Property Organization**
International Bureau

(43) **International Publication Date**
30 April 2009 (30.04.2009)

PCT

(10) **International Publication Number**
WO 2009/054001 A1

(51) **International Patent Classification:**
A61K 39/395 (2006.01) *A61P 37/00* (2006.01)
A61K 31/436 (2006.01) *A61P 37/06* (2006.01)
A61K 39/00 (2006.01) *A61P 35/04* (2006.01)

(21) **International Application Number:**
PCT/IN2008/000688

(22) **International Filing Date:** 20 October 2008 (20.10.2008)

(25) **Filing Language:** English

(26) **Publication Language:** English

(30) **Priority Data:**
02373/CHE/2007 22 October 2007 (22.10.2007) IN

(71) **Applicants** *(for all designated States except US)*: **BIOCON LIMITED** [IN/IN]; 20th KM, Hosur Road, Electronic City, Bangalore, Karnataka 560 100 (IN). **CENTRO DE INMUNOLOGIA MOLECULAR** [IN/IN]; Calle 216 y 15 Atabey Playa, Ciudad, Havana City 11600, Cuba 11600 (IN).

(72) **Inventors; and**
(75) **Inventors/Applicants** *(for US only)*: **MELARKODE, Ramakrishnan** [IN/IN]; No.288, 4th Main, Rainbow Drive Layout, Sarjapur Road, Bangalore, Karnataka 560 038 (IN). **NAIR, Pradip** [IN/IN]; # 101, Sarangi Residency, Near and Opposite India Packers Limited, Kammanahalli, Off Bennerughatta Road, Bangalore, Karnataka 560 076 (IN). **CHIVUKULA, Indira, Venkata** [US/US]; 6, Nye Road, Medfield, Usa, Massachusetts 02052 (US). **CASIMIRO, Jose, Enrique, Montero** [CU/CU]; Calle 314 # 2916, Fraga, La Lisa, Ciudad, Habana, Cuba 17100 (CU). **RODRIGUEZ, Rolando, Perez** [CU/CU]; Juan Delgado 567, entre Avenida de Acosta, y O'farril, Vibora, Municipio 10 de, Octubre, Ciudad de la Habana, Cuba (CU).

(74) **Agents: RANGANATH, Shivakumar** et al.; K & S Partners, # 134, First Floor, 60 Ft. Domlur Road, Indiranagar, Bangalore, Karnataka 560 008 (IN).

(81) **Designated States** *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) **Designated States** *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL, NO, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:
— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*
— *of inventorship (Rule 4.17(iv))*

Published:
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

(54) **Title:** A PHARMACEUTICAL COMPOSITION AND A PROCESS THEREOF

(57) **Abstract:** A combination therapy is provided of an EGFR-inhibiting agent that competitively inhibits native EGF binding to the receptor and an immunosuppressant to treat a mammalian host, preferably human by administration of a therapeutically effective amount of the drug for the prophylaxis and/or treatment of tumors. The Immunosuppressant selected from the group comprising rapamycin, tacrolimus, everolimus, pimecrolimus in the free form or in the form of a pharmaceutically acceptable salt or solvates. Novel anti-EGFR drug-conjugates, and compositions optionally containing at least one pharmaceutically acceptable carrier for simultaneous use is also contemplated.

WO 2009/054001 A1

A PHARMACEUTICAL COMPOSITION AND A PROCESS THEREOF

FIELD OF THE INVENTION

The invention is directed towards novel synergistic combinations of anti-EGFR antibodies and immunosuppressive agents used to treat a mammalian host by administration of a therapeutically effective amount of the drug in the course of a treatment for cancer or suspected tumor cell growth. The drug combination that is cytotoxic to tumor cells comprises an EGFR inhibiting antibody that competitively inhibits native EGF ligand binding to the EGFR receptor. The combination also comprises atleast one further immunosuppressant selected from the group comprising rapamycin, tacrolimus, everolimus, pimecrolimus in the free form or in the form of a pharmaceutically acceptable salt. The combination drug used is preferably an immunosuppressive agent and most preferably rapamycin. Novel anti-EGFR-drug conjugates, and compositions optionally containing at least one pharmaceutically acceptable carrier for simultaneous use is contemplated. The above described agents may be used for the prophylaxis and/or treatment of tumors by increasing cytotoxicity of the tumor cells towards the described combinatorial therapeutic agent.

BACKGROUND AND PRIOR ART OF THE INVENTION:

Despite the swift progress made in the field of cancer therapies, cancer recurrence still remains a problem. Issues concerning relapse and non-responsiveness to chemotherapeutic agents in cancer patients are a major problem improperly addressed. Further, overdoses of targeted antibody therapies results in increased blood toxicities such as neutropenia and thrombocytopenia. There remains a need to identify new methods of treatment that would alleviate and/or prevent relapse of tumors, whilst keeping the effective drug doses as low as possible

Combination therapies are among the current experimental regimens focusing on combining chemotherapy with immunotherapy in an attempt to overcome such resistance issues. Combination therapy refers to simultaneous administration of two or more medications or treatments, such as chemotherapy (CT), radiation therapy (RT) or a drug, to treat a single disease. The rationale for this tactic is to use drugs that work on different signal transduction pathways, thereby increasing the likelihood that more cells

will be affected. For example, antibodies and RT treat cancer locally, while CT also kills metastatic cancer cells.

A new trend in combination therapy is the use of immunoconjugates, which are antibody-drug conjugates used to deliver drugs specifically to target cells, as opposed to being freely available systemically. These immunoconjugates show an augmented combined efficacy *in vivo*. It has been found that therapeutic monoclonal antibodies (mAb) might be more effective when conjugated with cytotoxic drugs. For example, the anti-HER2 mAb Herceptin-geldanamycin chemical conjugate showed a greater anti-tumor effect than Herceptin alone in tumor xenograft mouse models.

The present invention provides a rationally designed synergistic combination that offers clinically meaningful survival advantages over a single monotherapy. The combinations described in the invention helps achieve this clinical benefit with a manageable toxicity profile, making them more attractive options in the metastatic setting.

The invention is directed towards novel synergistic combinations of anti-EGFR antibodies antibodies binding to EGFR and immunosuppressive agents used to treat a mammalian host by administration of a therapeutically effective amount of the drug in the course of a treatment for cancer or suspected of tumor cell growth. The drug combination cytotoxic to tumor cells comprise an EGFR inhibiting agent that competitively inhibits EGF ligand binding to the EGFR receptor such as nimotuzumab (hR3). The combination also comprises at least one further immunosuppressive agent selected from the group comprising rapamycin, tacrolimus, everolimus, pimecrolimus in the free form or in the form of a pharmaceutically acceptable salt. The combination drug used is preferably an immunosuppressive agent and most preferably rapamycin.

hR3 is an anti-EGFR (epidermal growth factor receptor) humanized monoclonal antibody (mAb). EGFR activation stimulates cell proliferation, angiogenesis, dedifferentiation, and migration, as well as protects from apoptosis,which are all unregulated in cancer. hR3 binds to EGFR, preventing EGF-EGFR binding and inhibiting downstream signaling through the reduction of cytoplasmic tyrosine phosphorylation thereby blocking the signal transduction for cell proliferation. hR3 is the subject of product patents US 5,891,996 and US6,506,883.

Rapamycin is a macrolide antibiotic, produced from the bacterium *Streptomyces hygroscopicus*, with potent immunosuppressive and anti-proliferative effects, used mainly to prevent organ transplant rejection. Rapamycin binds to the cytosolic protein FK-binding protein 12 (FKBP12) and this complex inhibits the mammalian target of Rapamycin (mTOR) pathway, specifically the mTOR-mediated S6K1 and 4E-BP1 arms by directly binding to mTOR Complex-1 (mTORC1). One result of mTOR inhibition is selectively blocking the transcriptional activation of interleukin-2 (IL-2), making IL-2 unavailable to bind to IL-2 receptors expressed by lymphocytes, thereby blocking T- and B-cell activation. Rapamycin was first described in US 3,929,992.

Goudar R, et al (2005)[i] describe a combination therapy of inhibitors of epidermal growth factor receptor/vascular endothelial growth factor receptor 2 (AEE788) and the mammalian target of rapamycin (RAD001) everolimus offering improved glioblastoma tumor growth inhibition.

Buck E, et al (2006), describes rapamycin synergizing with the epidermal growth factor receptor inhibitor erlotinib in non-small-cell lung, pancreatic, colon and breast tumors.

Li D, et al (2007), describes rapamycin synergizing with the irreversible EFGR tyrosine kinase inhibitor HKI-272 in erlotinib-resistant murine lung adenocarcinomas.

The proposed combination of hR3 and rapamycin would be novel to the above approaches because it involves an anti-EGFR monoclonal antibody, as opposed to a small molecule, to be combined with rapamycin for an epidermoid carcinoma target. A monoclonal antibody has a significantly longer half-life than a small molecule. Hence the dosing and therapeutic method would be significantly different from a small molecule inhibitor of the EGFR tyrosine kinase domain.

The application WO 2004/056847 (US 2003/194403) discloses combination of an anti-EGFR monoclonal antibody like hR3 and immunosuppressive agents like rapamycin.

The advantage of the present invention over the cited prior art is the synergistic benefit achieved by the concurrent administration of the EGFR inhibiting agent and a chemotherapeutic drug such as rapamycin. It has now surprisingly been found that a

synergistic combination of hR3 and rapamycin shows increased cytoxicity towards tumors.

OBJECTIVES OF THE INVENTION:

The main objective of the present invention is to obtain a synergistic combination of a therapeutic drug comprising a therapeutically effective mixture of an anti-EGFR antibody and an immunosuppressant.

Another main objective of the present invention is to obtain a pharmaceutical composition for therapeutic or prophylactic treatment of cancer

Yet another main objective of the present invention is to obtain a process of manufacturing a pharmaceutical composition for therapeutic or prophylactic treatment of cancer comprising a therapeutically effective mixture of an anti-EGFR antibody and an immunosuppressant, comprising step of mixing anti-EGFR antibody and an immunosuppressant along with pharmaceutically acceptable excipients.

Still another main objective of the present invention is to obtain a method of detecting a presence of the EGFR antigen or a cell expressing EGFR comprising contacting the sample with the diagnostic composition of claim 13 to allow a formation of a complex and detecting the formation of the complex.

Still another main objective of the present invention is to obtain a kit comprising the diagnostic combination.

STATEMENT OF THE INVENTION:

Accordingly, the present invention relates to a synergistic combination of a therapeutic drug comprising a therapeutically effective mixture of an anti-EGFR antibody and an immunosuppressant; a pharmaceutical composition for therapeutic or prophylactic treatment of cancer comprising administration of a therapeutically effective mixture of an anti-EGFR antibody and an immunosuppressant and a pharmaceutically acceptable carrier; a process of manufacturing a pharmaceutical composition for therapeutic or prophylactic treatment of cancer comprising a therapeutically effective mixture of an anti-EGFR antibody and an immunosuppressant, comprising step of mixing anti-EGFR antibody and an immunosuppressant along with pharmaceutically acceptable excipients; a method of detecting a presence of the EGFR antigen or a cell expressing EGFR comprising contacting the sample with the diagnostic composition of claim 13 to

allow a formation of a complex and detecting the formation of the complex; and a kit comprising the diagnostic combination.

