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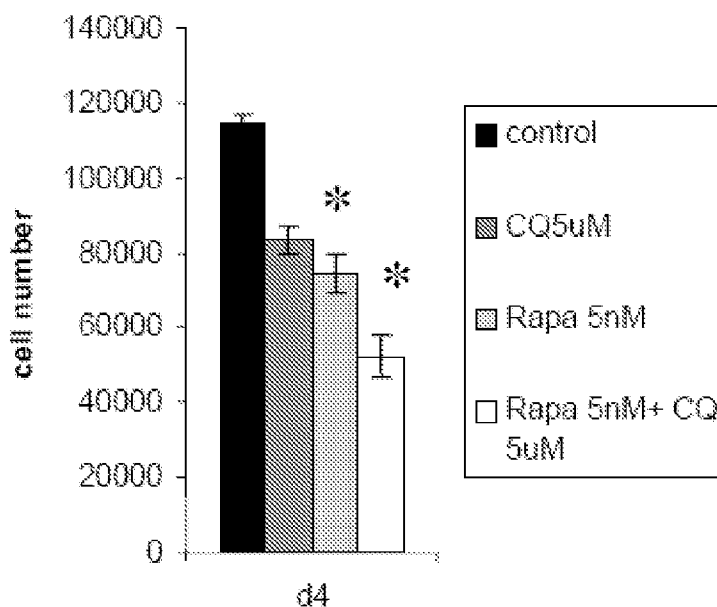


FIG. 1

(57) Abstract: The present invention is directed to methods of using a sirolimus drug and chloroquine or a quinoline derivative in combination. Methods of use are also described.



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COMBINATION ANTINEOPLASTIC THERAPY

CROSS REFERENCE TO RELATED APPLICATIONS

This applications claims the benefit of U.S. Provisional Application No. 61/177,697, filed May 13, 2009, the entirety of which is incorporated herein.

5 TECHNICAL FIELD

The invention is directed to methods of treating cancer using a sirolimus compound and a chloroquine derivative.

BACKGROUND

Sirolimus, also known as rapamycin and marketed in the United States under the
10 tradename RAPAMUNE® (Wyeth, Collegeville, PA), is currently indicated for the prevention of organ rejection after kidney transplant. Rapamycin derivatives useful for prevention of organ rejection have also been developed. For example, everolimus, marketed in the United States under the tradename CERTICAN® (Novartis, East Hanover, NJ) is indicated for the prevention of organ rejection after heart or kidney transplantation.

15 Rapamycin has demonstrated anti-proliferative effects and has been shown to inhibit the progression of dermal Kaposi's sarcoma in patients with kidney transplants. S. DiPaolo, et al. Monitoring Antitumor Efficacy of Rapamycin in Kaposi Sarcoma, *American Journal of Kidney Diseases*, vol. 49, issue 3, 462-470. Rapamycin, in combination with doxorubicin, has been investigated for the treatment of melanoma. M. Romano, et al. Rapamycin inhibits doxorubicin-
20 induced NF-kB/Rel nuclear activity and enhances the apoptosis of melanoma cells, *European Journal of Cancer*, vol. 40, issue 18, 2829-2836. Such data has prompted researchers to investigate the anti-proliferative potential of rapamycin and its derivatives. For example, temsirolimus, marketed in the United States under the tradename TORISEL® (Wyeth, Collegeville, PA), is currently indicated for the treatment of advanced renal cell carcinoma.
25 Deforolimus is currently in Phase 2 clinical studies to evaluate deforolimus in patients with breast, endometrial, prostate cancer, and non-small cell lung cancer. While these rapamycin derivatives have demonstrated efficacy in treating some types of cancer, more research and development is needed to improve efficacy and response and survival rates.

Chloroquine is a 4-amino-substituted quinoline that has been used for over 60 years for
30 malaria prophylaxis and treatment, rheumatoid arthritis treatment, and HIV treatment.

