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(54) Titre : COMBINAISONS ANTINEOPLASIQUES A BASE D'INHIBITEUR DE MTOP, D'HERCEPTINE ET/OU DE KHI-272

(54) Title: ANTINEOPLASTIC COMBINATIONS WITH MTOR INHIBITOR, HERCEPTIN, AND/OR HKI-272

(57) Abrégé/Abstract:
A combination of temsirolimus and herceptin in the treatment of cancer is provided. A combination of temsirolimus and HKI-272 is provided. A combination of herceptin and HKI-272 is also provided. Regimens and kits for treatment of metastatic breast cancer, containing herceptin, temsirolimus and/or HKI-272, optionally in combination with other anti-neoplastic agents, or immune modulators are described.

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(54) **Title:** ANTINEOPLASTIC COMBINATIONS WITH MTOR INHIBITOR, HERCEPTIN, AND/OR HKI-272

(57) **Abstract:** A combination of temsirolimus and herceptin in the treatment of cancer is provided. A combination of temsirolimus and HKI-272 is provided. A combination of herceptin and HKI-272 is also provided. Regimens and kits for treatment of metastatic breast cancer, containing herceptin, temsirolimus and/or HKI-272, optionally in combination with other anti-neoplastic agents, or immune modulators are described.

ANTINEOPLASTIC COMBINATIONS WITH MTOR INHIBITOR, HERCEPTIN, AND/OR HKI-272

BACKGROUND OF THE INVENTION

This invention relates to the use of combinations of Herceptin™ with an mTOR inhibitor and/or HKI-272, for the treatment of neoplasms associated with overexpression or amplification of HER2.

CCI-779, is rapamycin 42-ester with 3-hydroxy-2-(hydroxymethyl)-2-methylpropionic acid, an ester of rapamycin which has demonstrated significant inhibitory effects on tumor growth in both *in vitro* and *in vivo* models. This compound is now known generically under the name temsirolimus. The preparation and use of hydroxyesters of rapamycin, including temsirolimus, are described in US Patents 5,362,718 and 6,277,983.

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

Metastatic breast cancer (MBC) is essentially incurable with standard therapy, and patients with MBC have a median survival of about 2 years after documentation of metastasis. As a consequence, the goals of treatment are to improve patients' symptoms while trying to maintain (or improve, in certain cases) quality of life. Prolonging survival remains a clear goal, particularly in breast cancer that has overexpression or amplification of the her-2 oncogene.

Herceptin® (Trastuzumab) is an FDA-approved therapeutic monoclonal antibody for HER2 protein overexpressing metastatic breast cancer. A murine monoclonal antibody was described [see, US Patent 5,705,151]. The murine MAb4D5 molecule described in that document has been humanized in an attempt to improve its clinical efficacy by reducing immunogenicity and allowing it to support human effector functions. WO 92/22653. Later documents describe the development of a lyophilized formulation comprising full length humanized antibody huMAb4D5-8 described in WO 92/22653. Herceptin is currently approved by the FDA for the treatment of metastatic breast cancer that overexpresses HER2, (1) as a single agent after previous treatment of the metastatic breast cancer with one or more chemotherapy regimens and (2) in combination with paclitaxel in such patients without prior chemotherapy for their metastatic breast cancer. Moreover, there is evidence that the addition of herceptin to taxane adjuvant or neoadjuvant chemotherapy improves to patients with earlier stage breast cancer.

HKI-272, (E)-N-{4-[3-chloro-4-(2-pyridinyl methoxy) anilino]-3-cyano-7-ethoxy-6-quinolinyl}-4-(dimethylamino)-2-butenamide, has been described as a promising anticancer drug candidate for the treatment of breast cancers and other HER-2-dependent cancers. Because it also inhibits the EGFR kinase with similar potency, HKI-272 may be useful to treat tumors that overexpress both HER-2 and EGFR and be more efficacious than a specific EGFR or HER-2 antagonist. S. K. Rabindran *et al*, "Antitumor Activity of HKI-272, an Orally Active, Irreversible Inhibitor of the HER-2 Tyrosine Kinase", *Cancer Research* **64**, 3958-3965, June 1, 2004. See, US Patent No. 6,288,082; US Patent No. 6,297,258.

What is needed is an improved antineoplastic therapy.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a 3-dimensional contour plot with the plane at 0 % representing additive interaction, and peaks and valleys representing areas of synergy or antagonism, respectively, between the herceptin and HKI-272 in BT474 [HER-2+ (amplified); ATCC HTB-20] cells.

Fig. 2 is a 3-dimensional contour plot with the plane at 0 % representing additive interaction, and peaks and valleys representing areas of synergy or antagonism between the herceptin and HKI-272 in MCF-7 [HER-2^-, EGFR-; adenocarcinoma; ATCC HTB22] cells.

Fig. 3 is a 3-dimensional contour plot with the plane at 0 % representing additive interaction, and peaks and valleys representing areas of synergy or antagonism between the herceptin and HKI-272 in MDA-MB-361 [HER-2^+ (non-amplified); adenocarcinoma; ATCC HTB 27] cells.

DETAILED DESCRIPTION OF THE INVENTION

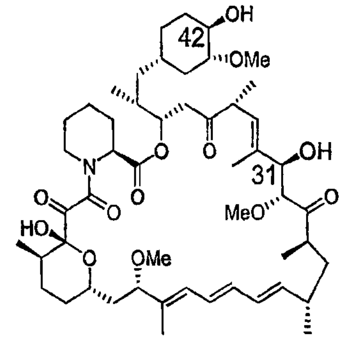
This invention provides the use of combinations comprising herceptin, an mTOR inhibitor and/or HKI-272 in the treatment of neoplasms. Thus, the invention provides for the combined use of a herceptin with an mTOR inhibitor, the combined use of herceptin with an HKI-272, the combined use of an mTOR inhibitor with HKI-272, or the combined use of a herceptin with mTOR inhibitor and an HKI-272. The invention further provides products containing a herceptin in combination with an mTOR inhibitor and/or HKI-272 formulated for simultaneous, separate or sequential use in treating neoplasms in a mammal. The invention is also useful as an adjuvant and/or neoadjuvant therapy of earlier stages of breast cancer. The following detailed description illustrates temsirolimus. However, other mTOR inhibitors may be substituted for temsirolimus in the methods, combinations and products described herein.

These methods, combinations and products are useful in the treatment of a variety of neoplasms associated with overexpression or amplification of HER2, including, for example, lung cancers, including bronchioalveolar carcinoma and non small cell lung cancer, breast cancers, prostate cancers, myeloma, head and neck cancer, or transitional cell carcinoma; small cell and large cell neuroendocrine carcinoma of the uterine cervix.

In one embodiment, the combination of temsirolimus and herceptin is particularly well suited for treatment of metastatic breast cancer. In another embodiment, the combination of herceptin and a mTOR inhibitor and/or an HKI-272, are well suited for treatment of breast, kidney, bladder, mouth, larynx, esophagus, stomach, colon, ovary, and lung), and polycystic kidney disease.

As used herein, the term **mTOR inhibitor** means a compound or ligand, or a pharmaceutically acceptable salt thereof, that inhibits cell replication by blocking the progression of the cell cycle from G1 to S. The term includes the neutral tricyclic compound rapamycin (sirolimus) and other rapamycin compounds, including, *e.g.*, rapamycin derivatives, rapamycin analogues, other macrolide compounds that inhibit mTOR activity, and all compounds included within the definition below of the term "a rapamycin". These include compounds with a structural similarity to "a rapamycin", *e.g.*, compounds with a similar macrocyclic structure that have been modified to enhance therapeutic benefit. FK-506 can also be used in the method of the invention.

As used herein, the term **a rapamycin** defines a class of immunosuppressive compounds that contain the basic rapamycin nucleus as shown below.



The rapamycins of this invention include compounds that are chemically or biologically modified as derivatives of the rapamycin nucleus, while still retaining immunosuppressive properties. Accordingly, the term **a rapamycin** includes rapamycin, and esters, ethers, carbamates, oximes, hydrazones, and hydroxylamines of rapamycin, as well as rapamycins in which functional groups on the rapamycin nucleus have been modified, for example through reduction or oxidation. Also included in the term **a rapamycin** are pharmaceutically acceptable salts of rapamycins.

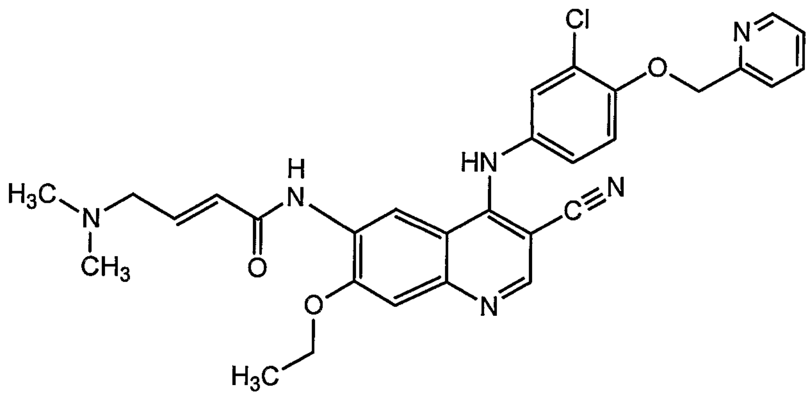
The term **a rapamycin** also includes 42- and/or 31-esters and ethers of rapamycin as described in the following patents, alkyl esters (U.S. Patent No. 4,316,885); aminoalkyl esters (U.S. Patent No. 4,650,803); fluorinated esters (U.S. Patent No. 5,100,883); amide esters (U.S. Patent No. 5,118,677);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

Further included within the definition of the term **a rapamycin** are 27-esters and ethers of rapamycin, which are disclosed in U.S. Patent No. 5,256,790. Also described are C-27 ketone rapamycins which are reduced to the corresponding alcohol, which is in turn converted to the corresponding ester or ether. The preparation of these esters and ethers is disclosed in the patent listed above. Also included are oximes, hydrazones, and hydroxylamines of rapamycin are disclosed in U.S. Patent Nos. 5,373,014, 5,378,836, 5,023,264, and 5,563,145. The preparation of these oximes, hydrazones, and hydroxylamines is disclosed in the above-listed patents. The preparation of 42-oxorapamycin is disclosed in 5,023,263.