BRIEF DESCRIPTION OF THE FIGURES:

FIG 1: SRB Assay: Percentage cytotoxicity of A431 cells exposed for 72 hours to hR3 in combination with Rapamycin for a serially diluted concentration range from 5.2nM to 83nM for hR3 and 1.6nM to 25nM for Rapamycin.

FIG 2: MTS Assay: Percentage cytotoxicity of A431 cells exposed for 72 hours to hR3 in combination with Rapamycin for a serially diluted concentration range from 5.2nM to 83nM for hR3 and 1.6nM to 25nM for Rapamycin.

FIG 3: SRB Assay: Percentage cytotoxicity of A431 cells exposed for 72 hours to hR3 in combination with Rapamycin for a serially diluted concentration range from 2.6nM to 1328nM for hR3 and 0.78nM to 400nM for Rapamycin. The analyses of Figures 3-A and 3-B correspond to Figures 4-A and 4-B (below), respectively.

FIG 4: Bliss Independence Analysis of the hR3-Rapamycin combination

FIG 5: Western blot image composite. The blots show key signal transduction molecules downstream to hR3 and rapamycin and evaluates the combination of these drugs to these molecules. The figure also has quantification of the blot as compared to TFIID or Akt control. The values shown are relative to the Cells + EGF or Cells+EGF+DMSO control.

FIG 6: (A) Data illustrates the daily tumor volumes of the tumor xenografts in nude mice during the treatment course, starting from Day 12 (dose commencement) until Day 28 after cell implantation (date of sacrifice).

(B) Data expresses fold difference in tumor growth at Day 28 as compared to Day 12 for each drug arm.

Error bars express SD of mean tumor measurements for mice in each drug arm.

FIG7: Data represents relative values assigned according to mitotic activity frequency in H&E (Hematoxylin and eosin) stained tumor sections. Data shows a decrease in

mitotic activity in the 606.5 hR3 + 12.5nM Rapamycin combination group, as compared to the placebo group.

DETAILED DESCRIPTION OF THE INVENTION:

The present invention relates to a synergistic combination of a therapeutic drug comprising a therapeutically effective mixture of an anti-EGFR antibody and an immunosuppressant.

In another embodiment of the present invention the antibody competitively inhibits native EGF ligand binding to the EGFR receptor.

In yet another embodiment of the present invention, the anti-EGFR antibody is hR3.

In still another embodiment of the present invention, the immunosuppressant is selected from the group comprising macrolides selected from rapamycin, tacrolimus, everolimus, pimecrolimus or cyclosporins.

In still another embodiment of the present invention, the anti-EGFR antibody is conjugated to the immunosuppressant.

In still another embodiment of the present invention, the drug elicits increased cytoxicity against tumor cells.

In still another embodiment of the present invention, the combination can be administered in the form of solid, semi-solid, or liquid, tablets, pills, powders, capsules, gels, ointments, liquids, suspensions or aerosols.

The present invention relates to a pharmaceutical composition for therapeutic or prophylactic treatment of cancer comprising administration of a therapeutically effective mixture of an anti-EGFR antibody and an immunosuppressant and a pharmaceutically acceptable carrier.

In another embodiment of the present invention, wherein the therapeutically effective mixture of an anti-EGFR antibody and an immunosuppressant further comprises pharmaceutically acceptable excipients.

In yet another embodiment of the present invention the composition is administered intravenously, intramuscularly, subcutaneously, intrasynovially, by infusion, sublingually, transdermally, orally, topically or by inhalation.

In still another embodiment of the present invention the combination drug is used in the treatment of breast cancer, bladder cancer, colon cancer, esophageal cancer, pancreatic

cancer, stomach cancer, lung cancer, uterine cancer, cervical cancer, kidney cancer, ovarian cancer, prostrate cancer, renal cancer and head and neck cancer.

The present invention relates to a process of manufacturing a pharmaceutical composition for therapeutic or prophylactic treatment of cancer comprising a therapeutically effective mixture of an anti-EGFR antibody and an immunosuppressant, comprising step of mixing anti-EGFR antibody and an immunosuppressant along with pharmaceutically acceptable excipients.

In another embodiment of the present invention, the immunosuppressant is selected from the group comprising macrolides selected from rapamycin, tacrolimus, everolimus, pimecrolimus or cyclosporins.

The present invention relates to the use of the combination of an anti-EGFR antibody and an immunosuppressant according to any of preceding claims for the preparation of a diagnostic composition for the immunohistological detection of cancers.

In another embodiment of the present invention, a method of detecting a presence of the EGFR antigen or a cell expressing EGFR comprising contacting the sample with the diagnostic composition of claim 13 to allow a formation of a complex and detecting the formation of the complex.

The present invention relates to a kit comprising the diagnostic combination.

This invention is directed towards novel synergistic combinations of EGFR inhibiting agents specific to the EGFR receptor and immunosuppressive drugs or agents, which can be used to treat a mammalian host, usually a human, suffering from cancer by administering the combination in a therapeutically-effective amount exhibiting cytotoxicity.

Preferred EGFR inhibiting agents are molecules which can competitively inhibit the binding of the native ligands for the receptor, the binding of which elicits signal transduction pathways that regulate cell proliferation, differentiation and apoptosis.

Preferred immunosuppressive agents may be selected from the group comprising rapamycin, tacrolimus, everolimus, pimecrolimus in the free form or in the form of a pharmaceutically acceptable salt. The combination drug used is preferably an immunosuppressive agent and most preferably rapamycin.

The effective dosage of each of the combination partners employed in the method of the invention may vary depending on the particular compound or pharmaceutical composition employed, the mode of administration, the severity of the condition being treated.

Another aspect of the present invention relates to anti-EGFR drug- conjugates, and compositions optionally containing at least one pharmaceutically acceptable carrier for simultaneous use.

Other objects and advantages of the present invention will be more fully apparent to those of ordinary skill in art, in light of the ensuing disclosure and appended claims.

Another aspect of the present invention relates to anti-EGFR drug- conjugates, and compositions optionally containing at least one pharmaceutically acceptable carrier for simultaneous use.

The synergistic drug combination of the present invention comprises at least two components, an immunosuppressant drug or agent and an EGFR inhibiting molecule that is characterized as a tyrosine kinase inhibitors displaying cytoxicity towards tumor cells.

DEFINITION OF TERMS:

The present invention provides novel combinatorial therapies for treatment of cancer and methods as set forth within this specification. In general, all technical and scientific terms used herein have the same meaning as commonly understood to one of ordinary skill in the art to which this invention belongs, unless clearly indicated otherwise. For clarification, listed below are definitions for certain terms used herein to describe the present invention. These definitions apply to the terms as they are used throughout this specification, unless otherwise clearly indicated.

"EGF receptor" is a transmembrane glycoprotein which has a molecular weight of 175 kDa and is found on many epithelial cell types. It is activated by at least three ligands, EGF (epidermal growth factor), TGF-.alpha. (transforming growth factor alpha) and amphiregulin. Both EGF and TGF-.alpha. have been demonstrated to bind to EGFR and lead to cellular proliferation and tumor growth.

"EGFR inhibiting agent" may be any monoclonal antibody directed against the epidermal growth factor receptor (EGFR) with potential antineoplastic activity. The antibody binds to the extracellular domain of EGFR, thereby preventing the activation and subsequent dimerization of the receptor; the decrease in receptor activation and dimerization that may result in signal transduction inhibition and anti-proliferative effects. Preferably the anti-EGFR agent is Nimotuzumab that binds to and inhibits EGFR, resulting in growth inhibition of tumor cells that overexpress EGFR. The antibodies may be murine, chimeric or humanized.

"hR3" is an anti-EGFR (epidermal growth factor receptor) humanized monoclonal antibody (mAb). EGFR activation stimulates cell proliferation, angiogenesis, dedifferentiation, and migration, as well as protects from apoptosis,which are all unregulated in cancer. hR3 binds to EGFR, preventing EGF-EGFR binding and inhibiting downstream signaling through the reduction of cytoplasmic tyrosine phosphorylation thereby blocking the signal transduction for cell proliferation.

The term "immunosuppressant" as used herein is meant to include compounds or compositions which suppress immune responses. Exemplary immunosuppressants include macrolides and cyclosporins, in particular macrolides (such as pimecrolimus, tacrolimus and sirolimus) and cyclosporins (such as phosphorine A) in the free form or in the form of a pharmaceutically acceptable salt or solvates. In one embodiment of the invention the immunosuppressant is a macrolide, in particular rapamycin.

The expression "synergistic" or "synergistically effective amounts" refers to amounts of each component of the combination which together are effective in producing more than an additive effect of the components alone. Therefore, the combination's effect is greater than the sum of the effects of the two components.

According to one aspect of the invention the anti-EGFR antibody is conjugated to the immunosuppressant. The immunosuppressant is preferably hR3 in context of the present invention.

Preferred EGFR inhibiting agents are molecules which can competitively inhibit the binding of the native ligands for the receptor, the binding of which elicits signal transduction pathways that regulate cell proliferation, differentiation and apoptosis.

The immunosuppressive drugs or agents contemplated for use in this invention are selected from the group comprising rapamycin, tacrolimus, everolimus, pimecrolimus in the free form or in the form of a pharmaceutically acceptable salt. The combination drug used is preferably an immunosuppressive agent and most preferably rapamycin.

Those of skill will readily appreciate that dose levels can vary as a function of the specific compound, the severity of the symptoms and the susceptibility of the subject to side effects. Preferred dosages for a given compound are readily determinable by those of skill in the art by a variety of means. A preferred means is to measure the physiological potency of a given compound.

In a further embodiment the invention provides for a kit comprising a combination of antibodies comprising anti-EGFR antibody and an immunosuppressant or a diagnostic composition of the invention. Advantageously, the kit of the present invention further comprises, optionally (a) buffer (s), storage solutions and/or remaining reagents or materials required for the conduct of medical, scientific or diagnostic assays and purposes. Furthermore, parts of the kit of the invention can be packaged individually in vials or bottles or in combination in containers or multicontainer units.

The kit of the present invention may be advantageously used, inter alia, for carrying out the (diagnostic) methods of the invention and could be employed in a variety of applications referred herein, e. g. , as diagnostic kits, as research tools or medical tools. Additionally, the kit of the invention may contain means for detection suitable for scientific, medical and/or diagnostic purposes. The manufacture of the kits follows preferably standard procedures which are known to the person skilled in the art.