Chloroquinoline derivatives such as hydroxylchloroquine are used to treat rheumatoid arthritis and systemic lupus erythematosus. Recent studies indicate that chloroquine may inhibit cell growth and may induce cell death in lung cancer cells. C. Fan, et al. *Bioorg. Med. Chem.* 2006 May 1;14(9):3218-22. In 2006, researchers reported that adding chloroquine to standard
5 chemotherapy and radiotherapy regimes for glioblastoma multiforme might improve survival rates of cancer patients receiving standard chemotherapy and radiotherapy. *Annals of Internal Medicine*, 7 March 2006, vol. 144, issue 5, 1-31.

To date, there have been no reports of treating cancer in patients using a combination of a sirolimus drug and chloroquine or substituted quinoline.

10 SUMMARY

The present invention is directed to methods of simultaneously inducing and impairing the completion of cancer cell autophagy in a patient having or suspected of having a neoplasm. The invention comprises co-administering to the patient a sirolimus drug together with a substituted quinoline, the amounts of said sirolimus drug and substituted quinoline, in
15 combination, being effective for limiting the growth and proliferation. This combination effect can be measured, for example, by an increase in autophagic vesicles in tumor tissue or surrogate tissue as compared to pretreatment samples or treatment with either a sirolimus drug or substituted quinoline alone. Also within the scope of the invention are methods of treating cancer in a patient comprising administering to the patient a therapeutically effective amount of a
20 sirolimus drug and chloroquine or a substituted quinoline. Pharmaceutical compositions and kits are also described.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 depicts cell numbers of LN229 cells expressing GFP-LC3 after treatment with sirolimus ("rapa"), chloroquine (CQ), and the combination of rapamycin and chloroquine.

25 FIG. 2 depicts digital fluorescence images of LN229 GFP-LC3 cells exposed to increasing doses of rapamycin (top) and chloroquine (CQ) (bottom). Punctate fluorescence indicates the accumulation of LC3-positive autophagosomes.

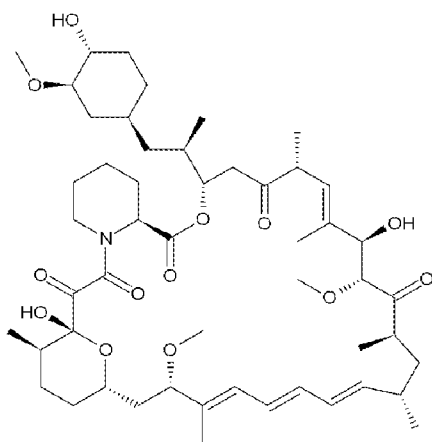
FIG. 3 depicts electron micrographs of LN229 GFP-LC3 cells untreated and treated with rapamycin, chloroquine (CQ), and a combination of rapamycin and chloroquine. Top left panel
30 depicts untreated sample. Top right panel depicts treatment with rapamycin only. Lower left panel depicts treatment with chloroquine only. Lower right panel depicts treatment with both rapamycin and chloroquine. "↑" identify autophagosomes.

DETAILED DESCRIPTION OF ILLUSTRATIVE EMBODIMENTS

While both sirolimus and chloroquine have been reported to independently exhibit antitumor activity, prior to the present disclosure, there have been no reports of methods of treating cancer using a combination of a sirolimus drug and chloroquine (or a substituted quinoline). As set forth herein, the effects of the combination of a sirolimus drug and chloroquine (or a substituted quinoline) results in an inhibition of cancer cell growth that is greater than the sum of the inhibition of the individual molecules. That is, the combination of a sirolimus drug and chloroquine (or a substituted quinoline) results in cancer cell growth inhibition that is greater than one skilled in the art would have expected, based on the cancer cell inhibition effects of the sirolimus drug and chloroquine (or substituted quinoline) independently.