As used herein, the term **a CCI-779** means rapamycin 42-ester with 3-hydroxy-2-(hydroxymethyl)-2-methylpropionic acid (temsirolimus), and encompasses prodrugs, derivatives, pharmaceutically acceptable salts, or analogs thereof.

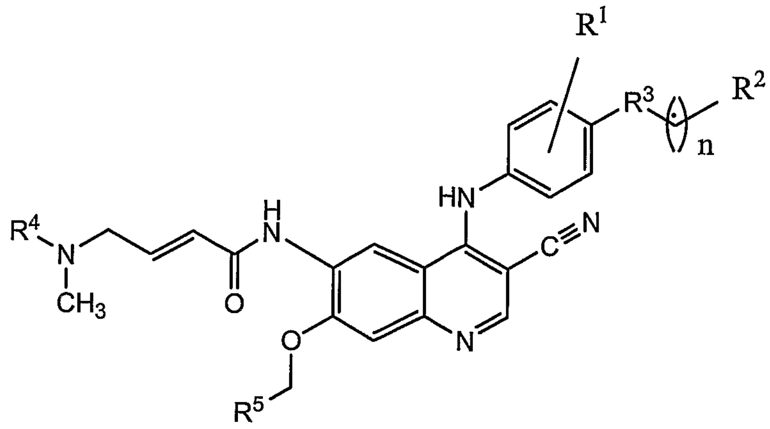
Examples of a rapamycin include, *e.g.*, rapamycin, 32-deoxorapamycin, 16-pent-2-ynyloxy-32-deoxorapamycin, 16-pent-2-ylyloxy-32(S)-dihydro-rapamycin, 16-pent-2-ylyloxy-32(S)-dihydr-o-40-O-(2-hydroxyethyl)-rapamycin, 40-O-(2-hydroxyethyl)-rapamycin, rapamycin 42-ester with 3-hydroxy-2-(hydroxymethyl)-2-methylpropionic acid (CCI-779), 40-[3-hydroxy-2-(hydroxymethyl)-2-meth-ylpropanoate]-rapamycin, or a pharmaceutically acceptable salt thereof, as disclosed in U.S. Patent No. 5,362,718, ABT578, or 40-(tetrazolyl)-rapamycin, 40-epi-(tetrazolyl)-rapamycin, *e.g.*, as disclosed in International Patent Publication No. WO 99/15530, or rapamycin analogs as disclosed in International Patent Publication No. WO 98/02441 and WO 01/14387, *e.g.*, AP23573. In another embodiment, the compound is Certican™ (everolimus, 2-O-(2-hydroxy)ethyl rapamycin, Novartis, U.S. Patent No. 5,665,772).

As used herein, "an **HKI-272**" refers to a compound having the following core,



or a derivative or pharmaceutically acceptable salt thereof. Suitable derivatives may include, *e.g.*, an ester, ether, or carbamate. The core structure, HKI-272, has the chemical name (E)-N-{4-[3-chloro-4-(2-pyridinylmethoxy) anilino] -3-cyano-7-ethoxy-6-quinolinyl}-4-(dimethylamino)-2- -butenamide.

In one embodiment, the invention also provides for use of substituted 3-cyano quinolines having structure:



where R_1 is halogen;

R_2 is pyridinyl, thiophene, pyrimidine, thiazole, or phenyl optionally substituted with up to three substituents;

R_3 is –O- or –S-;

R_4 is methyl or $CH_2CH_2OCH_3$;

R_5 is ethyl or methyl; and

n is O or 1.

These compounds, of which HKI-272 is a species, are characterized by the ability to act as potent HER-2 inhibitors. *See, e.g.*, US Patent 6,288,082 and US Patent 6,297,258. These compounds and their preparation are described in detail in US Published Patent Application No. 2005/0059678. For convenience, HKI-272 is used throughout this specification. However, it will be understood that the compound of the structure provided above can be substituted for HKI-272 in the combinations with an mTOR inhibitor and/or herceptin which are described in detail below.

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

It is preferred that the mTOR inhibitor used in the antineoplastic combinations of this invention is a rapamycin, and more preferred that the mTOR inhibitor is rapamycin, temsirolimus, or 42-O-(2-hydroxy)ethyl rapamycin. The preparation of 42-O-(2-hydroxy)ethyl rapamycin is described in U.S. Patent 5,665,772.

The preparation of temsirolimus is described in U.S. Patent 5,362,718. A regiospecific synthesis of temsirolimus is described in US Patent 6,277,983. Still another regiospecific method for synthesis of temsirolimus is described in US Patent Publication No. 2005-0033046-A1, published Feb 10, 2005 (Application No. 10/903,062, filed July 30, 2004), and its counterpart, International Patent Publication No. WO 2005/016935, published Apr 7, 2005.

Herceptin, and methods of making and formulating same have been described. See, e.g., US Patent 6,821,515; US Patent No. 6,399,063 and US Patent No. 6,387,371. Herceptin is available commercially from Genentech. As used herein, the term “a herceptin” includes includes trastuzumab and altered forms of, and derivatives of, trastuzumab. The term “a herceptin” includes agents that target the same epitope on the Her-2 receptor as targeted by trastuzumab. The epitope is known from H.S. Cho *et al.*, Structure of the extracellular region of HER2 alone and in complex with the Herceptin Fab, *Nature* 421 (2003), pp. 756–760.

HKI-272 and methods of making and formulating same have been described. See, e.g., US Published Patent Application No. 2005/0059678; US Patent No. 6,002,008, can also be used to prepare the substituted 3-quinoline compounds used this invention . In addition to the methods described in these documents, WO-9633978 and WO-9633980 describe methods that are useful for the preparation of these compounds. Although these methods describe the preparation of certain quinazolines, they are also applicable to the preparation of correspondingly substituted 3-cyanoquinolines .

As used in accordance with this invention, the term "treatment" means treating a mammal having a neoplasm by providing said mammal an effective amount of a combination of a two or three-way combination of the components selected from an mTOR inhibitor, a herceptin and/or HKI-272 with the purpose of inhibiting progression of the neoplastic disease, growth of a tumor in such mammal, eradication of the neoplastic disease, prolonging survival of the mammal and/or palliation of the mammal.

As used in accordance with this invention, the term "providing," with respect to providing an mTOR inhibitor with herceptin and/or HKI-272, means either directly administering the mTOR inhibitor, or administering a prodrug, derivative, or analog which will form an effective amount of the mTOR inhibitor within the body, along with herceptin and/or HKI-272 directly, or administering a prodrug, derivative, or analog which will form an effective amount of herceptin or HKI-272 in the body.

Use of a combination of an mTOR inhibitor (*e.g.*, temsirolimus), a herceptin and/or HKI-272 also provides for the use of combinations of each of the agents in which one, two, or all three agents is used at subtherapeutically effective dosages. Subtherapeutically effective dosages may be readily determined by one of skill in the art, in view of the teachings herein. In one embodiment, the subtherapeutically effective dosage is a dosage which is effective at a lower dosage when used in the combination regimen of the invention, as compared to the dosage that is effective when used alone. The invention further provides for one or more of the active agents in the combination of the invention to be used in a supratherapeutic amount, *i.e.*, at a higher dosage in the combination than when used alone. In this embodiment, the other active agent(s) may be used in a therapeutic or subtherapeutic amount.

The combinations of the invention may be in the form of a kit of parts. The invention therefore includes a product containing an mTOR inhibitor, a herceptin and/or HKI-272 as a combined preparation for simultaneous, separate or sequential delivery for the treatment of a neoplasm in a mammal in need thereof. In one embodiment, a product contains temsirolimus and a herceptin as a combined preparation for simultaneous, separate or sequential use in treating a neoplasm in a mammal in need thereof. Optionally, the product further contains an HKI-272. HKI-272 may be separately formulated, e.g., for oral delivery. In another embodiment, a product contains temsirolimus and an HKI-272 as a combined preparation for simultaneous, separate or sequential use in a neoplasm in a mammal in need thereof. Optionally, the product further contains herceptin. In yet another embodiment, the product contains a herceptin and an HKI-272. Optionally, the product further contains an mTOR inhibitor. In one embodiment, the neoplasm is metastatic breast cancer.

In one embodiment, a pharmaceutical pack contains a course of treatment of a neoplasm for one individual mammal, wherein the pack contains units of an mTOR inhibitor in unit dosage form and units of herceptin in unit dosage form, optionally further in combination with units of an HKI-272 in unit dosage form. In another embodiment, a pharmaceutical pack contains a course of treatment of a neoplasm for one individual mammal, wherein the pack contains units of an mTOR inhibitor in unit dosage form and units of HKI-272 in unit dosage form, optionally further in combination with units of herceptin in unit dosage form. In yet another embodiment, a pharmaceutical pack contains a course of treatment of a neoplasm for one individual mammal, wherein the pack contains units of a herceptin in unit dosage form and units of HKI-272 in unit dosage form, optionally further in combination with units of an mTOR inhibitor in unit dosage form. In one embodiment, a pharmaceutical pack as described herein contains a course of treatment of metastatic breast cancer for one individual mammal.