The susceptibility of a particular tumor cell to killing with the combined therapy may be determined by in vitro testing. For example, a culture of the tumor cell is combined with combinations at varying concentrations for a period of time sufficient to allow the active agents to inhibit cell proliferation. For in vitro testing, cultured cells from a biopsy sample of the tumor may be used.

The compositions herein are preferably administered to human patients via oral, intravenous or parenteral administrations and other systemic forms. The pharmaceutical formulations or compositions of this invention may be in the dosage form of solid,

semi-solid, or liquid such as, e.g., tablets, pills, powders, capsules, gels, ointments, liquids, suspensions, aerosols or the like. Preferably the compositions are administered in unit dosage forms suitable for single administration of precise dosage amounts. The compositions may also include, depending on the formulation desired, pharmaceutically-acceptable, non-toxic carriers or diluents, which are defined as vehicles commonly used to formulate pharmaceutical compositions for animal or human administration.

The EGFR inhibiting agent and the immunosuppressant may be administered alone or in combination with adjuvants that enhance stability of the ingredients, facilitate administration of pharmaceutical compositions containing them in certain embodiments, provide increased dissolution or dispersion, increase activity, provide adjunct therapy, and the like, including other active ingredients that may further lower toxic dosage levels of the immunosuppressants.

According to this invention, the components of the therapy and pharmaceutical compositions containing them may be administered to a patient in any conventional manner and in any pharmaceutically acceptable dosage form, including, but not limited to, intravenously, intramuscularly, subcutaneously, intrasynovially, by infusion, sublingually, transdermally, orally, topically or by inhalation. Preferred modes of administration are oral and intravenous.

The total amount of therapeutic compound, hR3 + Rapamycin combination as per the instant invention to be used can be determined by those skilled in the art. The amount of therapuetic agent is an amount effective to accomplish the purpose of the particular active agents. The amount in the composition is a therapeutically effective dose, i.e., a pharmacologically or biologically effective amount. However, the amount can be less than a pharmacologically or biologically effective amount when the composition is used in a dosage unit form, such as a capsule, a tablet or a liquid, because the dosage unit form may contain a multiplicity of delivery agent/biologically or chemically active agent compositions or may contain a divided pharmacologically or biologically effective amount. The total effective amounts can then be administered in cumulative units containing, in total, pharmacologically or biologically or chemically active amounts of biologically or pharmacologically active agent.

The composition may include one or more adjuvants, one or more carriers, one or more excipients, one or more stabilizers, one or more imaging reagents, one or more effectors; one or more photodynamic agents; and/or physiologically acceptable saline.

The composition of the invention can additionally comprise inert constituents including carriers, diluents, fillers, salts, and other materials well-known in the art, the selection of which depends upon the particular purpose to be achieved and the properties of such additives which can be readily determined by one skilled in the art.

According to one aspect of the invention, the composition includes pharmaceutically acceptable carriers. Pharmaceutically accepted carriers include but are not limited to saline, sterile water, phosphate buffered saline, and the like. Other buffering agents, dispersing agents, and inert non-toxic substances suitable for delivery to a patient may be included in the compositions of the present invention. The compositions may be solutions suitable for administration, and are typically sterile and free of undesirable particulate matter.

The compositions may be sterilized by conventional sterilization techniques. It may be desirable to add a preservative to the formulation of the present invention.

It is contemplated that the polypeptide or antibodies of the present invention may be used to treat a mammal. In one embodiment, the antibody is administered to a nonhuman mammal for the purposes of obtaining preclinical data, for example. Exemplary nonhuman mammals to be treated include nonhuman primates, dogs, cats, rodents and other mammals in which preclinical studies are performed. Such mammals may be established animal models for a disease to be treated with the antibody or may be used to study toxicity of the antibody of interest. In each of these embodiments, dose escalation studies may be performed on the mammal to ascertain the optimal dosage.

It is well known in the medical arts that dosages for any one patient depend on many factors, including the general health, sex, weight, body surface area, and age of the patient, as well as the particular compound to be administered, the time and route of administration, and other drugs being administered concurrently.

As used herein, "an effective amount" means an amount required to achieve a desired end result. The amount required to achieve the desired end result will depend on the

nature of the disease or disorder being treated, and can be determined by standard clinical techniques. In addition, in vitro assays may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed will also depend on the route of administration and the seriousness of the disease or disorder, and should be decided according to the judgment of the practitioner and each subject's circumstances. Effective doses may be extrapolated from dose-response curves derived from in vitro or animal model test systems.

The detailed components of the combination may be characterized in several ways which will become more apparent from the description which follows. The following examples are offered by way of illustration and shall not be construed as being limitations on the scope or spirit of the instant invention.

EXAMPLE 1:

Sulforhodamine B (SRB) Colorimetric Assay

The cell line A431 was maintained in Dulbecco's modified eagle medium (DMEM) containing 10% FBS, 1% Penicillin-Streptomycin, and 20mM HEPES. The principle of this assay is based on the ability of the protein dye sulforhodamine B to bind electrostatically and pH dependently on amino acid residues of trichloroacetic acid (TCA)-fixed cells. Under mild acidic conditions SRB binds to the protein of cells and under mild basic conditions it can be eluted from cells for measurement. Results of the SRB assay are linear with respect to cell number and cellular protein amount.

A431 (human epidermoid carcinoma cells overexpressing EGFR) were seeded at a concentration of 5,000 cells per well in five 96-well flat-bottom plates, leaving twelve wells per plate without cells as a background reference. Plates were incubated in a humidified CO_2 incubator at 37°C for 24 hours. One 96-well plate was fixed using 10% TCA to be used as the 0-hour baseline value. Serial dilutions of Rapamycin (R) and hR3 were performed using 96-well round-bottom plates. Control per plate: twelve wells as background reference and twelve wells as negative controls. Experimental per plate (in triplicates): hR3 serially diluted from 83nM to 5.19nM, Rapamycin serially diluted from 25nM to 1.56nM, 83nM hR3 combined with 25nM to 1.56nM Rapamycin, and 25nM Rapamycin combined with 83nM to 5.19nM hR3. The spent medium was

tapped off from each plate and the total volume from each round-bottom plate was transferred to the flat-bottom plate containing cells. The final volume per well was 200μL. Plates were placed in a humidified CO_2 incubator at 37°C for 48 and 72 hours, with one hR3 and Rapamycin plate fixed at each time point. After fixation, all plates were stained using 0.4% SRB, washed with 1% acetic acid , eluted using 10mM unbuffered Tris base, and read at 570nm in an ELISA plate reader.

As depicted in Figure 1-a, R + 83 hR3 shows a significantly higher percentage of cytotoxicity as compared with R alone. In Figure 1-b, hR3 + 25 R also shows significantly higher cytotoxicity as compared with hR3 alone. Data suggests that hR3 combined with R has an amplified cytotoxic effect over hR3 or R alone.

EXAMPLE 2:

CellTiter 96® Aqueous Non-Radioactive Cell Proliferation Assay (Promega Corp., Madison WI)

The cell line A431 was maintained in DMEM containing 10% FBS, 1% Penicillin-Streptomycin, and 20mM HEPES. This colorimetric assay uses a soluble form of MTT, known as MTS, to determine the cell viability in a sample. Metabolically active cells bioreduce MTS into a soluble formazan product, so this conversion is directly related to the number of viable cells. It is the absorbance of the formazan product that is measured spectrophotometrically.

The cell line A431 was maintained in DMEM containing 10% FBS, 1% Penicillin-Streptomycin, and 20mM HEPES. This colorimetric assay uses a soluble form of MTT, known as MTS, to determine the cell viability in a sample. Metabolically active cells bioreduce MTS into a soluble formazan product, so this conversion is directly related to the number of viable cells. It is the absorbance of the formazan product that is measured spectrophotometrically.

A431 cells were seeded at a concentration of 10,000 cells per well in four 96-well flat-bottom plates, leaving twelve wells per plate without cells as a background reference. Plates were incubated in a humidified CO_2 incubator at 37°C for 24 hours. The identical plate set-up and dilutions as the SRB assay were used, but in this MTS-based assay, the total volume per well was 100μL. Plates were incubated in a humidified CO_2

values normalized. Here, Rapamycin alone displays greater cytotoxicity than hR3 in its lower doses and begins to show similar cytotoxicity as the dose concentration increases. The Bliss curve shows the dose response of R + hR3 if the combination effect was exactly additive. The combinations of varying hR3 with constant R (Hv / Rc) and varying R with constant hR3 (Rv / Hc) both show dose-response curves above the Bliss curve, indicating that R + hR3 have a synergistic cytotoxic effect. In lower doses Rv / Hc shows greater synergy than Hv / Rc, but as the concentration of hR3 increases both combinations show similar synergism.

EXAMPLE 4:

Preparation of Protein Lysates and Western Blotting

Cell extracts were prepared by RadioImmuno Precipitation Assay (RIPA) Buffer containing PMSF, protease inhibitor cocktail and sodium orthovanadate (sc-24948, Santa Cruz Biotechnology, Santa Cruz, CA). The soluble protein concentration was determined by the Bradford method as a macro-bovine serum albumin assay (#105570, Bangalore Genei, Bangalore, India). Protein immuno-detection was done by electrophoretic transfer of 10% SDS-PAGE separated proteins to Polyvinylidene Fluoride (PVDF #162-0177, Biorad Laboratories,Hercules,CA), incubation with primary/secondary antibody, and chemiluminescent substrate ECL plus (RPN 2132,GE Healthcare, Buckinghamshire,UK). The primary antibodies included phospho-Tyrosine[#9411] (pTYR), total EGFR[#2232], phospho-Akt(S473)[#9271] (pAKT), Akt pan11E7[#4685], phospho-MAPK (p42/p44)[#9101] (pMAPK), and phospho-Stat3(Y705)[#9131] (pSTAT3), mTOR[#2972], phosphor-S6 Ribosomal Protein(S235/236) [#2211] (pS6RP). All these primary antibodies were obtained from Cell Signaling Technologies (Danvers, MA). TFIID (TBP) (SI-1) [sc-273] was used as loading control which was obtained from Santa Cruz Biotechnology (Santa Cruz, CA).