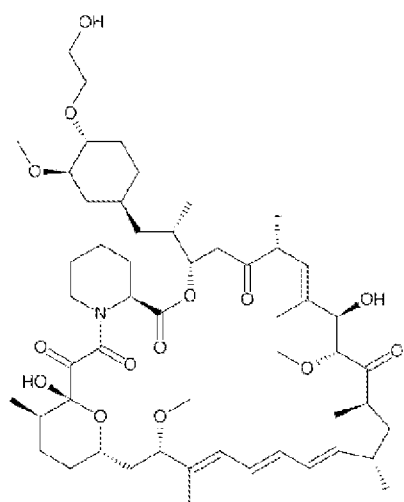
While not wishing to be bound to any particular theory, it is postulated that the combination of the sirolimus drug and chloroquine (or a substituted quinoline) results in simultaneous induction of autophagy (likely induced by the sirolimus drug) and impairment of the clearance of accumulating autophagic vesicles (likely impaired by the chloroquine or substituted quinoline) leading to the accumulation of a greater number of ineffective autophagosomes, followed by cell death.

As used herein, “a sirolimus drug” refers to a drug that is sirolimus, also known as rapamycin, or a sirolimus derivative:

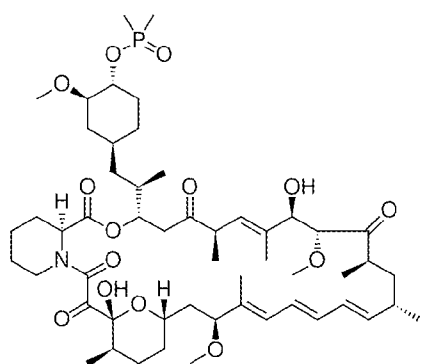


Sirolimus (Rapamycin)

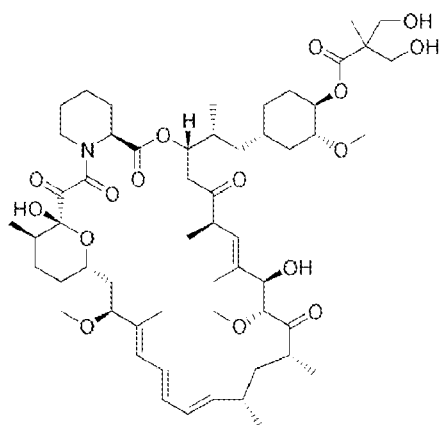
Sirolimus derivatives are known in the art and include, for example, everolimus, deforolimus, temisirolimus, and zotarolimus:



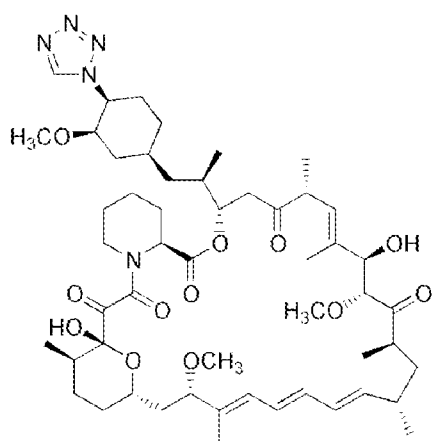
Everolimus



Deforolimus



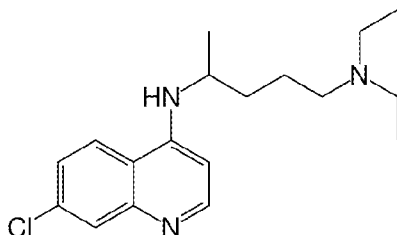
Temisirolimus



Zotarolimus

Sirolimus drugs are inhibitors of the mammalian target of rapamycin (mTOR), also known as FK506 binding protein 12-rapamycin associated protein 1 (FRAP1). mTor is a serine/threonine protein kinase that regulates, for example, cell growth, cell proliferation, cell motility, cell survival, protein synthesis, and transcription.

5 As used herein, "chloroquine" refers to the following compound:



Chloroquine

As used herein, "substituted quinoline" refers to chloroquine derivatives wherein one or more hydrogens or functional groups of the chloroquine molecule have been replaced by one or more substituents that is, for example, halogen, C₁₋₁₀alkyl, -OC₁₋₁₀alkyl, -OH, C₆₋₁₀aryl, heteroaryl, heterocycloalkyl, alkylheterocycloalkyl, heteroalkyl, alkylheteroalkyl. Exemplary embodiments of such substituted quinolines include, for example, hydroxychloroquine, amodiaquine, amopyroquine, cycloquine, tebuquine, bis pyroquine, sontoquine, primaquine, quinacrine, a mixture thereof, or pharmaceutically acceptable salts, enantiomers, or prodrugs thereof.