Administration of the compositions may be oral, intravenous, respiratory (*e.g.*, nasal or intrabronchial), infusion, parenteral (besides i.v., such as intralesional, intraperitoneal and subcutaneous injections), intraperitoneal, transdermal (including all administration across the surface of the body and the inner linings of bodily passages including epithelial and mucosal tissues), and vaginal (including intrauterine administration). Other routes of administration are also feasible, such as via liposome-mediated delivery; topical, nasal, sublingual, uretheral, intrathecal, ocular or otic delivery, implants, rectally, intranasally.

While the components of the invention may be delivered via the same route, a product or pack according to the invention may contain a rapamycin, such as temsirolimus, for delivery by a different route than that of the herceptin or the HKI-272, *e.g.*, one or more of the components may be delivered orally, while one or more of the others are administered intravenously. In one embodiment, temsirolimus is prepared for oral delivery, HKI-272 is prepared for oral delivery and herceptin is prepared for intravenous delivery. In another embodiment, both temsirolimus and herceptin are prepared for intravenous delivery. In still another embodiment, all of the components are prepared for oral delivery. Optionally, other active components may be delivered by the same or different routes as the mTOR inhibitor (*e.g.*, temsirolimus) or herceptin. Other variations would be apparent to one skilled in the art and are contemplated within the scope of the invention.

The mTOR inhibitor plus herceptin combination may be administered in the absence of HKI-272. In one embodiment, these are the sole active antineoplastic agents utilized in the regimen. In another embodiment, the mTOR inhibitor/herceptin combination is administered in combination with HKI-272.

The mTOR inhibitor plus HKI-272 combination may be administered in the absence of herceptin. In another embodiment, the mTOR inhibitor/HKI-272 combination is administered in combination with herceptin. In one embodiment, these two and three-way combinations are the sole active antineoplastic agents utilized in the regimen. In another embodiment, these two and three-way combinations may be utilized in further combination with other active agents.

The herceptin plus HKI-272 combination may be administered in the absence of an mTOR inhibitor. In another embodiment, the herceptin/HKI-272 combination is administered in combination with an mTOR inhibitor. In one embodiment, these two and three-way combinations are the sole active antineoplastic agents utilized in the regimen. In another embodiment, these two and three-way combinations may be utilized in further combination with other active agents.

As is typical with oncology treatments, dosage regimens are closely monitored by the treating physician, based on numerous factors including the severity of the disease, response to the disease, any treatment related toxicities, age, and health of the patient. Dosage regimens are expected to vary according to the route of administration.

It is projected that initial i.v. infusion dosages of the mTOR inhibitor (*e.g.*, temsirolimus) will be from about 5 to about 175 mg, or about 5 to about 25 mg, when administered on a weekly dosage regimen. It is projected that the oral dosage of an mTOR useful in the invention will be 10 mg/ week to 250 mg/week, about 20 mg/week to about 150 mg/week, about 25 mg/week to about 100 mg/week, or about

30 mg/week to about 75 mg/week. For rapamycin, the projected oral dosage will be between 0.1 mg/day to 25 mg/day. Precise dosages will be determined by the administering physician based on experience with the individual subject to be treated.

Other dosage regimens and variations are foreseeable, and will be determined through physician guidance. It is preferred that the mTOR inhibitor is administered by i.v. infusion or orally, preferably in the form of tablets or capsules.

For herceptin, single doses and multiple doses are contemplated. In one embodiment, a single loading dose of herceptin is administered as a 90-minute intravenous infusion in a range of about 4- 5 mg/kg on day 1, followed by about 2 mg/kg per week starting on day 8. Typically, 3 weeks is 1 cycle. From 1, to 2 to 3, weeks may be provided between cycles. Herceptin may also be given at a dose of 6 mg/kg once every 3 – 4 weeks. In addition, herceptin may also be given after completion of chemotherapy as maintenance therapy.

For an HKI-272, it is desired that a compound of the invention is in the form of a unit dose. Suitable unit dose forms include tablets, capsules and powders in sachets or vials. Such unit dose forms may contain from 0.1 to 300 mg of a compound of the invention and preferably from 2 to 100 mg. Still further preferred unit dosage forms contain 5 to 50 mg of a compound of the present invention. The compounds of the present invention can be administered at a dose range of about 0.01 to 100 mg/kg or preferably at a dose range of 0.1 to 10 mg/kg. In one embodiment, the compounds are administered orally from 1 to 6 times a day, more usually from 1 to 4 times a day. Alternatively, the compounds may be administered through another suitable route, e.g., intravenous. In still another embodiment, the compounds are administered once a week. In certain situations, dosing with the HKI-272 may be delayed or discontinued for a brief period (e.g., 1, 2 or three weeks) during the course of treatment. Such a delay or discontinuation may occur once, or more, during the course of treatment. The effective amount will be known to one of skill in the art; it will also be dependent upon the form of the compound. One of skill in the art could routinely perform empirical activity tests to determine the bioactivity of the compound in bioassays and thus determine what dosage to administer.

These regimens may be repeated, or alternated, as desired. Other dosage regimens and variations are foreseeable, and will be determined through physician guidance.

For example, in one embodiment, the regimen further comprises administration of a taxane, *e.g.*, docetaxel and paclitaxel [*e.g.*, a suspension of paclitaxel bound to albumen nanoparticles, which is available as Abraxane]. Paclitaxel may also be administered on a weekly schedule, at doses 60 - 100 mg/m2 administered over 1 hour, weekly, or 2 – 3 weekly doses followed by a one week rest. In one embodiment, paclitaxel is administered intravenously over 3 hours at a dose of 175 mg/m^2, optionally followed by cisplatin at a dose of 75 mg/m^2; or paclitaxel administered intravenously over 24 hours at a dose of 135 mg/m^2, optionally followed by cisplatin at a dose of 75 mg/m^2. In patients previously treated with therapy for carcinoma, paclitaxel can be injected at several doses and schedules. However, the optimal regimen is not yet clear. The recommended regimen is paclitaxel 135 mg/m^2 or 175 mg/m^2 administered intravenously over 3 hours every 3 weeks. These doses may be altered as needed or desired.

Still other active agents may be included in a combination with an mTOR inhibitor and herceptin, including, *e.g.*, chemotherapeutic agents, such as alkylating agents; hormonal agents (*i.e.*, estramustine, tamoxifen, toremifene, anastrozole, or letrozole); antibiotics (*i.e.*, plicamycin, bleomycin, mitoxantrone, idarubicin, dactinomycin, mitomycin, or daunorubicin); antimitotic agents (*i.e.*, vinblastine, vincristine, teniposide, or vinorelbine, available as Navelbine); topoisomerase inhibitors (*i.e.*, topotecan, irinotecan, etoposide, or doxorubicin, *e.g.*, CAELYX or Doxil, pegylated liposomal doxorubicin hydrochloride); and other agents (*i.e.*, hydroxyurea, altretamine, rituximab, paclitaxel, docetaxel, L-asparaginase, or gemtuzumab ozogamicin); biochemical modulating agents, imatib, EGFR inhibitors such as EKB-569 or other multi-kinase inhibitors, *e.g.*, those that targets serine/threonine and receptor tyrosine kinases in both the tumor cell and tumor vasculature, or immunomodulators (*i.e.*, interferons, IL-2, or BCG). Examples of suitable interferons include interferon α, interferon β, interferon γ, and mixtures thereof.

In one embodiment, the combination of an mTOR inhibitor and herceptin may be further combined with antineoplastic alkylating agents, *e.g.*, those described in US 2002-0198137A1. Antineoplastic alkylating agents are roughly classified, according to their structure or reactive moiety, into several categories which include nitrogen mustards, such as MUSTARGEN (meclorethamine), cyclophosphamide, ifosfamide, melphalan, and chlorambucil; azidines and epoxides, such as thiotepa, mitomycin C, dianhydrogalactitol, and dibromodulcitol; alkyl sulfinates, such as busulfan; nitrosoureas, such as bischloroethylnitrosourea (BCNU), cyclohexyl-chloroethylnitrosourea (CCNU), and methylcyclohexylchloroethylnitrosourea (MeCCNU); hydrazine and triazine derivatives, such as procarbazine, dacarbazine, and temozolomide; streptazoin, melphalan, chlorambucil, carmustine, methclorethamine, lomustine)and platinum compounds. Platinum compounds are platinum containing agents that react preferentially at the N7 position of guanine and adenine residues to form a variety of monofunctional and bifunctional adducts. (Johnson S W, Stevenson J P, O'Dwyer P J. Cisplatin and Its Analogues. In Cancer Principles & Practice of Oncology 6th Edition. ed. DeVita V T, Hellman S, Rosenberg S A. Lippincott Williams & Wilkins. Philadelphia 2001. p. 378.) These compounds include cisplatin, carboplatin, platinum IV compounds, and multinuclear platinum complexes.

The following are representative examples of alkylating agents of this invention. Meclorethamine is commercially available as an injectable (MUSTARGEN). Cyclophosphamide is commercially available as an injectable (cyclophosphamide, lyophilized CYTOXAN, or NEOSAR) and in oral tablets (cyclophosphamide or CYTOXAN). Ifosfamide is commercially available as an injectable (IFEX). Melphalan is commercially available as an injectable (ALKERAN) and in oral tablets (ALKERAN). Chlorambucil is commercially available in oral tablets (LEUKERAN). Thiotepa is commercially available as an injectable (thiotepa or THIOPLEX). Mitomycin is commercially available as an injectable (mitomycin or MUTAMYCIN). Busulfan is commercially available as an injectable (BUSULFEX) and in oral tablets (MYLERAN). Lomustine (CCNU) is commercially available in oral capsules (CEENU). Carmustine (BCNU) is commercially available as an intracranial implant (GLIADEL) and as an injectable (BICNU). Procarbazine is commercially available in oral capsules (MATULANE). Temozolomide is commercially available in oral capsules (TEMODAR). Cisplatin is commercially available as an injectable (cisplatin, PLATINOL, or PLATINOL-AQ). Carboplatin is commercially available as an injectable (PARAPLATIN). Oxiplatin is commercially available as ELOXATIN.