For analysis of the effects of Rapamycin and hR3 on the phosphorylation of downstream signaling proteins, A431 cells were seeded at $1x10^6$ cells per well in a 6-well plate in DMEM 10%FBS, 1% Penicillin-Streptomycin, 20mM HEPES. Plates were incubated in a humidified CO_2 incubator at 37°C for 24 hours. Following which they were washed once with 2mL 1XDPBS (Gibco #14190). Subsequently the following drug treatments were done in DMEM containing 1% FBS, 1% Penicillin-

Streptomycin, 20mM HEPES: cells alone; cells + EGF; cells + T1h + EGF; cells + Rapamycin 25nM + EGF; cells + Rapamycin 1.56nM+ EGF; cells + hR3 12.5ug/mL + EGF; cells + hR3 3.12ug/mL+ EGF; cells + hR3 1.56ug/mL + EGF; cells +DMSO+EGF; cells + hR3 12.5ug/mL+Rapamycin25nM+EGF; cells + hR3 12.5ug/mL+Rapamycin 1.56nM+EGF; cells + hR3 3.12ug/mL+Rapamycin 25nM+EGF; cells + hR3 3.12ug/mL+Rapamycin1.56nM+EGF; cells + hR3 1.56ug/mL+Rapamycin 25nM+EGF; cells + hR3 1.56ug/mL+Rapamycin 1.56nM+EGF. T1h a non-specific IgG antibody was used here as a negative control. Plates were incubated in a humidified CO_2 incubator at 37°C for an additional 24 hours without EGF. Subsequently 10ng/mL of EGF was added and kept in humified CO_2 incubator for 10mins to stimulate the cells. The medium was removed, the plate wells were washed once with 1X DPBS and cells were lysed as previously described. The total protein in the lysate was estimated by Bradfords method as mentioned above. 50ug of total protein was loaded and separated using 10%SDS-PAGE and it was transferred to a PVDF membrane by a overnight wet transfer method at 4^0C. The membrane was blocked with 3% Bovine Serum Albumin (# RM 3151, Himedia Laboratories,Mumbai,India) for one hour at room temperature. The blots were then incubated overnight at 4^0C with respective primary antibody diluted as per the manufacturer's dilution recommendation. Subsequently the blots were washed thrice with 1X Tris buffered saline-Tween20 (TBS-T). The secondary antibody was added and incubated for one hour at room temperature. The blots were washed again in 1X TBS-T and to the membrane ECL plus reagent was added. The blots were exposed to scientific imaging film (Biomax^{TM} MS film (#829 4985 Kodak, Rochester,NY) and the film was developed using hyperprocessor (SRX-101A, GE Amersham Biosciences). The blots were quantified using Alpha view software (Alpha Innotech, San Leandro, CA).

Figure 5 shows a relative expression of proteins downstream to hR3 and rapamycin by western blotting. The samples 1-8 have been normalized to control 3 (cells + T1h + EGF) and samples 9-15 have been normalized to the sample 9 (cells + DMSO + EGF). The numbers below the blot are relative values to their respective controls. TFIID values were observed to be almost equivalent and hence not mentioned. The proteins like pTyr (170kDa), pMAPK and pSTAT3 that are downstream effectors of EGFR

were downregulated by BioMab EGFR (hR3) while, rapamycin treatment did not have any effect on these proteins except for pTyr at the highest concentration of rapamycin. The combination of rapamycin (25nM) and hR3 (12.5 and 3.12 μg/ml) showed a significant reduction of phosphorylated downstream molecules including pTyr, pMAPK and pSTAT3. PS6RP protein which is downstream to mTOR pathway was affected by rapamycin treatment especially with 25nM of rapamycin and remains unaffected with hR3 treatment. pAKT expression was not affected much at the time point of analysis in this assay. Overall, the study proved that at certain concentrations the drug combination suppresses pMAPK and pTyr expression better than the individual drugs alone. The inhibition with pSTAT3 is sustained.

EXAMPLE 5:

Study of drug combination in a sc-tumor xenograft model in nude mice

Each 9 week-old female BALB/c nude mouse was implanted with $5x10^6$ A431 epidermoid carcinoma cells in a single s.c. site on the left flank. Tumors were allowed to grow to at least 200 mm^3, at which time the animals were sorted into treatment groups of six animals per group based on even distribution of body weight and marked on the tail-base for identification. Tumor volumes and clinical observations were measured daily, and body weights were determined weekly. The tumor volume was determined by measuring in three directions with vernier calipers and calculated using the following formula: tumor volume = $4/3\pi(radius^3)$, with radius determined using averaged length and width measurements. hR3 was prepared in PBS and Rapamycin was prepared in DMSO as separate injection solutions. Six doses were administered over a two-week period, given by i.p. injection using an insulin syringe. hR3 was introduced first, followed by Rapamycin one hour later. All control animals were dosed with equal volumes of the vehicles. Treatment combinations were as follows: 12.5nM Rapamycin, 2.5nM Rapamycin, 1213nM hR3, 606.5nM hR3, 121.3nM hR3, 121.3nM hR3 + 2.5nM Rapamycin, 606.5nM hR3 + 12.5nM Rapamycin, 121.3nM hR3 + 12.5nM Rapamycin, Placebo. After dosing all mice were sacrificed, with tumors and organs harvested. Snap-frozen tumor samples, formalin-fixed tumor and organs, and murine blood serum were collected and stored.

Figure 6 shows tumor volumes in mean values, fold difference in tumor growth as compared to date of treatment commencement, and mouse weight over the study period.

EXAMPLE 6:

Hematoxylin and Eosin staining on paraffin-embedded formalin-fixed tumor tissue

The formalin fixed tumor tissues from the mice xenografts were processed and mounted in wax. Microtome sections at 5 micron thick were taken on poly L lysine coated slides. The sections were deparaffinized in three changes Xylene (Merck) of 15 minutes, 10 minutes and finally 5 minutes. The slides are subsequently transferred in to alcohol grades of 100, 90 and 80% for three minutes each. Finally the sections are washed in running tap water for around two minutes. The slides are then transferred to Hematoxyline (Harris Hematoxlyin solution Merck) for 7 – 10 minutes. The sections are then brought to tap water and washed till the sections are blue. The section is then differentiated using 1% acid alcohol with 1-2 dips. The sections are subsequently dipped in ammonia water and washed in tap water till the sections are blue. The sections are then counter stained with Eosin (Eosin Yellowish Merck) for 30 seconds and washed in running tap water for two minutes. The sections are dehydrated in ascending grades of alcohol cleared in Xylene and coverslip mounted with DPX (Merck).

The sections were observed by two persons one of them being a pathologist using an upright BX 51 Bi headed microscope TE 2000 S. Number of metaphase and anaphase cells were counted in each section. They were scored as none = 0; low= 1; moderate = 2; high =3; very high =4.

Figure 7 shows the representation of the scores in each group, with one of the combinations showing substantially lower mitotic activity.

It is to be understood that this invention is not limited to the particular methodology, protocols, cell lines, animal species or genera, and reagents described, as such may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the

present invention which will be limited only by the appended claims. The above description is for the purpose of teaching the person of ordinary –skill in the art how to practice the present invention, and it is not intended to detail all those obvious modifications and variations of it which will become apparent to the skilled worker upon reading the description.

All publications mentioned herein are incorporated herein by reference for the purpose of describing and disclosing, for example, the cell lines, and methodologies that are described in the publications which might be used in connection with the presently described invention. The publications discussed above and throughout the text are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the inventors are not entitled to antedate such disclosure by virtue of prior invention.

We Claim

1. A synergistic combination of a therapeutic drug comprising a therapeutically effective mixture of an anti-EGFR antibody and an immunosuppressant.
2. The combination of claim 1, wherein the antibody competitively inhibits native EGF ligand binding to the EGFR receptor.
3. The combination of claim 1, wherein the anti-EGFR antibody is hR3.
4. The combination of claim 1, wherein the immunosuppressant is selected from the group comprising macrolides selected from rapamycin, tacrolimus, everolimus, pimecrolimus or cyclosporins.
5. The combination of claim 1, wherein the anti-EGFR antibody is conjugated to the immunosuppressant.
6. The combination of claim 1, wherein the drug elicits increased cytoxicity against tumor cells.
7. The combination of claim 1 can be administered in the form of solid, semi-solid, or liquid, tablets, pills, powders, capsules, gels, ointments, liquids, suspensions or aerosols.
8. A pharmaceutical composition for therapeutic or prophylactic treatment of cancer comprising administration of a therapeutically effective mixture of an anti-EGFR antibody and an immunosuppressant and a pharmaceutically acceptable carrier.
9. The pharmaceutical composition of claim 8, wherein the therapeutically effective mixture of an anti-EGFR antibody and an immunosuppressant further comprises pharmaceutically acceptable excipients.
10. The pharmaceutical composition of claim 8, wherein the composition is administered intravenously, intramuscularly, subcutaneously, intrasynovially, by infusion, sublingually, transdermally, orally, topically or by inhalation.
11. The pharmaceutical composition of claim 8, wherein the combination drug is used in the treatment of breast cancer, bladder cancer, colon cancer, esophageal cancer, pancreatic cancer, stomach cancer, lung cancer, uterine cancer, cervical cancer, kidney cancer, ovarian cancer, prostrate cancer, renal cancer and head and neck cancer.
12. A process of manufacturing a pharmaceutical composition for therapeutic or prophylactic treatment of cancer comprising a therapeutically effective mixture

of an anti-EGFR antibody and an immunosuppressant, comprising step of mixing anti-EGFR antibody and an immunosuppressant along with pharmaceutically acceptable excipients.

13. The process as claimed in claim 12, wherein the immunosuppressant is selected from the group comprising macrolides selected from rapamycin, tacrolimus, everolimus, pimecrolimus or cyclosporins.

14. The use of the combination of an anti-EGFR antibody and an immunosuppressant according to any of preceding claims for the preparation of a diagnostic composition for the immunohistological detection of cancers.

15. A method of detecting a presence of the EGFR antigen or a cell expressing EGFR comprising contacting the sample with the diagnostic composition of claim 13 to allow a formation of a complex and detecting the formation of the complex.