15 Also within the scope of the term "substituted quinoline" are quinoline-based compounds substituted by one or more substituents that is, for example, halogen, C₁₋₁₀alkyl, -C₂₋₆alkenyl, -OC₁₋₁₀alkyl, -OH, C₆₋₁₀aryl, heteroaryl, heterocycloalkyl, heterocycloalkylalkyl, heteroalkyl, heteroalkylalkyl, haloalkyl. Exemplary embodiments of such substituted quinolines include, for example, mefloquine, quinine, a mixture thereof, or pharmaceutically acceptable salts, enantiomers, or prodrugs thereof.

As used herein, halogen refers to -F, -Cl, or -Br.

As used herein, "C₁₋₁₀alkyl" refers to a saturated straight, branched, cyclic, or multicyclic hydrocarbon having from 1 to 10 carbon atoms (and all combinations and subcombinations of ranges and specific numbers of carbon atoms therein). Alkyl groups include, but are not limited to, methyl, ethyl, n-propyl, isopropyl, cyclopropyl, n-butyl, isobutyl, t-butyl, cyclobutyl, n-pentyl, cyclopentyl, isopentyl, neopentyl, n-hexyl, isohexyl, cyclohexyl, cycloheptyl, cyclooctyl, decahydronaphthalenyl, adamantyl, 3-methylpentyl, 2,2-dimethylbutyl, and 2,3-dimethylbutyl. Alkyl groups can be substituted or unsubstituted.

As used herein, "heterocycloalkyl" refers to a non-aromatic heterocyclyl group where one or more of the ring forming carbon atoms is replaced by a heteroatom such as O, N, or S. Ring-

forming carbon and heteroatoms such as S and N can further be oxidized in a heterocycloalkyl moiety. For example, the heterocycloalkyl can bear one or two oxo, sulfido, or nitroxide moieties. In some embodiments, heterocycloalkyl groups have about 3 to about 20 ring-forming atoms, preferably, 3, 4, 5, 6, or 7 ring forming atoms. Heterocycloalkyl groups include, but are not limited to piperazinyl, morpholinyl, tetrahydropyranyl, tetrahydrofuranyl, piperidinyl, pyrrolidinyl, oxazolinyl, and the like. Heterocycloalkyl groups can be substituted or unsubstituted.

As used herein, "heteroalkyl" refers to an alkyl group in which one or more hydrogen atoms is replaced by a heteroatom that is N, O, P or S. Heteroalkyl groups can be substituted or unsubstituted.

As used herein, "C₂₋₆alkenyl" refers to an alkyl group having one or more double bonds.

As used herein, "C₆₋₁₄aryl" refers to mono-, di-, tri-, or other multicyclic aromatic ring system having from 6 to 14 carbon atoms (and all combinations and subcombinations of ranges and specific numbers of carbon atoms therein). Non-limiting examples include phenyl, naphthyl, anthracenyl, and phenanthrenyl. Aryl groups can be substituted or unsubstituted.

As used herein, "heteroaryl" refers to a mono-, di-, tri-, or other multicyclic aromatic ring system that includes at least one, and preferably from 1 to about 4 sulfur, oxygen, or nitrogen heteroatom ring members. Heteroaryl groups can have, for example, from about 3 to about 50 carbon atoms (and all combinations and subcombinations of ranges and specific numbers of carbon atoms therein), with from about 4 to about 10 carbons being preferred. Non-limiting examples of heteroaryl groups include, for example, pyrrolyl, furyl, pyridyl, 1,2,4-thiadiazolyl, pyrimidinyl, isothiazolyl, thiazolyl, triazolyl, imidazolyl, tetrazolyl, pyrazinyl, quinolyl, isoquinolyl, thiophenyl, benzothienyl, isobenzofuryl, pyrazolyl, indolyl, purinyl, carbazolyl, benzimidazolyl, oxazolyl, and isoxazolyl. Heteroaryl groups can be substituted or unsubstituted.