In another embodiment, a combination of the invention may further include treatment with an antineoplastic antimetabolite, such as is described in US Patent Publication No. US 2005-0187184A1 or US 2002-0183239 A1. As used in accordance with this invention, the term "antimetabolite" means a substance which is structurally similar to a critical natural intermediate (metabolite) in a biochemical pathway leading to DNA or RNA synthesis which is used by the host in that pathway, but acts to inhibit the completion of that pathway (*i.e.*, synthesis of DNA or RNA). More specifically, antimetabolites typically function by (1) competing with metabolites for the catalytic or regulatory site of a key enzyme in DNA or RNA synthesis, or (2) substitute for a metabolite that is normally incorporated into DNA or RNA, and thereby producing a DNA or RNA that cannot support replication. Major categories of antimetabolites include (1) folic acid analogs, which are inhibitors of dihydrofolate reductase (DHFR); (2) purine analogs, which mimic the natural purines (adenine or guanine) but are structurally different so they competitively or irreversibly inhibit nuclear processing of DNA or RNA; and (3) pyrimidine analogs, which mimic the natural pyrimidines (cytosine, thymidine, and uracil), but are structurally different so thy competitively or irreversibly inhibit nuclear processing of DNA or RNA.

The following are representative examples of antimetabolites of this invention. 5-Fluorouracil (5-FU; 5-fluoro-2,4(1H,3H)-pyrimidinedione) is commercially available in a topical cream (FLUOROPLEX or EFUDEX), a topical solution (FLUOROPLEX or EFUDEX), and as an injectable containing 50 mg/mL 5-fluorouracil (ADRUCIL or flurouracil). Floxuradine (2'-deoxy-5-fluorouridine) is commercially available as an injectable containing 500 mg/vial of floxuradine (FUDR or floxuradine). Thioguanine (2-amino-1,7-dihydro-6-H-purine-6-thione) is commercially available in 40 mg oral tablets (thioguanine). Cytarabine (4-amino-1-

(beta)-D-arabinofuranosyl-2(1H)-pyrimidinone) is commercially available as a liposomal injectable containing 10 mg/mL cytarabine (DEPOCYT) or as a liquid injectable containing between 1 mg - 1 g/vial or 20 mg/mL (cytarabine or CYTOSAR-U). Fludarabine (9-H-Purin-6-amine,2-fluoro-9-(5-O-phosphono-(beta)-D-a-rabinofuranosyl) is commercially available as a liquid injectable containing 50 mg/vial (FLUDARA). 6-Mercaptopurine (1,7-dihydro-6H-purine-6-thione) is commercially available in 50 mg oral tablets (PURINETHOL). Methotrexate (MTX; N-[4-[[(2,4-diamino-6-pteridinyl)methyl]methylamino]benzoyl]-L-glutamic acid) is commercially available as a liquid injectable containing between 2.5 - 25 mg/mL and 20 mg - 1 g/vial (methotrexate sodium or FOLEX) and in 2.5 mg oral tablets (methotrexate sodium). Gemcitabine (2'-deoxy-2',2'-difluorocytidine monohydrochloride ((beta)-isomer)), is commercially available as a liquid injectable containing between 200 mg - 1 g/vial (GEMZAR). Capecitabine (5'-deoxy-5-fluoro-N-[(pentyloxy)carbonyl]-cytidine) is commercially available as a 150 or 500 mg oral tablet (XELODA). Pentostatin ((R)-3-(2-deoxy-(beta)-D-erythro-pentofuranosyl)-3,6,7,- 8-tetrahydroimidazo[4,5-d][1,3]diazepin-8-ol) is commercially available as a liquid injectable containing 10 mg/vial (NIPENT). Trimetrexate (2,4-diamino-5-methyl-6-[(3,4,5-trimethoxyanilino)methyl]quinazoline mono-D-glucuronate) is commercially available as a liquid injectable containing between 25 - 200 mg/vial (NEUTREXIN). Cladribine (2-chloro-6-amino-9-(2-deoxy-(beta)-D-erythropento-furanosyl) purine) is commercially available as a liquid injectable containing 1 mg/mL (LEUSTATIN).

The term "biochemical modulating agent" is well known and understood to those skilled in the art as an agent given as an adjunct to anti-cancer therapy, which serves to potentate its antineoplastic activity, as well as counteract the side effects of the active agent, *e.g.*, an antimetabolite. Leucovorin and levofolinate are typically used as biochemical modulating agents for methotrexate and 5-FU therapy. Leucovorin (5-formyl-5,6,7,8-tetrahydrofolic acid) is commercially available as an injectable liquid containing between 5 - 10 mg/mL or 50 - 350 mg/vial (leucovorin calcium or WELLCOVORIN) and as 5 - 25 mg oral tablets (leucovorin calcium). Levofolinate (pharmacologically active isomer of 5-formyltetrahydrofolic acid) is commercially available as an injectable containing 25 - 75 mg levofolinate (ISOVORIN) or as 2.5 - 7.5 mg oral tablets (ISOVORIN).

In another embodiment, the combination of the invention further includes an active agent selected from among a kinase inhibitor. Particularly desirable are multi-kinase inhibitors target serine/threonine and receptor tyrosine kinases in both the tumor cell and tumor vasculature. Examples of suitable kinase inhibitors are Sorafenib (BAY 43-9006, Bayer, commercially available as NEXAVAR), which has been granted Fast Track status by the FDA for metastatic renal cell cancer. Another suitable farnesyltransferase inhibitor is Zarnestra (R115777, tipifarnib). Yet another compound is suntinib (SUTENT). Still other suitable compounds that target Ras/Raf/MEK and/or MAP kinases include, *e.g.*, avastin, ISIS 5132, and MEK inhibitors such as CI-1040 or PD 0325901.

As described herein, subtherapeutically effective amounts of herceptin and temsirolimus may be used to achieve a therapeutic effect when administered in combination. For example, herceptin may be provided at dosages of 5 to 50% lower, 10 to 25% lower, or 15 to 20% lower, when provided along with temsirolimus. For example, a resulting herceptin dosage can be from about 8 to 40 mg, or about 8 to 30 mg, or 8 to 25 mg. Subtherapeutically effective amounts of herceptin are expected to reduce the side-effects of herceptin treatment. The invention further provides for one or more of the active agents in the combination of the invention to be used in a supratherapeutic amount, i.e., at a higher dosage in the combination than when used alone. In this embodiment, the other active agent(s) may be used in a therapeutic or subtherapeutic amount.

The mTOR inhibitor, herceptin, HKI-272 or other active compounds used in the combination and products of the invention may be formulated in any suitable manner. For example, oral formulations containing the mTOR inhibitor (and optionally, other active compounds) useful in combination and products of this invention may comprise any conventionally used oral forms, including tablets, capsules, buccal forms, troches, lozenges and oral liquids, suspensions or solutions. Capsules may contain mixtures of the active compound(s) with inert fillers and/or diluents such as the pharmaceutically acceptable starches (*e.g.* corn, potato or tapioca starch), sugars, artificial sweetening agents, powdered celluloses, such as crystalline and microcrystalline celluloses, flours, gelatins, gums, etc. Useful tablet formulations may be made by conventional compression, wet granulation or dry granulation methods and utilize pharmaceutically acceptable diluents, binding agents, lubricants, disintegrants, surface modifying agents (including surfactants), suspending or stabilizing agents, including, but not limited to, magnesium stearate, stearic acid, talc, sodium lauryl sulfate, microcrystalline cellulose, carboxymethylcellulose calcium, polyvinylpyrrolidone, gelatin, alginic acid, acacia gum, xanthan gum, sodium citrate, complex silicates, calcium carbonate, glycine, dextrin, sucrose, sorbitol, dicalcium phosphate, calcium sulfate, lactose, kaolin, mannitol, sodium chloride, talc, dry starches and powdered sugar. Preferred surface modifying agents include nonionic and anionic surface modifying agents. Representative examples of surface modifying agents include, but are not limited to, poloxamer 188, benzalkonium chloride, calcium stearate, cetostearyl alcohol, cetomacrogol emulsifying wax, sorbitan esters, colloidal silicon dioxide, phosphates, sodium dodecylsulfate, magnesium aluminum silicate, and triethanolamine. Oral formulations herein may utilize standard delay or time release formulations to alter the absorption of the active compound(s). The oral formulation may also consist of administering the active ingredient in water or a fruit juice, containing appropriate solubilizers or emulsifiers as needed. Preferred oral formulations for rapamycin 42-ester with 3-hydroxy-2-(hydroxymethyl)-2-methylpropionic acid are described in US Patent Publication No. 2004/0077677 A1, published April 22, 2004.

In some cases it may be desirable to administer the compounds directly to the airways in the form of an aerosol.

The compounds may also be administered parenterally or intraperitoneally. Solutions or suspensions of these active compounds as a free base or pharmacologically acceptable salt can be prepared in water suitably mixed with a surfactant such as hydroxy-propylcellulose. Dispersions can also be prepared in glycerol, liquid polyethylene glycols and mixtures thereof in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases, the form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (*e.g.*, glycerol, propylene glycol and liquid polyethylene glycol), suitable mixtures thereof, and vegetable oils. Preferred injectable formulations for rapamycin 42-ester with 3-hydroxy-2-(hydroxymethyl)-2-methylpropionic acid are described in US Patent Publication No. 2004/0167152 A1, published August 26, 2004.