16. A kit comprising the diagnostic combination of claim 14.

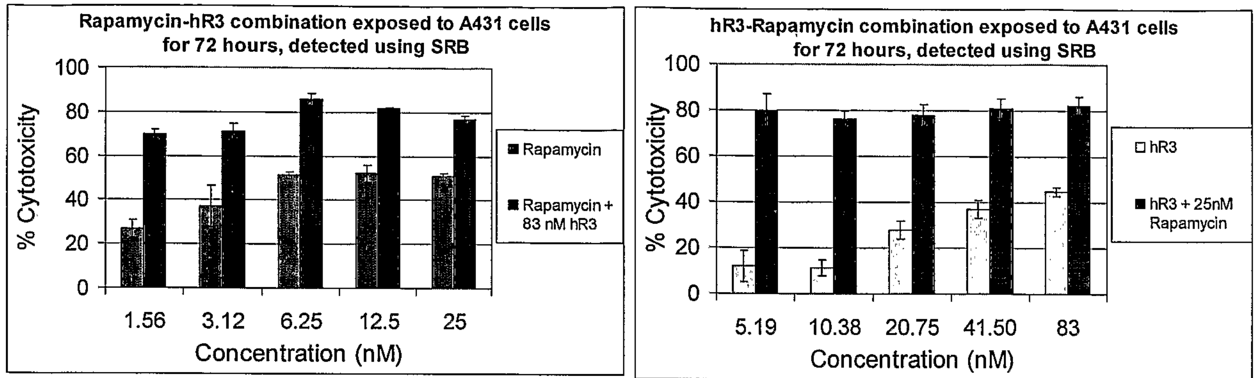


FIG 1

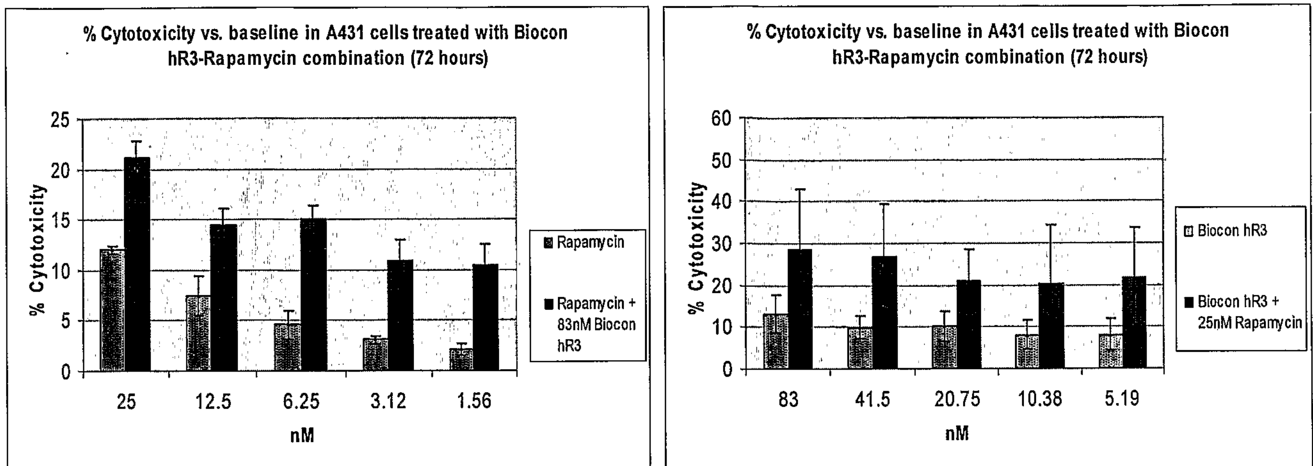


FIG 2

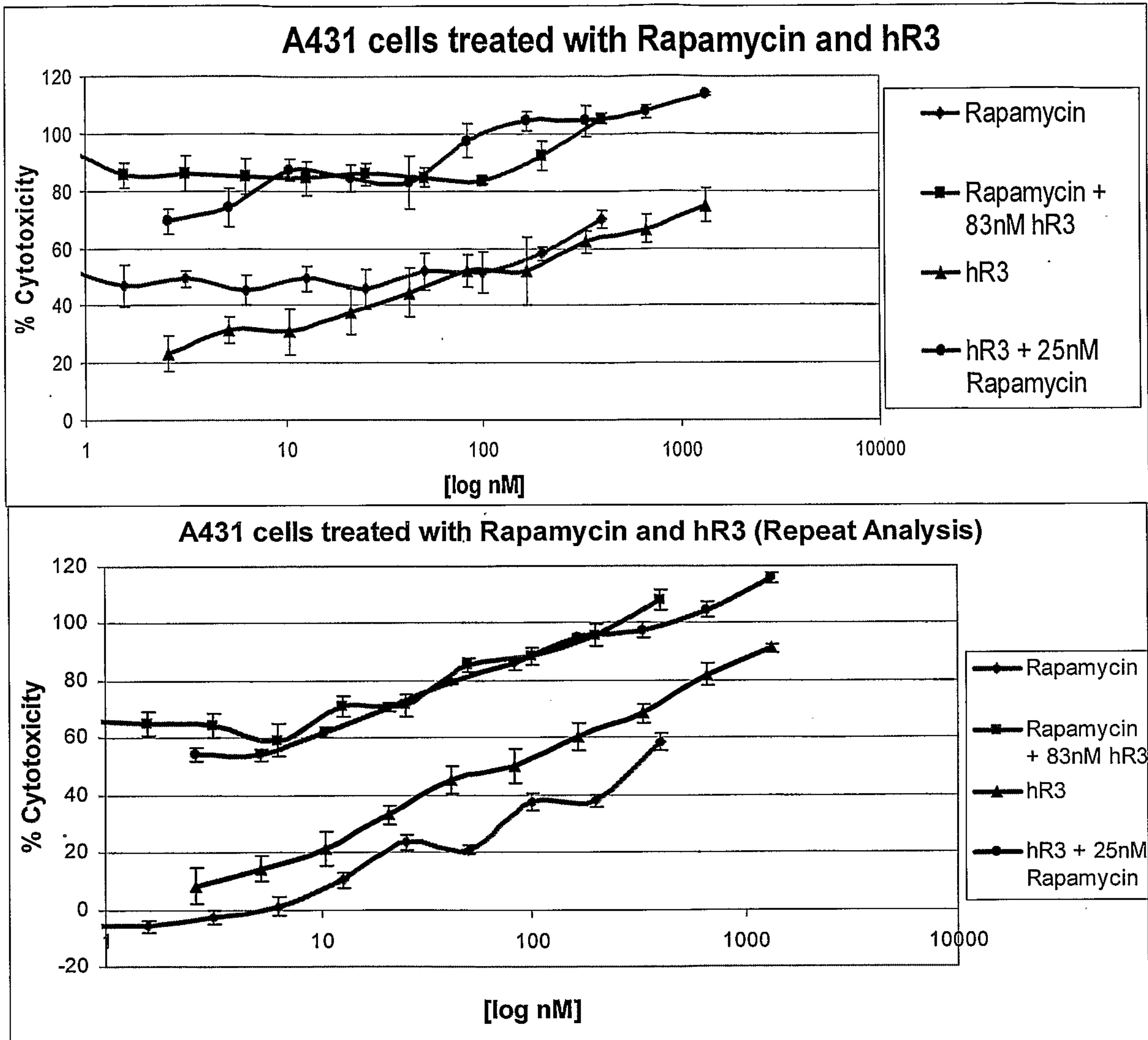
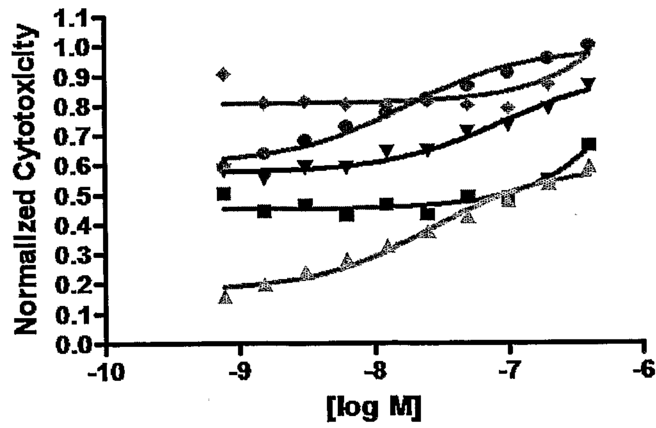