As used herein, "haloalkyl" means an alkyl group substituted with one or more halogen groups selected from -F or -Cl.

Typically, substituted chemical moieties include one or more substituents that replace hydrogen. Exemplary substituents include, for example, halo (*e.g.*, -F, -Cl, -Br), (provided that when halo is -Br, the -Br is attached to an sp² carbon such as on a carbon of an alkenyl or a ring carbon of aryl or heteroaryl group), alkoxy, haloalkoxy, -OCF₃, alkylthio, monohaloalkylthio, polyhaloalkylthio, -SCF₃, alkyl, -CF₃, haloalkyl, lower alkyl, spiroalkyl, alkenyl, alkynyl, aralkyl, aryl, heteroaryl, heterocyclyl, hydroxyl (-OH), nitro (-NO₂), cyano (-CN), sulfonyl (-SO₂R⁴), sulfamoyl (-SO₂NR⁵R⁶), -SR⁴, amino (-NH₂, NHR⁵, NHR⁶, N(R⁵R⁶) and the like.

As used herein, “effective amount” refers to an amount of a compound as described herein that may be effective to inhibit, or treat the symptoms of cancer.

As used herein, “treating” refers to the preventative, curative, and/or palliative treatment of a condition, for example, cancer, and includes, in particular, not only the prevention and/or
5 treatment of the condition *per se*, but also the prevention of the progression of the condition.

As used herein, “pharmaceutically acceptable” refers to those compounds, materials, compositions, and/or dosage forms that are, within the scope of sound medical judgment, suitable for contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem complications commensurate with a reasonable
10 benefit/risk ratio.

As used herein, “pharmaceutically acceptable salts” refer to derivatives of the disclosed compounds wherein the parent compound is modified by making acid or base salts thereof. Examples of pharmaceutically acceptable salts include, but are not limited to, mineral or organic acid salts of basic residues such as amines; alkali or organic salts of acidic residues such as
15 carboxylic acids; and the like. Thus, the term “acid addition salt” refers to the corresponding salt derivative of a parent compound that has been prepared by the addition of an acid. The pharmaceutically acceptable salts include the conventional salts or the quaternary ammonium salts of the parent compound formed, for example, from inorganic or organic acids. For example, such conventional salts include, but are not limited to, those derived from inorganic
20 acids such as hydrochloric, hydrobromic, sulfuric, sulfamic, phosphoric, nitric and the like; and the salts prepared from organic acids such as acetic, propionic, succinic, glycolic, stearic, lactic, malic, tartaric, citric, ascorbic, pantoic, maleic, hydroxymaleic, phenylacetic, glutamic, benzoic, salicylic, sulfanilic, 2-acetoxybenzoic, fumaric, toluenesulfonic, methanesulfonic, ethane disulfonic, oxalic, isethionic, and the like. Certain acidic or basic compounds of the present
25 invention may exist as zwitterions. All forms of the compounds, including free acid, free base, and zwitterions, are contemplated to be within the scope of the present invention.

“Neoplasm” refers to lump, tumor, or leukemia characterized by an abnormal proliferation of cells.

“Patient” refers to an animal, including a mammal, preferably a human.

30 In some embodiments, the pharmaceutical compositions of the invention further comprise a pharmaceutically acceptable carrier or diluent. Such compositions are prepared in accordance with acceptable pharmaceutical procedures, such as described in *Remington's Pharmaceutical Sciences*, 17th edition, ed. Alfonso R. Gennaro, Mack Publishing Company, Easton, PA (1985).

Pharmaceutically acceptable carriers and/or diluents are those that are compatible with the other ingredients in the formulation and biologically acceptable.