For the purposes of this disclosure, transdermal administrations are understood to include all administrations across the surface of the body and the inner linings of bodily passages including epithelial and mucosal tissues. Such administrations may be carried out using the present compounds, or pharmaceutically acceptable salts thereof, in lotions, creams, foams, patches, suspensions, solutions, and suppositories (rectal and vaginal).

Transdermal administration may be accomplished through the use of a transdermal patch containing the active compound and a carrier that is inert to the active compound, is non toxic to the skin, and allows delivery of the agent for systemic absorption into the blood stream via the skin. The carrier may take any number of forms such as creams and ointments, pastes, gels, and occlusive devices. The creams and ointments may be viscous liquid or semisolid emulsions of either the oil-in-water or water-in-oil type. Pastes comprised of absorptive powders dispersed in petroleum or hydrophilic petroleum containing the active ingredient may also be suitable. A variety of occlusive devices may be used to release the active ingredient into the blood stream such as a semi-permeable membrane covering a reservoir containing the active ingredient with or without a carrier, or a matrix containing the active ingredient. Other occlusive devices are known in the literature.

Suppository formulations may be made from traditional materials, including cocoa butter, with or without the addition of waxes to alter the suppository's melting point, and glycerin. Water soluble suppository bases, such as polyethylene glycols of various molecular weights, may also be used.

As used in this invention, the combination regimen can be given simultaneously or can be given in a staggered regimen, with the mTOR inhibitor being given at a different time during the course of chemotherapy than the herceptin. This time differential may range from several minutes, hours, days, weeks, or longer between administration of the at least two agents. Therefore, the term combination (or combined) does not necessarily mean administered at the same time or as a unitary dose, but that each of the components are administered during a desired treatment period. The agents may also be administered by different routes.

Pharmaceutical Packs/Kits:

The invention includes a product or pharmaceutical pack containing a course of an anti-neoplastic treatment for one individual mammal comprising one or more container(s) having one, one to four, or more unit(s) of an mTOR inhibitor (*e.g.*, temsirolimus) in unit dosage form and, optionally, one, one to four, or more unit(s) of herceptin, and optionally, another active agent.

In another embodiment, pharmaceutical packs contain a course of anti-neoplastic treatment for one individual mammal comprising a container having a unit of a rapamycin in unit dosage form, a containing having a unit of herceptin, and optionally, a container with another active agent. In other embodiments, the rapamycin is rapamycin, an ester (including a 42-ester, ether (including a 42-ether), oxime, hydrazone, or hydroxylamine of rapamycin. In another embodiment, the rapamycin is 42-O-(2-hydroxy)ethyl rapamycin.

In another embodiment, the rapamycin is temsirolimus, and the pack contains one or more container(s) comprising one, one to four, or more unit(s) of temsirolimus with the components described herein.

In some embodiments, the compositions of the invention are in packs in a form ready for administration. In other embodiments, the compositions of the invention are in concentrated form in packs, optionally with the diluent required to make a final solution for administration. In still other embodiments, the product contains a compound useful in the invention in solid form and, optionally, a separate container with a suitable solvent or carrier for the compound useful in the invention.

In still other embodiments, the above packs/kits include other components, *e.g.*, instructions for dilution, mixing and/or administration of the product, other containers, syringes, needles, etc. Other such pack/kit components will be readily apparent to one of skill in the art.

The following examples illustrate of the uses of the combinations of the invention. It will be readily understood that alterations or modifications, e.g., in the formulation of the components, the routes of delivery, and the dosing, can be made for reasons known to those of skill in the art.

EXAMPLE 1: COMBINATION REGIMEN OF TEMSIROLIMUS (CCI-779) and HERCEPTIN IN TREATMENT OF NEOPLASMS

Dosing begins at month 1, day 1 with weekly intravenous (IV) temsirolimus and herceptin (IV) at the dosages provided below.

Temsirolimus and herceptin can be administered simultaneously, consecutively, or on alternative days.

Temsirolimus is administered IV weekly over a 30-minute period using an in-line filter and an automatic dispensing pump. Optionally, antihistamine (diphenhydramine, 25 to 50 mg IV or the equivalent) is administered about 30 minutes prior to temsirolimus infusion.

A herceptin loading dose is administered IV weekly over a 90 minute period. Weekly doses are administered, which are typically half the amount of the loading dose. For example, a 4 mg/kg loading dose is typically followed by 2 mg/kg weekly doses. These amounts may be adjusted. In one embodiment, no loading dose is required and the same dose is administered throughout the course of treatment.

HERCEPTIN (mg/kg)	Temsirolimus Dose (mg)
2	15
4	25
6	50

Dose adjustments and/or delays for temsirolimus, and/or herceptin are permitted. For example, treatment may continue as described herein for six months, with weekly doses of temsirolimus. The herceptin may be provided on a weekly basis for a cycle, e.g., three weeks. Typically, 2 to 3 weeks is provided between cycles.

In certain situations, dosing with the temsirolimus may be delayed or discontinued for a brief period (e.g., 1, 2 or three weeks) during the regimen. Similarly, a cycle of treatment with herceptin may be shortened by one or more weeks, lengthened by one or more weeks, or the period between cycles delayed or eliminated. Such a delay or discontinuation may occur once, or more, during the course of treatment.

EXAMPLE 2: USE OF A COMBINATION REGIMEN OF HKI-272 AND TEMSIROLIMUS (CCI-779) IN TREATMENT OF NEOPLASMS

Dosing begins at month 1, day 1 with daily HKI-272 and weekly intravenous (IV) temsirolimus at the dosages provided below.

On month 1, day 1, HKI-272 is administered orally prior to temsirolimus. Temsirolimus is administered following HKI-272, preferably within 30 minutes.

Temsirolimus is administered IV weekly over a 30-minute period using an in-line filter and an automatic dispensing pump. Optionally, antihistamine (diphenhydramine, 25 to 50 mg IV or the equivalent) is administered about 30 minutes prior to temsirolimus infusion.

Thereafter, HKI-272 is taken orally once daily with food, preferably in the morning.

HKI-272 (mg)	**Temsirolimus Dose (mg)**
80	15
160	25
240	50

Dose adjustments and/or delays for HKI-272 and temsirolimus are permitted. For example, treatment may continue as described herein for six months, with daily doses of HKI-272 and weekly doses of temsirolimus. However, in certain situations, dosing with one or both drugs may be delayed or discontinued for a brief period (e.g., 1, 2 or three weeks) during the regimen course of treatment. Such a delay or discontinuation may occur once, or more, during the course of treatment.

EXAMPLE 3: USE OF A COMBINATION REGIMEN OF HKI-272, TEMSIROLIMUS (CCI-779), and HERCEPTIN IN TREATMENT OF NEOPLASMS

Dosing begins at month 1, day 1 with daily HKI-272 and weekly intravenous (IV) temsirolimus and herceptin (IV) at the dosages provided below.

On month 1, day 1, HKI-272 is administered orally prior to temsirolimus. Temsirolimus and herceptin are administered following HKI-272, preferably within 30 minutes.

Temsirolimus is administered IV weekly over a 30-minute period using an in-line filter and an automatic dispensing pump. Optionally, antihistamine (diphenhydramine, 25 to 50 mg IV or the equivalent) is administered about 30 minutes prior to temsirolimus infusion.

A herceptin loading dose is administered IV weekly over a 90 minute period. Weekly doses are administered, which are typically half the amount of the loading dose. For example, a 4 mg/kg loading dose is typically followed by 2 mg/kg weekly doses. These amounts may be adjusted. In one embodiment, no loading dose is required and the same dose is administered throughout the course of treatment.

Thereafter, HKI-272 is taken orally once daily with food, preferably in the morning.

HKI-272 (mg)	**HERCEPTIN (mg/kg)**	**Temsirolimus Dose (mg)**
80	2	15
160	4	25
240	6	50

Dose adjustments and/or delays for HKI-272, temsirolimus, and/or herceptin are permitted. For example, treatment may continue as described herein for six months, with daily doses of HKI-272 and a weekly dose of temsirolimus. The herceptin may be provided on a weekly basis for a cycle, e.g., three weeks. Typically, 2 to 3 weeks is provided between cycles. However, in certain situations, dosing with the HKI-272 and/or temsirolimus may be delayed or discontinued for a brief period (*e.g.*, 1, 2 or three weeks) during the regimen or course of treatment. Such a delay or discontinuation may occur once, or more, during the course of treatment.

Similarly, a cycle of treatment with herceptin may be shortened by one or more weeks, lengthened by one or more weeks, or the period between cycles delayed or eliminated. Such a delay or discontinuation may occur once, or more, during the course of treatment.

EXAMPLE 4: USE OF A COMBINATION REGIMEN OF HKI-272 and HERCEPTIN IN TREATMENT OF NEOPLASMS

The antineoplastic activity of the HKI-272 plus herceptin combination was confirmed in *in vitro* standard pharmacological test procedure. The following briefly describes the procedure used and the results obtained.

The combination was tested in three breast cancer cell lines of differing genotypes. More particularly, BT474 [HER-2+ (amplified); ATCC HTB-20] and is highly sensitive to both HKI-272 and Herceptin. MDA-MB-361 [HER-2^+ (non-amplified); adenocarcinoma; ATCC HTB 27] has lower levels of HER-2 without amplification and less sensitive to both herceptin and HKI-272. MCF-7 [HER-2$^-$, EGFR-; adenocarcinoma; ATCC HTB22] has no HER-2 and is resistant to both Herceptin and HKI-272.