FIG 3



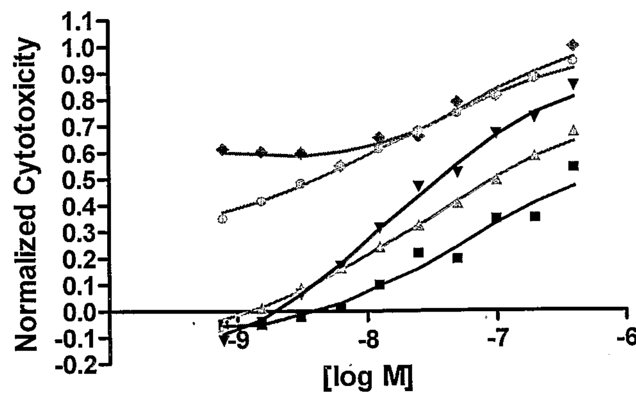


FIG 4

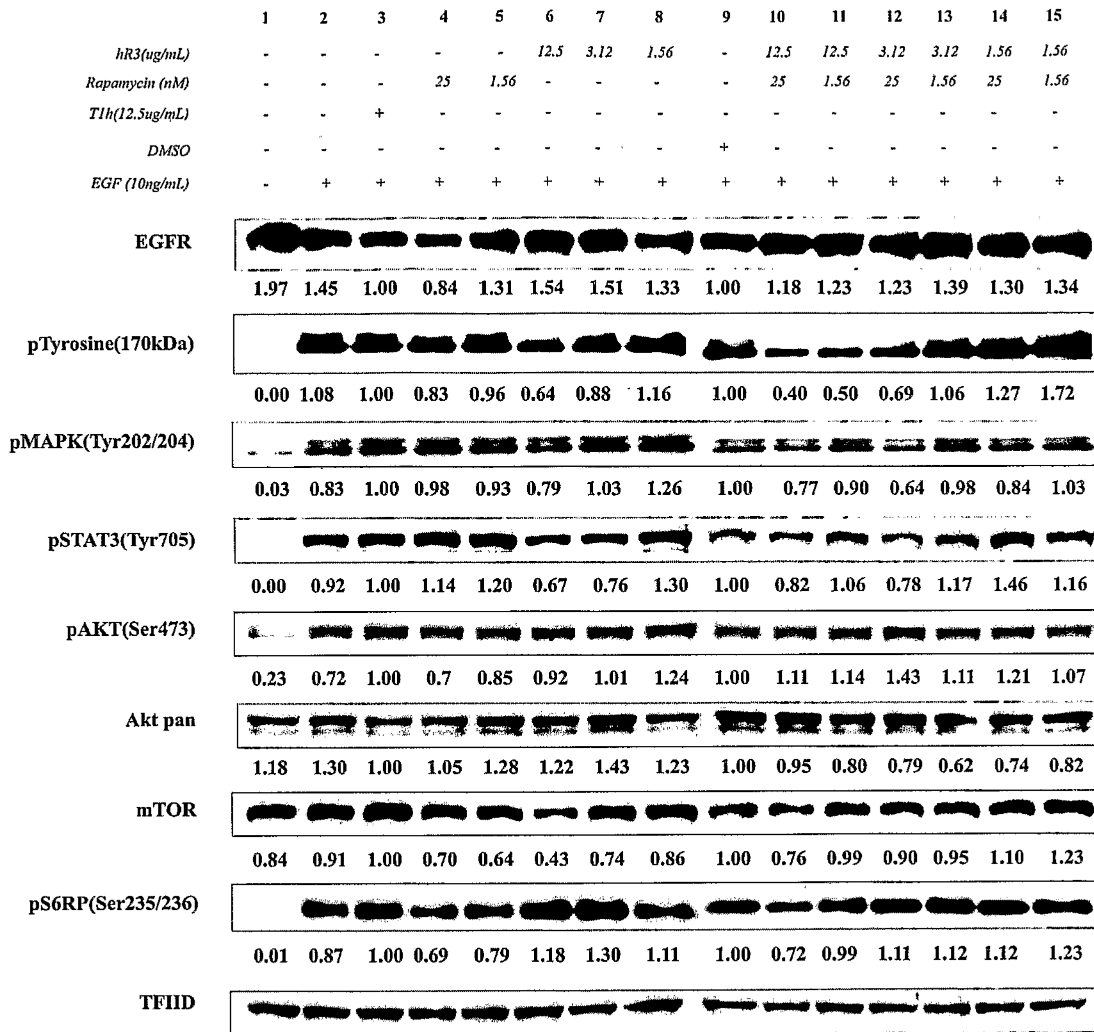
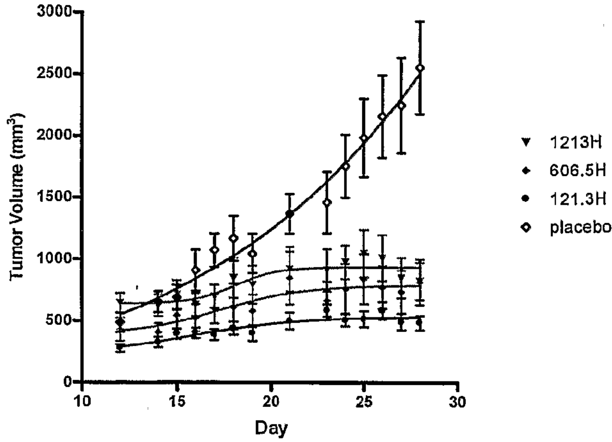
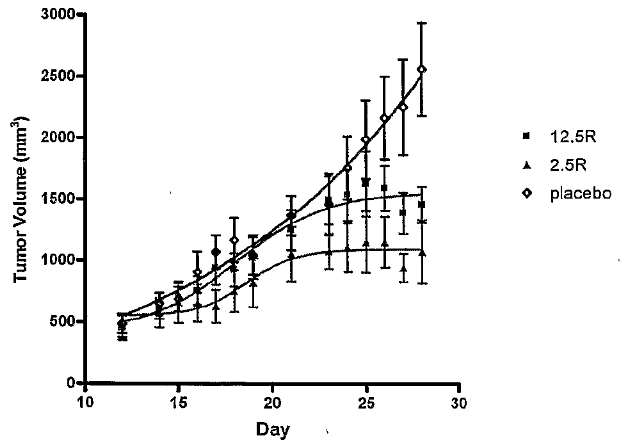
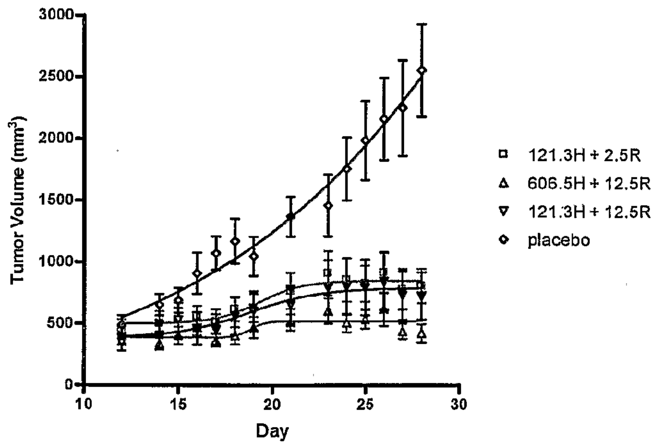


FIG 5

A







B

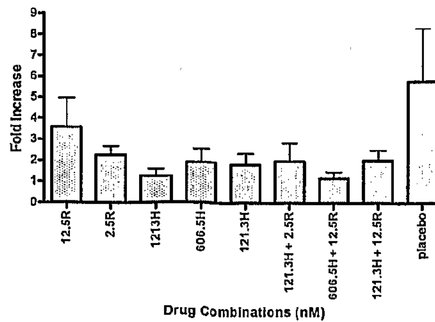


FIG 6

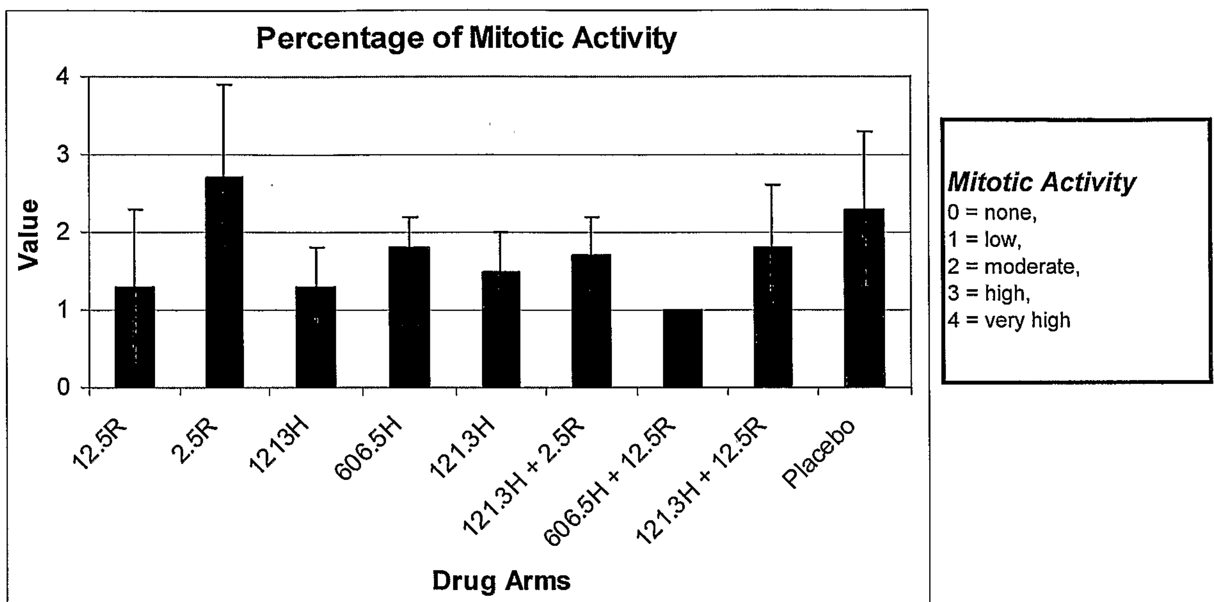


FIG 7

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IN2008/000688

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl.

A61K 39/395 (2006.01) ***A61K 31/436*** (2006.01) ***A61K 39/00*** (2006.01) ***A61P 37/00*** (2006.01)
A61P 37/06 (2006.01) ***A61P 35/04*** (2006.01)

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)

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 practicable, search terms used)
EPODOC, WPIDS, MEDLINE, BIOSIS, CAPLUS: EGFR, epidermal growth factor receptor, EGFR antibody, hR3, nimotuzumab, immunosuppress, suppress immun, modulat immun, inhibit immun, macrolide, rapamycin, sirolimus, FK 506, cancer, neoplasm, tumour and related words

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2005/0276812 A1 (EBENS J R et al.) 15 December 2005 (See entire document, in particular abstract, paragraphs [0002], [0007], [0013], [0017], [0020], [0024], [0050], [0056], [0107], [0194], [0467]-[0469], claims 1, 16 – 17, 26 – 27, 35 – 36 and 48)	1, 2, 4 – 16
X	WO 2007/106503 A2 (OSI PHARMACEUTICALS, INC.) 20 September 2007 (See entire document, in particular abstract, paragraphs [18], [19], [74], [76], [77], [80], [81], [101], [113], [117], [119], [127], [152], claims 1, 2, 7, 9 – 11, 19, 26 and 53)	1, 2, 4, 6 – 13, 15, 16
X	WO 2007/059782 A1 (GENMAB A/S) 31 May 2007 (See entire document, in particular abstract, page 1 lines 4 – 7, page 11 lines 13 – 16 and 20 – 21, page 43 lines 16 – 18, page 44 lines 30 – 34, page 46 lines 5 – 7 and lines 23 – 24, page 61 lines 12 – 32, page 73 lines 10 – 17, page 75 lines 25 – 33, page 78 lines 29 – 32, page 93 lines 4 – 13 and page 99 lines 11 – 23)	1, 2, 5 – 12, 14 – 16

[X] Further documents are listed in the continuation of Box C [X] See patent family annex

* Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier application or patent 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

Date of the actual completion of the international search	Date of mailing of the international search report
16 March 2009	23 MAR 2009
Name and mailing address of the ISA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. +61 2 6283 7999	Authorized officer **ROSELLE MAILVAGANAM** AUSTRALIAN PATENT OFFICE (ISO 9001 Quality Certified Service) Telephone No : +61 2 6225 6112

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IN2008/000688

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2007/0104721 A1 (MOORE et al.) 10 May 2007 (See entire document, in particular abstract, paragraphs [0002], [0012], [0013], [0015]-[0016], [0030], [0037], [0039], [0046] and [0051])	1, 4, 6, 7, 8 – 11
X	WO 2005/056606 A2 (XENCOR, INC.) 23 June 2005 (See entire document, in particular abstract, paragraphs [005], [030]-[032], [057], [072]-[073], [079], [0115], [0180]-[0182] and [0192])	1 – 13
X	WO 2004/056847 A2 (GENMAB A/S AND MEDAREX, INC.) 8 July 2004 (See entire document, in particular abstract, page 2 lines 10 – 14 and lines 23 – 24, page 3 lines 1 – 2, page 11 lines 31 – 38, page 12 lines 5 – 7 and lines 19 – 21, page 13 lines 1 – 6, page 14 lines 12 – 14 and lines 35 – 36, page 15 lines 10 – 15, page 16 lines 3 – 8, page 68 lines 16 – 18, page 69 lines 12 – 17, claims 1, 15 – 19, 22, 31 and 33 – 35)	1, 2, 4 – 16
X	WO 2003/064383 A2 (ARIAD GENE THERAPEUTICS, INC.) 7 August 2003 (See entire document, in particular abstract, page 2 lines 30 – 34, page 15 line 12 – page 16 line 8 and page 27 lines 32 – 39)	1 – 3, 6 – 10, 12, 13