The compounds of this invention may be administered orally or parenterally, neat or in combination with conventional pharmaceutical carriers. Applicable solid carriers can include one or more substances that may also act as flavoring agents, lubricants, solubilizers, suspending agents, fillers, glidants, compression aids, binders or tablet-disintegrating agents or an encapsulating material. In powders, the carrier is a finely divided solid that is in admixture with the finely divided active ingredient. In tablets, the active ingredient is mixed with a carrier having the necessary compression properties in suitable proportions and compacted in the shape and size desired. The powders and tablets preferably contain up to 99% of the active ingredient. Suitable solid carriers include, for example, calcium phosphate, magnesium stearate, talc, sugars, lactose, dextrin, starch, gelatin, cellulose, methyl cellulose, sodium carboxymethyl cellulose, polyvinylpyrrolidone, low melting waxes and ion exchange resins.

Oral formulations are preferred. Formulations for oral or injected use are based on sufficient solubility as to allow the therapeutic agent to enter solution in the stomach or in an injectable medium. Suitable drug formulations include, but are not limited to, tablets, pills, capsules, sachets, granules, powders, chewing gums, suspensions, emulsions, suppositories, and solutions. Particularly preferred for oral use are tablets and capsules of all varieties and microbe-free solutions for injection or infusion. Where appropriate and necessary the formulations may include diluents, binding agents, dispersing agents, surface-active agents, lubricating agents, coating materials, flavoring agents, coloring agents, controlled release formulations, sweeteners or any other pharmaceutically acceptable additives, for example, gelatin, sodium starch glycolate, lactose, starch, talc, magnesium stearate, microcrystalline cellulose, Povidone, hydrogenated or unsaturated oils, polyglycols, syrups or other aqueous solutions. Where the formulations are tablets or capsules and the like the formulations may be presented as premeasured unit doses or in multidose containers from which the appropriate unit dose may be withdrawn.

Liquid carriers may be used in preparing solutions, suspensions, emulsions, syrups, and elixirs. The active ingredient of this invention can be dissolved or suspended in a pharmaceutically acceptable liquid carrier such as water, an organic solvent, a mixture of both or pharmaceutically acceptable oils or fat. The liquid carrier can contain other suitable pharmaceutical additives such as solubilizers, emulsifiers, buffers, preservatives, sweeteners, flavoring agents, suspending agents, thickening agents, colors, viscosity regulators, stabilizers, or osmo-regulators. Suitable examples of liquid carriers for oral and parenteral administration

include water (particularly containing additives as above, *e.g.* cellulose derivatives, preferably sodium carboxymethyl cellulose solution), alcohols (including monohydric alcohols and polyhydric alcohols *e.g.* glycols) and their derivatives, and oils (*e.g.* fractionated coconut oil and arachis oil). For parenteral administration the carrier can also be an oily ester such as ethyl oleate and isopropyl myristate. Sterile liquid carriers are used in sterile liquid form compositions for parenteral administration.

Liquid pharmaceutical compositions, which are sterile solutions or suspensions, can be administered by, for example, intramuscular, intraperitoneal or subcutaneous injection. Sterile solutions can also be administered intravenously. Oral administration may be either liquid or solid composition form.

The injectable form may be an aqueous or nonaqueous solution, suspension or emulsion in a pharmaceutically acceptable liquid, *e.g.* sterile pyrogen-free water or parenterally acceptable oils or mixture of liquids which may contain bacteriostatic agents, antioxidants or other preservatives and stabilizers, buffers (preferably but not limited to a physiological pH range of 6.5-7.7, solutes to render the solution isotonic with the blood, thickening agents, suspending agents or other pharmaceutically acceptable additives. Such forms will be presented in unit dose form such as ampules or disposable injection devices or in multi-dose forms such as a bottle from which the appropriate dose may be withdrawn, or as a solid form or concentrate that can be used to quickly prepare an injectable formulation. All formulations for injection are preferable as sterile and pyrogen free. Suppositories containing the compound will also contain suitable carriers, *e.g.* cocoa butter, polyglycols or other state-of-the-art carriers.