Cells from each of these cell lines were incubated in the presence of a range of concentrations (0.0041, 0.012, 0.037, 0.11, 0.33, 0.1, 3 μg/mg) for each drug. The cells were maintained in RPMI 1640 medium (Life Technologies, Inc., Gaithersburg, Md.) supplemented with 10% fetal bovine serum (FBS, Life Technologies) and 50 μg/ml gentamicin (Life Technologies) under 7% CO_2 at 37 °C. Cells were plated in 96-well microtiter dishes (12,000 cells/well for BT474 Cells, 6000 cells/well MCF-7 Cells and 10,000 cells/well for MDA-MB-361 Cells) in 100 μl RPMI 1640 medium containing 5% FBS and 50 μg/ml gentamicin and incubated overnight at 37 °C. Compound dilutions were prepared in the same medium, at 2X final concentration, and 100 μl of the drug dilution was added to the cell-containing wells.

Serial dilutions of one compound were prepared in the presence of a fixed dose of a second compound. Alternatively, a checkerboard dilution series was employed. Cells were cultured for three days in the presence of the drugs. Untreated cells were included as controls. The percentage of surviving cells was determined using sulforhodamine B (SRB, Sigma-Aldrich, St Louis, Mo.), a protein binding dye. Cellular protein was precipitated in each well by the addition of 50 μl 50% cold trichloroacetic acid. After 1 hour, the plates were washed extensively in water and dried. SRB dye reagent (0.4% SRB in 1% acetic acid, 80 μl per well) was added and plates were kept at room temperature for ten minutes. Plates were then washed thoroughly in 1% acetic acid and dried. Cell-associated dye was dissolved in 10 mM Tris (150 μl) and the absorbance was read at 540 nm in a microtiter plate reader. The concentration of compound that caused a fixed percentage inhibition of growth was determined by plotting cell survival (relative to untreated cells) against the compound dose.

A model for studying drug interactions has been described by Prichard and Shipman [Antiviral Research. 14:181-206 (1990); Prichard, MN, et al., 1993. MacSynergy II. Version 1.0. User's manual. University of Michigan, Ann Arbor.] This is a 3-dimensional model: one for each drug and the third for the biological effect. Theoretical additive interactions are calculated from the individual dose-response curves, based on a dissimilar sites model of additivity (Bliss independence). The calculated additive surface, representing predicted cytotoxicity is subtracted from the experimental surface to reveal areas of enhanced toxicity (synergy) or reduced toxicity (antagonism). The resulting surface appears as a horizontal plane at 0% inhibition above the calculated additive surface, if the interaction is additive. Peaks and valleys deviating from this plane are indicative of synergy and antagonism, respectively. MacSynergy II, a Microsoft Excel-based software was used to perform all calculations automatically. This spreadsheet calculates the theoretical additive interactions, and locates and quantifies synergistic or antagonistic interactions that are significant at the 95% confidence levels. The results were plotted as a 3-dimensional plot, or as a contour plot with the plane at 0% representing additive interaction, and peaks and valleys representing areas of synergy or antagonism, respectively, between the two drugs.

For purposes of this study, the Pritchard and Shipman method was modified to allow determination of the combination effects at different levels of statistical significance (p-values 0.05, 0.01, 0.001). A p-value of 0.05 is considered significant. The method of estimating statistical variability within each experiment was also modified. Variability was determined across all compound combinations, whereas in the original version, variability was estimated separately for each compound combination. It is believed that better estimates of the variability are obtained with the modified approach. In general, single points of synergy or antagonism are not considered representative of either synergistic or antagonistic activity. Thus, single point peaks or valleys are disregarded in the analysis. Furthermore, peaks or valleys that occur only along single concentration of one of the compounds are also disregarded, if no synergy or antagonism is observed at the adjacent, flanking concentrations. Finally, all experiments are repeated at least twice and determinations of synergy and antagonism are made by examination of all the data.

Figs. 1 – 3 provide the results from a single set of experiments. In MDA-MB-361 cells, there is an area of antagonism at 0.11 – 3 μg/mL herceptin for at a concentration of 0.012 μg/mL HKI-272 at the 95% confidence level. In MCF7 cells, there is an area of synergy at 0.037 – 0.33 μg/mL herceptin for at a concentration of 0.11 μg/mL HKI-272 at the 95% confidence level. For the BT474 cells, there is an area of antagonism at 0.33 – 1 μg/mL herceptin for at a concentration of 0.11 μg/mL HKI-272 at the 95% confidence level. When repeated at the 99% confidence level, no statistically significant areas of antagonism or synergy were found. Based on the above criteria, the combination of herceptin and HKI-272 is considered additive across all concentrations.

The results of these standard pharmacological test procedures derived from multiple independent experiments, indicate that combinations of HKI-272 are not significantly antagonistic or synergistic, but are additive over a range of concentrations. These data support the use of the combinations in the treatment of HER2+ cancers. As these combinations contain at least two active antineoplastic agents, the use of such combinations also provides for the use of combinations of each of the agents in which one or both of the agents is used at subtherapeutically effective dosages, thereby lessening toxicity associated with the individual chemotherapeutic agent.

It will be clear to one of skill in the art that modifications can be made to the specific embodiments described herein without departing from the scope of the invention.

What is claimed is:

1. Use of an effective amount of a combination of active components consisting of:

i) CCI 779 (temsirolimus); and

ii) (E)-N-{4-[3-chloro-4-(2-pyridinylmethoxy)anilino]-3-cyano-7-ethoxy-6-quinolinyl}-4-(dimethylamino)-2-butenamide, or a pharmaceutically acceptable salt thereof, for treating a HER2 positive lung cancer or a HER2 positive breast cancer.

2. The use of claim 1, wherein the effective amount of the CCI-779 (temsirolimus) is in the range of between 5-175 mg.

3. The use of any one of claims 1-2, wherein the effective amount of the (E)-N-{4-[3-chloro-4-(2-pyridinylmethoxy)anilino]-3-cyano-7-ethoxy-6-quinolinyl}-4-(dimethylamino)-2-butenamide, or pharmaceutically acceptable salt thereof, is in the range of between 0.1-300 mg.

4. The use of any one of claims 1-3, wherein the effective amount of the (E)-N-{4-[3-chloro-4-(2-pyridinylmethoxy)anilino]-3-cyano-7-ethoxy-6-quinolinyl}-4-(dimethylamino)-2-butenamide, or pharmaceutically acceptable salt thereof, is 80 mg.

5. The use of any one of claims 1-3, wherein the effective amount of the (E)-N-{4-[3-chloro-4-(2-pyridinylmethoxy)anilino]-3-cyano-7-ethoxy-6-quinolinyl}-4-(dimethylamino)-2-butenamide, or pharmaceutically acceptable salt thereof, is 160 mg.

6. The use of any one of claims 1-3, wherein the effective amount of the (E)-N-{4-[3-chloro-4-(2-pyridinylmethoxy)anilino]-3-cyano-7-ethoxy-6-quinolinyl}-4-(dimethylamino)-2-butenamide, or pharmaceutically acceptable salt thereof, is 240 mg.

7. The use of any one of claims 1-6, wherein the effective amount of the CCI-779 (temsirolimus) is 15 mg, 25 mg, or 50 mg.

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8. The use of any one of claims 1-7, wherein the active components are provided as a combined preparation for simultaneous use in treating the HER2 positive lung cancer or the HER2 positive breast cancer in a patient in need thereof, or as a preparation for separate, sequential or staggered use in treating the HER2 positive lung cancer or the HER2 positive breast cancer in a patient in need thereof.

9. The use of any one of claims 1-8, wherein the active components of the combination are provided to the patient in a formulation suitable for one or more of the following administration routes: oral, as an aerosol, parenteral, intraperitoneal, transdermal, or as a suppository.

10. The use of any one of claims 1-9, wherein the provided active components are adapted for administration to the patient using the same route of administration or using different routes of administration.

11. The use of any one of claims 1-10, wherein the combination of active components are adapted for intravenous delivery.

12. The use of any one of claims 1-10, wherein the combination of active components are adapted for oral delivery.

13. The use of any one of claims 1-12, wherein the active components are contained in a pharmaceutical pack or kit.

14. The use of any one of claims 1-13, wherein the HER2 positive lung cancer comprises bronchioalveolar carcinoma or non-small cell lung cancer.

15. The use of any one of claims 1-13, wherein the HER2 positive lung cancer is non-small cell lung cancer.

16. The use of any one of claims 1-13, wherein the HER2 positive breast cancer is metastatic breast cancer.

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17. Use of a combination of active components consisting of:

i) CCI 779 (temsirolimus); and

ii) (E)-N-{4-[3-chloro-4-(2-pyridinylmethoxy)anilino]-3-cyano-7-ethoxy-6-quinolinyl}-4-(dimethylamino)-2-butenamide, or a pharmaceutically acceptable salt thereof, in the manufacture of a medicament for treating a HER2 positive lung cancer or a HER2 positive breast cancer.

18. The use of claim 17, wherein the effective amount of the CCI 779 (temsirolimus) is in the range of between 5-175 mg.

19. The use of any one of claims 17-18, wherein the effective amount of the (E)-N-{4-[3-chloro-4-(2-pyridinylmethoxy)anilino]-3-cyano-7-ethoxy-6-quinolinyl}-4-(dimethylamino)-2-butenamide, or pharmaceutically acceptable salt thereof, is in the range of between 0.1-300 mg.

20. The use of any one of claims 17-19, wherein the effective amount of the (E)-N-{4-[3-chloro-4-(2-pyridinylmethoxy)anilino]-3-cyano-7-ethoxy-6-quinolinyl}-4-(dimethylamino)-2-butenamide, or pharmaceutically acceptable salt thereof, is 80 mg.