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member					
US	2005276812	AU	16610/02	AU	65311/01	AU	68028/01
		AU	70118/01	AU	71973/01	AU	73150/01
		AU	84906/01	AU	86785/01	AU	2001265311
		AU	2001268028	AU	2002254651	AU	2002367318
		AU	2003230874	AU	2003259913	AU	2003290848
		AU	2004253953	AU	2004291141	AU	2004308972
		AU	2004324192	AU	2005249490	AU	2006203137
		BR	PI0510883	CA	2401448	CA	2410162
		CA	2412211	CA	2416456	CA	2416538
		CA	2420140	CA	2420176	CA	2420193
		CA	2421056	CA	2439594	CA	2451239
		CA	2460120	CA	2471431	CA	2481085
		CA	2481507	CA	2495389	CA	2505705
		CA	2530393	CA	2546285	CA	2551813
		CA	2567520	CA	2584028	CA	2585822
		CA	2591583	CA	2591590	CA	2591630
		CA	2591656	CA	2591814	CA	2591841
		CA	2591929	CA	2591930	CA	2594659
		CA	2632702	CA	2633171	CA	2633413
		CA	2633595	CA	2648046	CA	2648048
		CA	2648051	CN	1993146	EP	1259614
		EP	1286749	EP	1309620	EP	1309685
		EP	1311662	EP	1311668	EP	1311674
		EP	1341814	EP	1366161	EP	1409549
		EP	1445317	EP	1445318	EP	1487877
		EP	1489095	EP	1494709	EP	1571968
		EP	1575571	EP	1578371	EP	1589933
		EP	1639000	EP	1659177	EP	1666491
		EP	1666492	EP	1666493	EP	1666497
		EP	1666594	EP	1666596	EP	1666597
		EP	1683864	EP	1686174	EP	1689432
		EP	1696963	EP	1753463	EP	1805220

EP	1873245	EP	1944317	EP	1992643
EP	2000148	EP	2000482	EP	2000545
EP	2011886	JP	2006104212	JP	2006223304
JP	2007049999	JP	2007077155	JP	2008133284
JP	2008137989	JP	2009001563	KR	20050048615
KR	20050086634	KR	20050103314	KR	20060015699
KR	20060030066	KR	20060055559	KR	20060074927
KR	20060077919	KR	20060125923	KR	20070037575
KR	20070087247	KR	20080106369	MX	PA03001643
MX	PA03001644	MX	PA03001645	MX	PA03011985
MX	PA04002593	MX	PA04006554	MX	PA04010091
MX	PA04010092	MX	PA05001933	MX	PA05005107
MX	PA05014153	MX	PA06014065	NO	20066075
NZ	533933	NZ	535925	US	6579520
US	6642360	US	6740520	US	6916648
US	6962797	US	6972325	US	6974696
US	7019115	US	7019116	US	7019124
US	7026449	US	7029874	US	7037710
US	7067628	US	7067636	US	7074593
US	7081514	US	7081520	US	7081521
US	7084255	US	7084258	US	7087421
US	7087737	US	7105640	US	7112656
US	7112657	US	7118887	US	7119177
US	7122375	US	7125959	US	7125962
US	7132283	US	7153939	US	7153941
US	7157247	US	7160985	US	7166282
US	7166700	US	7169912	US	7186801
US	7189529	US	7189530	US	7189532
US	7189534	US	7189563	US	7189564
US	7189566	US	7189803	US	7189804
US	7189805	US	7189814	US	7189821
US	7189822	US	7189838	US	7193045
US	7193046	US	7193047	US	7193048
US	7193049	US	7193050	US	7193057
US	7193059	US	7193060	US	7193061
US	7193062	US	7193063	US	7193074
US	7195760	US	7196165	US	7196167

US	7196174	US	7196176	US	7202336
US	7202337	US	7202338	US	7205389
US	7205391	US	7208575	US	7211645
US	7214656	US	7214777	US	7217787
US	7220830	US	7220831	US	7220835
US	7220841	US	7220850	US	7223838
US	7223841	US	7227000	US	7230076
US	7230077	US	7230082	US	7232882
US	7232889	US	7232892	US	7235630
US	7235633	US	7238787	US	7241862
US	7244428	US	7244816	US	7247710
US	7250490	US	7253255	US	7253256
US	7256039	US	7256261	US	7256262
US	7259238	US	7268217	US	7271247
US	7271250	US	7276577	US	7276578
US	7276586	US	7279553	US	7282561
US	7282566	US	7282569	US	7285623
US	7285624	US	7285625	US	7285626
US	7285629	US	7285645	US	7288625
US	7288628	US	7291329	US	7291701
US	7291703	US	7291704	US	7291705
US	7291706	US	7291707	US	7291708
US	7291712	US	7291718	US	7294335
US	7294690	US	7294700	US	7297764
US	7297767	US	7297776	US	7304131
US	7304132	US	7304133	US	7304134
US	7304135	US	7304143	US	7304145
US	7307144	US	7307151	US	7309761
US	7309762	US	7309766	US	7309769
US	7309770	US	7309771	US	7309775
US	7309776	US	7312307	US	7312309
US	7312310	US	7312315	US	7312316
US	7314913	US	7314918	US	7317079
US	7317080	US	7317082	US	7317083
US	7317092	US	7317093	US	7319137
US	7326773	US	7329404	US	7329730
US	7332573	US	7335728	US	7335730

US	7339024	US	7339025	US	7339033
US	7339034	US	7342097	US	7342104
US	7343721	US	7345145	US	7345147
US	7348415	US	7351789	US	7351795
US	7351804	US	7354997	US	7354999
US	7355000	US	7355013	US	7357926
US	7358339	US	7358340	US	7361732
US	7365156	US	7368250	US	7371814
US	7371815	US	7371836	US	7375179
US	7375184	US	7375196	US	7378486
US	7378487	US	7378491	US	7378501
US	7381791	US	7385033	US	7388073
US	7390876	US	7390879	US	7390882
US	7390886	US	7393917	US	7393931
US	7399824	US	7399828	US	7402661
US	7405269	US	7408032	US	7408033
US	7408034	US	7417125	US	7423120
US	7423130	US	7425605	US	7425613
US	7427667	US	7435793	US	7435798
US	7449549	US	7462692	US	7465785
US	7465786	US	7468424	US	7468427
US	7470774	US	7488795	US	7488796
US	7491529	US	7495083	US	7498298
US	2002058309	US	2002072067	US	2002072092
US	2002072496	US	2002072497	US	2002090681
US	2002102647	US	2002103125	US	2002110859
US	2002115145	US	2002119130	US	2002123463
US	2002127576	US	2002127584	US	2002127643
US	2002132252	US	2002132253	US	2002132768
US	2002132981	US	2002137909	US	2002142419
US	2002142958	US	2002142959	US	2002142961
US	2002150976	US	2002156004	US	2002156006
US	2002160384	US	2002161199	US	2002165143
US	2002168715	US	2002169284	US	2002173463
US	2002177164	US	2002177165	US	2002177188
US	2002177553	US	2002182638	US	2002182673
US	2002183493	US	2002183494	US	2002183505

US	2002192209	US	2002192706	US	2002192751
US	2002193299	US	2002193300	US	2002197612
US	2002197615	US	2002197674	US	2002198147
US	2002198148	US	2002198149	US	2003003507
US	2003003531	US	2003003546	US	2003004102
US	2003008297	US	2003008348	US	2003008352
US	2003008353	US	2003009012	US	2003009013
US	2003013153	US	2003013855	US	2003017476
US	2003017540	US	2003017541	US	2003017542
US	2003017543	US	2003017544	US	2003017563
US	2003017981	US	2003017982	US	2003018168
US	2003022187	US	2003022239	US	2003022293
US	2003022294	US	2003022295	US	2003022296
US	2003022297	US	2003022298	US	2003022300
US	2003022301	US	2003022328	US	2003022331
US	2003027162	US	2003027163	US	2003027212
US	2003027249	US	2003027262	US	2003027263
US	2003027264	US	2003027265	US	2003027266
US	2003027267	US	2003027268	US	2003027269
US	2003027270	US	2003027271	US	2003027272
US	2003027273	US	2003027274	US	2003027275
US	2003027276	US	2003027277	US	2003027278
US	2003027279	US	2003027280	US	2003027281
US	2003027324	US	2003027754	US	2003027985
US	2003027986	US	2003027992	US	2003027993
US	2003032023	US	2003032057	US	2003032061
US	2003032062	US	2003032063	US	2003032101
US	2003032102	US	2003032103	US	2003032104
US	2003032105	US	2003032106	US	2003032107
US	2003032108	US	2003032109	US	2003032110
US	2003032111	US	2003032112	US	2003032113
US	2003032114	US	2003032115	US	2003032116
US	2003032117	US	2003032118	US	2003032119
US	2003032120	US	2003032121	US	2003032122
US	2003032123	US	2003032124	US	2003032125
US	2003032126	US	2003032127	US	2003032128
US	2003032129	US	2003032130	US	2003032131

US	2003032132	US	2003032133	US	2003032134
US	2003032135	US	2003032136	US	2003032137
US	2003032138	US	2003032139	US	2003032140
US	2003032155	US	2003032156	US	2003034993
US	2003036114	US	2003036117	US	2003036118
US	2003036119	US	2003036120	US	2003036121
US	2003036122	US	2003036123	US	2003036124
US	2003036125	US	2003036126	US	2003036127
US	2003036128	US	2003036129	US	2003036130
US	2003036131	US	2003036132	US	2003036133
US	2003036134	US	2003036135	US	2003036136
US	2003036137	US	2003036138	US	2003036139
US	2003036140	US	2003036141	US	2003036142
US	2003036143	US	2003036144	US	2003036145
US	2003036146	US	2003036147	US	2003036148
US	2003036149	US	2003036150	US	2003036151
US	2003036152	US	2003036153	US	2003036154
US	2003036155	US	2003036156	US	2003036157
US	2003036158	US	2003036159	US	2003036160
US	2003036161	US	2003036162	US	2003036164
US	2003036165	US	2003036179	US	2003036180
US	2003036634	US	2003038826	US	2003038827
US	2003038828	US	2003039648	US	2003040013
US	2003040014	US	2003040053	US	2003040054
US	2003040055	US	2003040056	US	2003040057
US	2003040058	US	2003040059	US	2003040060
US	2003040061	US	2003040062	US	2003040063
US	2003040064	US	2003040065	US	2003040066
US	2003040067	US	2003040068	US	2003040069
US	2003040070	US	2003040071	US	2003040072
US	2003040073	US	2003040074	US	2003040075
US	2003040076	US	2003040077	US	2003040078
US	2003040079	US	2003040473	US	2003043176
US	2003044806	US	2003044841	US	2003044842
US	2003044844	US	2003044902	US	2003044916
US	2003044917	US	2003044918	US	2003044919
US	2003044920	US	2003044921	US	2003044922