21. The use of any one of claims 17-19, wherein the effective amount of the (E)-N-{4-[3-chloro-4-(2-pyridinylmethoxy)anilino]-3-cyano-7-ethoxy-6-quinolinyl}-4-(dimethylamino)-2-butenamide, or pharmaceutically acceptable salt thereof, is 160 mg.

22. The use of any one of claims 17-19, wherein the effective amount of the (E)-N-{4-[3-chloro-4-(2-pyridinylmethoxy)anilino]-3-cyano-7-ethoxy-6-quinolinyl}-4-(dimethylamino)-2-butenamide, or pharmaceutically acceptable salt thereof, is 240 mg.

23. The use of any one of claims 17-22, wherein the effective amount of the CCI-779 (temsirolimus) is 15 mg, 25 mg, or 50 mg.

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24. The use of any one of claims 17-23, wherein the active components are provided as a combined preparation for simultaneous use in treating the HER2 positive lung cancer or the HER2 positive breast cancer in a patient in need thereof, or as a preparation for separate, sequential or staggered use in treating the HER2 positive lung cancer or the HER2 positive breast cancer in a patient in need thereof.

25. The use of any one of claims 17-24, wherein the active components of the combination are provided to the patient in a formulation suitable for one or more of the following administration routes: oral, as an aerosol, parenteral, intraperitoneal, transdermal, or as a suppository.

26. The use of any one of claims 17-25, wherein the provided active components are adapted for administration to the patient using the same route of administration or using different routes of administration.

27. The use of any one of claims 17-26, wherein the combination of active components are adapted for intravenous delivery.

28. The use of any one of claims 17-26, wherein the combination of active components are adapted for oral delivery.

29. The use of any one of claims 17-28, wherein the active components are contained in a pharmaceutical pack or kit.

30. The use of any one of claims 17-29, wherein the HER2 positive lung cancer comprises bronchioalveolar carcinoma or non-small cell lung cancer.

31. The use of any one of claims 17-29, wherein the HER2 positive lung cancer is non-small cell lung cancer.

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32. The use of any one of claims 17-29, wherein the HER2 positive breast cancer is metastatic breast cancer.

33. A product, consisting of:

i) an effective amount of CCI 779 (temsirolimus); and

ii) an effective amount of (E)-N-{4-[3-chloro-4-(2-pyridinylmethoxy)anilino]-3-cyano-7-ethoxy-6-quinolinyl}-4-(dimethylamino)-2-butenamide or a pharmaceutically acceptable salt thereof;

as a combined preparation for simultaneous use in treating a HER2 positive cancer in a patient in need thereof, or as a preparation for separate or sequential use in treating a HER2 positive cancer in a patient in need thereof, wherein the HER2 positive cancer is a HER2 positive lung cancer or a HER2 positive breast cancer.

34. The product of claim 33, wherein the effective amount of the CCI-779 (temsirolimus) is in the range of between 5-175 mg.

35. The product of any one of claims 33-34, wherein the effective amount of the (E)-N-{4-[3-chloro-4-(2-pyridinylmethoxy)anilino]-3-cyano-7-ethoxy-6-quinolinyl}-4-(dimethylamino)-2-butenamide, or pharmaceutically acceptable salt thereof, is in the range of between 0.1-300 mg.

36. The product of any one of claims 33-35, wherein the effective amount of the (E)-N-{4-[3-chloro-4-(2-pyridinylmethoxy)anilino]-3-cyano-7-ethoxy-6-quinolinyl}-4-(dimethylamino)-2-butenamide, or pharmaceutically acceptable salt thereof, is 80 mg.

37. The product of any one of claims 33-35, wherein the effective amount of the (E)-N-{4-[3-chloro-4-(2-pyridinylmethoxy)anilino]-3-cyano-7-ethoxy-6-quinolinyl}-4-(dimethylamino)-2-butenamide, or pharmaceutically acceptable salt thereof, is 160 mg.

38. The product of any one of claims 33-35, wherein the effective amount of the (E)-N-{4-[3-chloro-4-(2-pyridinylmethoxy)anilino]-3-cyano-7-ethoxy-6-quinolinyl}-4-(dimethylamino)-2-butenamide, or pharmaceutically acceptable salt thereof, is 240 mg.

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39. The product of any one of claims 33-38, wherein the effective amount of the CCI-779 (temsirolimus) is 15 mg, 25 mg, or 50 mg.

40. The product any one of claims 33-39, wherein the HER2 positive lung cancer comprises bronchioalveolar carcinoma or non-small cell lung cancer.

41. The product of any one of claims 33-39, wherein the HER2 positive lung cancer is non-small cell lung cancer.

42. The product of any one of claims 33-39, wherein the HER2 positive breast cancer is metastatic breast cancer.

43. A pharmaceutical pack, comprising a course of an anti-neoplastic treatment for a HER2 positive cancer for one individual patient, wherein the pack consists of:

i) at least one unit of CCI 779 (temsirolimus) in unit dosage form; and

ii) at least one unit of (E)-N-{4-[3-chloro-4-(2-pyridinylmethoxy)anilino]-3-cyano-7-ethoxy-6-quinolinyl}-4-(dimethylamino)-2-butenamide or a pharmaceutically acceptable salt thereof, in unit dosage form;

wherein the HER2 positive cancer is a HER2 positive lung cancer or a HER2 positive breast cancer.

44. The pharmaceutical pack of claim 43, wherein the at least one unit dosage of the CCI-779 (temsirolimus) is in an amount in the range of between 5-175 mg.

45. The pharmaceutical pack of any one of claims 43-44, wherein the at least one unit dosage of the (E)-N-{4-[3-chloro-4-(2-pyridinylmethoxy)anilino]-3-cyano-7-ethoxy-6-quinolinyl}-4-(dimethylamino)-2-butenamide, or pharmaceutically acceptable salt thereof, is in an amount in the range of between 0.1-300 mg.

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46. The pharmaceutical pack of any one of claims 43-45, wherein the at least one unit dosage of the (E)-N-{4-[3-chloro-4-(2-pyridinylmethoxy)anilino]-3-cyano-7-ethoxy-6-quinolinyl}-4-(dimethylamino)-2-butenamide, or pharmaceutically acceptable salt thereof, is in an amount of 80 mg.

47. The pharmaceutical pack of any one of claims 43-45, wherein the at least one unit dosage of the (E)-N-{4-[3-chloro-4-(2-pyridinylmethoxy)anilino]-3-cyano-7-ethoxy-6-quinolinyl}-4-(dimethylamino)-2-butenamide, or pharmaceutically acceptable salt thereof, is in an amount of 160 mg.

48. The pharmaceutical pack of any one of claims 43-45, wherein the at least one unit dosage of the (E)-N-{4-[3-chloro-4-(2-pyridinylmethoxy)anilino]-3-cyano-7-ethoxy-6-quinolinyl}-4-(dimethylamino)-2-butenamide, or pharmaceutically acceptable salt thereof, is in an amount of 240 mg.

49. The pharmaceutical pack of any one of claims 43-48, wherein the at least one unit dosage of the CCI-779 (temsirolimus) is in an amount of 15 mg, 25 mg, or 50 mg.

50. The pharmaceutical pack of any one of claims 43-49, wherein the HER2 positive lung cancer comprises bronchioalveolar carcinoma or non-small cell lung cancer.

51. The pharmaceutical pack of any one of claims 43-49, wherein the HER2 positive lung cancer is non-small cell lung cancer.

52. The pharmaceutical pack of any one of claims 43-49, wherein the HER2 positive breast cancer is metastatic breast cancer.

53. A pharmaceutical composition for treating a HER2 positive cancer in a patient, wherein the HER2 positive cancer is a HER2 positive lung cancer or a HER2 positive breast cancer, and wherein the pharmaceutical composition consists of:

i) at least one unit of CCI 779 (temsirolimus) in unit dosage form;

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ii) at least one unit of (E)-N-{4-[3-chloro-4-(2-pyridinylmethoxy)anilino]-3-cyano-7-ethoxy-6-quinolinyl}-4-(dimethylamino)-2-butenamide or a pharmaceutically acceptable salt thereof, in unit dosage form; and

iii) at least one pharmaceutically acceptable carrier.

54. The pharmaceutical composition of claim 53, wherein the at least one unit dosage of the CCI 779 (temsirolimus) is in an amount in the range of between 5-175 mg.

55. The pharmaceutical composition of any one of claims 53-54, wherein the at least one unit dosage of the (E)-N-{4-[3-chloro-4-(2-pyridinylmethoxy)anilino]-3-cyano-7-ethoxy-6-quinolinyl}-4-(dimethylamino)-2-butenamide, or pharmaceutically acceptable salt thereof, is in an amount in the range of between 0.1-300 mg.

56. The pharmaceutical composition of any one of claims 53-55, wherein the at least one unit dosage of the (E)-N-{4-[3-chloro-4-(2-pyridinylmethoxy)anilino]-3-cyano-7-ethoxy-6-quinolinyl}-4-(dimethylamino)-2-butenamide, or pharmaceutically acceptable salt thereof, is in an amount of 80 mg.

57. The pharmaceutical composition of any one of claims 53-55, wherein the at least one unit dosage of the (E)-N-{4-[3-chloro-4-(2-pyridinylmethoxy)anilino]-3-cyano-7-ethoxy-6-quinolinyl}-4-(dimethylamino)-2-butenamide, or pharmaceutically acceptable salt thereof, is in an amount of 160 mg.

58. The pharmaceutical composition of any one of claims 53-55, wherein the at least one unit dosage of the (E)-N-{4-[3-chloro-4-(2-pyridinylmethoxy)anilino]-3-cyano-7-ethoxy-6-quinolinyl}-4-(dimethylamino)-2-butenamide, or pharmaceutically acceptable salt thereof, is in an amount of 240 mg.

59. The pharmaceutical composition of any one of claims 53-58, wherein the at least one unit dosage of the CCI-779 (temsirolimus) is in an amount of 15 mg, 25 mg, or 50 mg.

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60. The pharmaceutical composition of any one of claims 53-59, wherein the HER2 positive lung cancer comprises bronchioalveolar carcinoma or non-small cell lung cancer.

61. The pharmaceutical composition of any one of claims 53-59, wherein the HER2 positive lung cancer is non-small cell lung cancer.

62. The pharmaceutical composition of any one of claims 53-59, wherein the HER2 positive breast cancer is metastatic breast cancer.

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FIG. 1

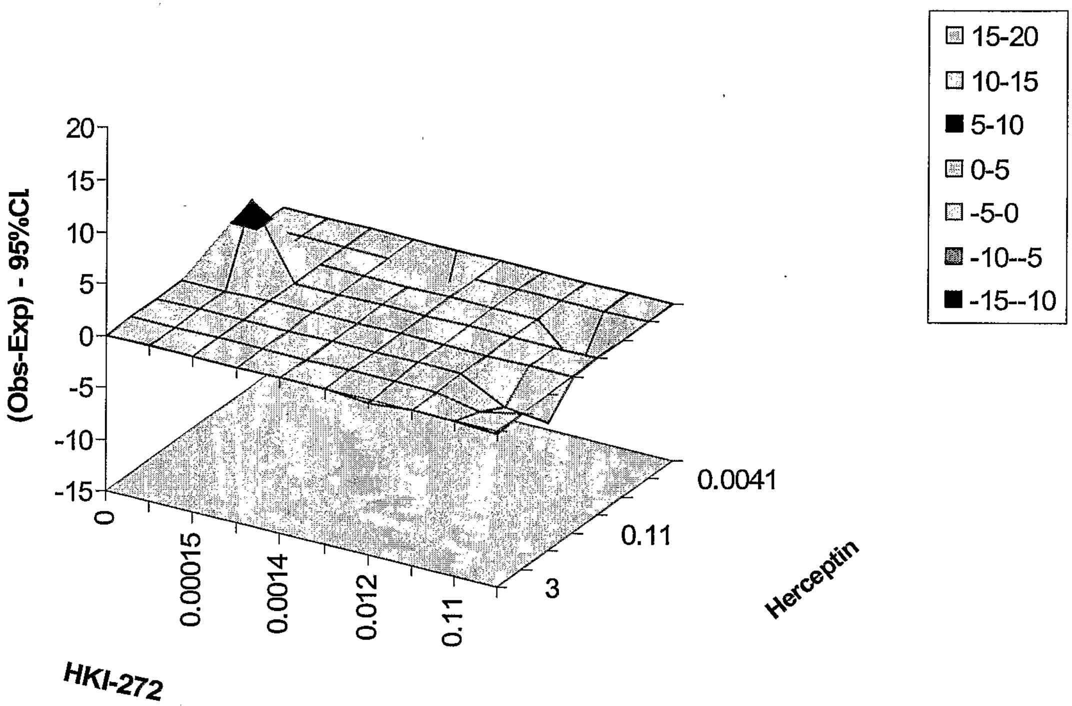
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FIG. 2

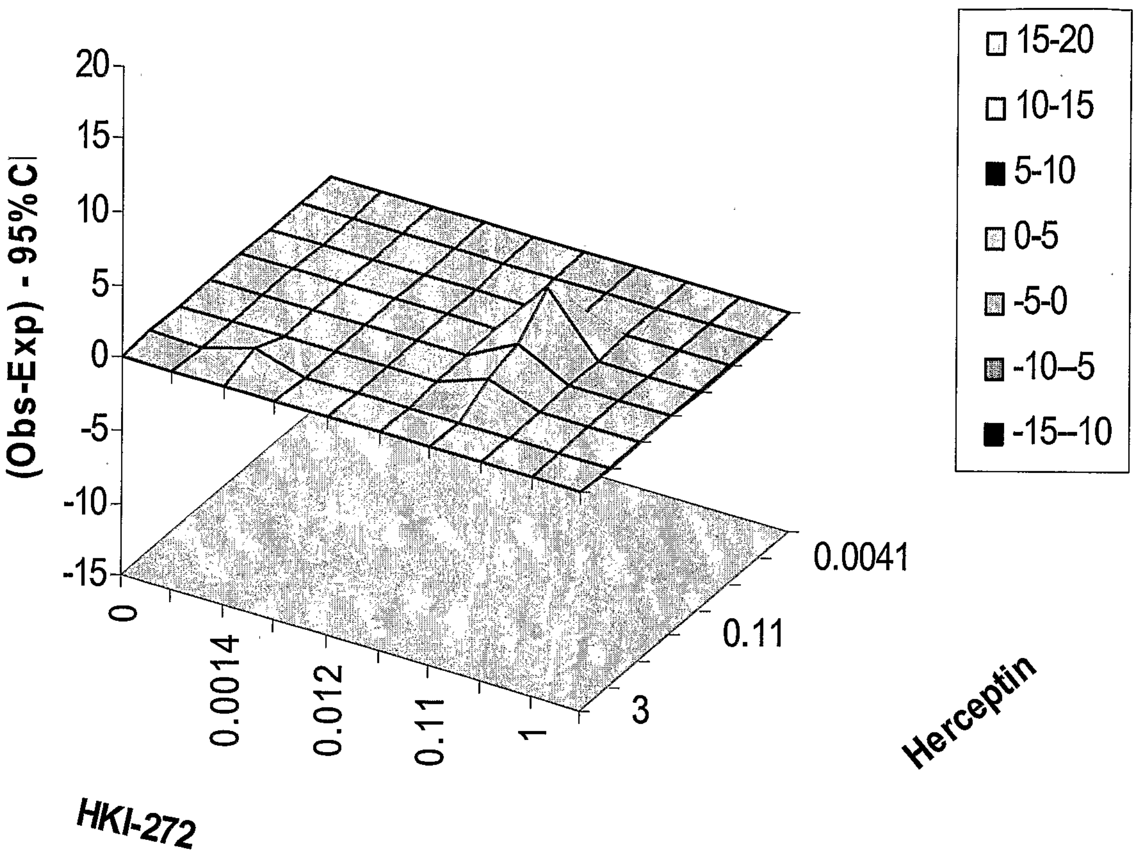
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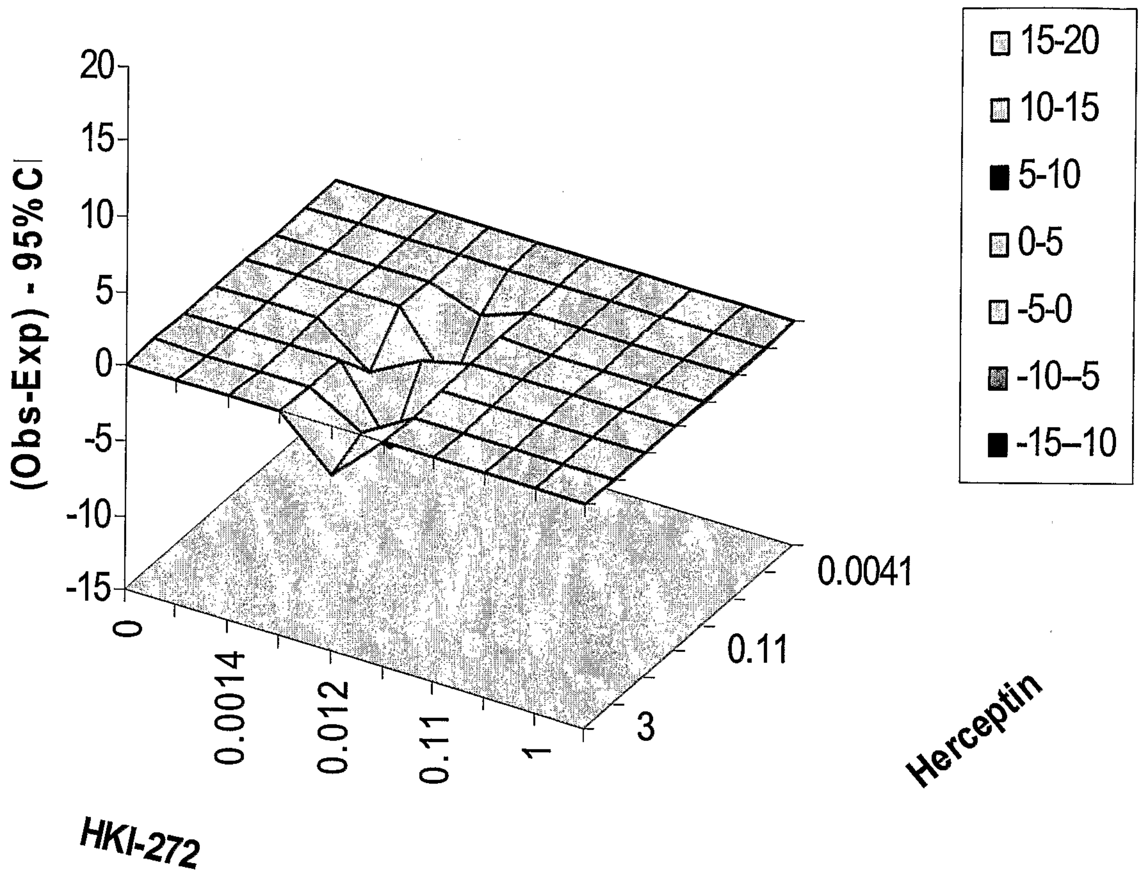
FIG. 3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0.012
0.11
1
HKI-272
0.0041
0.11
3
Herceptin