US	2003044923	US	2003044924	US	2003044925
US	2003044926	US	2003044927	US	2003044928
US	2003044929	US	2003044930	US	2003044931
US	2003044932	US	2003044945	US	2003045462
US	2003045463	US	2003045684	US	2003045700
US	2003049633	US	2003049638	US	2003049681
US	2003049682	US	2003049684	US	2003049733
US	2003049734	US	2003049735	US	2003049738
US	2003049739	US	2003049740	US	2003049741
US	2003049742	US	2003049743	US	2003049744
US	2003049745	US	2003049746	US	2003049747
US	2003049748	US	2003049749	US	2003049750
US	2003049751	US	2003049752	US	2003049753
US	2003049754	US	2003049755	US	2003049756
US	2003049757	US	2003049758	US	2003049759
US	2003049760	US	2003049761	US	2003049762
US	2003049763	US	2003049764	US	2003049765
US	2003049766	US	2003049767	US	2003049768
US	2003049769	US	2003049770	US	2003049771
US	2003049772	US	2003049773	US	2003049774
US	2003049775	US	2003049776	US	2003049777
US	2003049778	US	2003049779	US	2003049780
US	2003049781	US	2003049782	US	2003049783
US	2003049816	US	2003049817	US	2003050239
US	2003050240	US	2003050241	US	2003050457
US	2003050459	US	2003050462	US	2003054359
US	2003054403	US	2003054404	US	2003054405
US	2003054406	US	2003054442	US	2003054454
US	2003054455	US	2003054456	US	2003054457
US	2003054458	US	2003054459	US	2003054460
US	2003054461	US	2003054462	US	2003054463
US	2003054464	US	2003054465	US	2003054466
US	2003054467	US	2003054468	US	2003054469
US	2003054470	US	2003054471	US	2003054472
US	2003054473	US	2003054474	US	2003054475
US	2003054476	US	2003054477	US	2003054478
US	2003054479	US	2003054480	US	2003054481

US	2003054482	US	2003054483	US	2003054516
US	2003054517	US	2003054986	US	2003054987
US	2003055216	US	2003055222	US	2003055224
US	2003059780	US	2003059782	US	2003059783
US	2003059828	US	2003059831	US	2003059832
US	2003059833	US	2003059876	US	2003059879
US	2003059880	US	2003059882	US	2003059883
US	2003059884	US	2003059885	US	2003059886
US	2003059909	US	2003060406	US	2003060407
US	2003060600	US	2003060601	US	2003060602
US	2003060612	US	2003063112	US	2003064062
US	2003064375	US	2003064407	US	2003064440
US	2003064441	US	2003064442	US	2003064443
US	2003064444	US	2003064445	US	2003064446
US	2003064447	US	2003064448	US	2003064449
US	2003064450	US	2003064451	US	2003064452
US	2003064453	US	2003064454	US	2003064455
US	2003064456	US	2003064457	US	2003064458
US	2003064459	US	2003064460	US	2003064461
US	2003064462	US	2003064463	US	2003064464
US	2003064465	US	2003064466	US	2003064467
US	2003064468	US	2003064469	US	2003064925
US	2003065142	US	2003065143	US	2003065154
US	2003065159	US	2003067478	US	2003068623
US	2003068647	US	2003068648	US	2003068679
US	2003068680	US	2003068681	US	2003068682
US	2003068683	US	2003068684	US	2003068685
US	2003068686	US	2003068687	US	2003068688
US	2003068689	US	2003068692	US	2003068693
US	2003068694	US	2003068695	US	2003068696
US	2003068697	US	2003068698	US	2003068699
US	2003068700	US	2003068701	US	2003068702
US	2003068703	US	2003068704	US	2003068705
US	2003068706	US	2003068707	US	2003068708
US	2003068709	US	2003068710	US	2003068711
US	2003068712	US	2003068713	US	2003068714
US	2003068715	US	2003068716	US	2003068717

US	2003068718	US	2003068719	US	2003068720
US	2003068721	US	2003068722	US	2003068723
US	2003068724	US	2003068725	US	2003068726
US	2003068727	US	2003068728	US	2003068729
US	2003068730	US	2003068731	US	2003068732
US	2003068733	US	2003068734	US	2003068735
US	2003068736	US	2003068737	US	2003068738
US	2003068739	US	2003068740	US	2003068741
US	2003068742	US	2003068743	US	2003068744
US	2003068745	US	2003068746	US	2003068747
US	2003068748	US	2003068749	US	2003068750
US	2003068751	US	2003068752	US	2003068753
US	2003068754	US	2003068755	US	2003068756
US	2003068757	US	2003068758	US	2003068759
US	2003068760	US	2003068761	US	2003068762
US	2003068763	US	2003068764	US	2003068765
US	2003068766	US	2003068767	US	2003068768
US	2003068769	US	2003068770	US	2003068771
US	2003068772	US	2003068773	US	2003068774
US	2003068775	US	2003068776	US	2003068793
US	2003068794	US	2003068795	US	2003068796
US	2003068797	US	2003068798	US	2003069178
US	2003069179	US	2003069394	US	2003069403
US	2003069407	US	2003071834	US	2003071835
US	2003072745	US	2003073129	US	2003073130
US	2003073131	US	2003073169	US	2003073170
US	2003073171	US	2003073172	US	2003073173
US	2003073174	US	2003073175	US	2003073176
US	2003073177	US	2003073178	US	2003073179
US	2003073180	US	2003073181	US	2003073182
US	2003073183	US	2003073184	US	2003073185
US	2003073186	US	2003073208	US	2003073210
US	2003073211	US	2003073212	US	2003073213
US	2003073214	US	2003073215	US	2003073216
US	2003073624	US	2003073809	US	2003073810
US	2003073813	US	2003073821	US	2003077593
US	2003077594	US	2003077659	US	2003077698

US	2003077700	US	2003077718	US	2003077732
US	2003077733	US	2003077736	US	2003077776
US	2003077777	US	2003077778	US	2003077779
US	2003077780	US	2003077781	US	2003077782
US	2003077783	US	2003077784	US	2003077785
US	2003077786	US	2003077787	US	2003077788
US	2003077789	US	2003077790	US	2003077791
US	2003077792	US	2003078377	US	2003082546
US	2003082626	US	2003082627	US	2003082628
US	2003082689	US	2003082715	US	2003082716
US	2003082717	US	2003082759	US	2003082760
US	2003082767	US	2003083248	US	2003087373
US	2003087374	US	2003096353	US	2003096744
US	2003100061	US	2003104536	US	2003104538
US	2003104539	US	2003104540	US	2003104541
US	2003104542	US	2003104543	US	2003104998
US	2003119105	US	2003119106	US	2003124661
US	2003130181	US	2003134785	US	2003138439
US	2003139328	US	2003147901	US	2003148373
US	2003148376	US	2003148408	US	2003157615
US	2003157635	US	2003166104	US	2003166105
US	2003166106	US	2003166107	US	2003166108
US	2003166109	US	2003166110	US	2003166111
US	2003166112	US	2003166113	US	2003166114
US	2003166115	US	2003166117	US	2003166118
US	2003166119	US	2003166120	US	2003166121
US	2003166122	US	2003166123	US	2003166124
US	2003166125	US	2003166126	US	2003166127
US	2003166128	US	2003166129	US	2003166130
US	2003166131	US	2003166133	US	2003166134
US	2003166135	US	2003166188	US	2003170254
US	2003170793	US	2003170794	US	2003170795
US	2003170796	US	2003170797	US	2003170798
US	2003170799	US	2003170800	US	2003170801
US	2003170802	US	2003170803	US	2003170805
US	2003170806	US	2003175882	US	2003180310
US	2003180311	US	2003180867	US	2003185841

US	2003186365	US	2003186368	US	2003187241
US	2003190321	US	2003190701	US	2003190703
US	2003194410	US	2003194744	US	2003194780
US	2003194781	US	2003195148	US	2003195333
US	2003195344	US	2003195345	US	2003198994
US	2003199021	US	2003199435	US	2003199436
US	2003199437	US	2003199674	US	2003203402
US	2003203433	US	2003203434	US	2003203435
US	2003203436	US	2003203441	US	2003203442
US	2003203443	US	2003203444	US	2003203445
US	2003203446	US	2003204055	US	2003206188
US	2003206915	US	2003207392	US	2003207803
US	2003211091	US	2003211092	US	2003211572
US	2003211574	US	2003215905	US	2003215908
US	2003215909	US	2003216305	US	2003216560
US	2003216561	US	2003228305	US	2003228319
US	2004005312	US	2004005657	US	2004006219
US	2004029218	US	2004043927	US	2004048332
US	2004063921	US	2004087769	US	2004223964
US	2004229277	US	2004241703	US	2004242860
US	2005009105	US	2005014226	US	2005037458
US	2005037463	US	2005042216	US	2005060932
US	2005064492	US	2005079577	US	2005084935
US	2005106644	US	2005107595	US	2005123925
US	2005124789	US	2005163766	US	2005170368
US	2005196832	US	2005202526	US	2005226868
US	2005226869	US	2005227328	US	2005227342
US	2005238649	US	2005238650	US	2006073544
US	2006073545	US	2006074227	US	2006078964
US	2006147373	US	2006160997	US	2006216232
US	2006251662	US	2007020735	US	2007041983
US	2007048218	US	2007048326	US	2007053835
US	2007054361	US	2007087006	US	2007207142
US	2007212735	US	2007219350	US	2007243193
US	2007265436	US	2007269446	US	2008124331
US	2008160021	US	2008166294	US	2008171040
US	2008182275	US	2008200653	US	2008226657

		US	2008227106	US	2008248051	US	2008248053
		US	2008318239	US	2009017473	US	2009047296
		US	2009053226	WO	2003000113	WO	2003024392
		WO	2003057160	WO	2003088808	WO	2003088898
		WO	2004016225	WO	2004045516	WO	2005003154
		WO	2005049075	WO	2005063299	WO	2005117986
		WO	2006043954	ZA	200401949	ZA	200405011
WO	2007106503	EP	1996193	US	2007280928		
WO	2007059782	AU	2006317242	CA	2631184	CN	101370526
		EP	1973576	KR	20080090406		
US	2007104721	AR	057854	AU	2006311877	CA	2626326
		CN	101300029	EP	1942937	KR	20080064156
		WO	2007056118				
WO	2005056606	AU	2004297977	AU	2005295344	BR	0215759
		BR	PI0417312	CA	2494009	CA	2589731
		CN	1650471	CN	1926719	EP	1509967
		EP	1700358	EP	1701979	EP	1961071
		MX	PA04011992	NZ	537354	US	6897832
		US	7382332	US	2003020667	US	2004207566
		US	2005103329	US	2005142133	US	2006033674
		US	2008034492	WO	2002097917	WO	2005057620
		WO	2006044949				
WO	2004056847	AU	2003303237	BR	0210405	CA	2450285
		CN	1610695	CN	1966525	CZ	20040038
		EP	1417232	EP	1572746	HU	0600225
		MX	PA03011365	NZ	530212	RU	2004100834
		US	7247301	US	2003091561	US	2003194403
		WO	2002100348				
WO	2003064383	BR	0307544	CA	2472341	CN	1646537
		EP	1478648	EP	1648900	MX	PA04007402
		PL	371526	US	7091213	US	7186826
		US	7432277	US	2003220297	US	2004073024
		US	2005026868	US	2005032825	US	2006264405
		US	2006264456	US	2007190106	WO	2005016252
		WO	2007024745				

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.

END OF ANNEX