Preferably the pharmaceutical composition is in single unit dosage form, *e.g.* as tablets, capsules, powders, solutions, suspensions, emulsions, granules, or suppositories. In such form, the composition is sub-divided in unit dose containing appropriate quantities of the active ingredient; the unit dosage forms can be packaged compositions, for example packeted powders, vials, ampoules, prefilled syringes or sachets containing liquids. The unit dosage form can be, for example, a capsule or tablet itself, or it can be the appropriate number of any such compositions in package form.

When the combination products are not formulated together in a single dosage form, the sirolimus drug and chloroquine, or substituted quinoline, may be administered at the same time or simultaneously (that is, together), or in any order.

In addition to standard pharmaceutical additives there may be included within formulations of the compound other therapeutic agents.

The dosage of the composition of the present invention that will be most suitable for prophylaxis or treatment will vary with the form of administration, the particular compounds chosen and the physiological characteristics of the particular patient under treatment. Generally, small dosages may be used initially and, if necessary, increased by small increments until the
5 desired effect under the circumstances is reached. Generally speaking, oral administration may require higher dosages.

Preferably, administration of the combination products of the invention is oral, although other routes of administration, as described above, are contemplated to be within the scope of the present invention. Although it is preferable that the sirolimus drug and chloroquine, or
10 substituted quinoline, are all administered in the same fashion (that is, for example, both orally), if desired, they may each be administered in different fashions (that is, for example, one component of the combination product may be administered orally, and another component may be administered intravenously). The dosage of the combination products of the invention may vary depending upon various factors such as the pharmacodynamic characteristics of the
15 particular agent and its mode and route of administration, the age, health and weight of the recipient, the nature and extent of the symptoms, the kind of concurrent treatment, the frequency of treatment, and the effect desired.

Although the proper dosage of the pharmaceutical composition of this invention will be readily ascertainable by one skilled in the art, once armed with the present disclosure, by way of
20 general guidance, typically a daily dosage may range from about 0.015 to about 1500 milligrams of the chloroquine or the substituted quinoline (and all combinations and subcombinations of ranges therein) and about 0.001 to about 1000 milligrams of the sirolimus drug (and all combinations and subcombinations of ranges therein) per kilogram of patient body weight.

Particularly when provided as a single dosage form, the potential exists for a chemical
25 interaction between the combined active ingredients. For this reason, the preferred dosage forms of the combination products of this invention are formulated such that although the active ingredients are combined in a single dosage form, the physical contact between the active ingredients is minimized (that is, reduced).

In order to minimize contact, one embodiment of this invention where the product is
30 orally administered provides for a combination product wherein one active ingredient is enteric coated. By enteric coating one or more of the active ingredients, it is possible not only to minimize the contact between the combined active ingredients, but also, it is possible to control the release of one of these components in the gastrointestinal tract such that one of these components is not released in the stomach but rather is released in the intestines. Another

embodiment of this invention where oral administration is desired provides for a combination product wherein one of the active ingredients is coated with a sustained-release material that effects a sustained-release throughout the gastrointestinal tract and also serves to minimize physical contact between the combined active ingredients. Furthermore, the sustained-released component can be additionally enteric coated such that the release of this component occurs only in the intestine. Still another approach would involve the formulation of a combination product in which the one component is coated with a sustained and/or enteric release polymer, and the other component is also coated with a polymer such as a low-viscosity grade of hydroxypropyl methylcellulose (HPMC) or other appropriate materials as known in the art, in order to further separate the active components. The polymer coating serves to form an additional barrier to interaction with the other component.

Dosage forms of the combination products of the present invention wherein one active ingredient is enteric coated can be in the form of tablets such that the enteric coated component and the other active ingredient are blended together and then compressed into a tablet or such that the enteric coated component is compressed into one tablet layer and the other active ingredient is compressed into an additional layer. Optionally, in order to further separate the two layers, one or more placebo layers may be present such that the placebo layer is between the layers of active ingredients. In addition, dosage forms of the present invention can be in the form of capsules wherein one active ingredient is compressed into a tablet or in the form of a plurality of microtablets, particles, granules or non-pareils, which are then enteric coated. These enteric-coated microtablets, particles, granules or non-pareils are then placed into a capsule or compressed into a capsule along with a granulation of the other active ingredient.

In addition to providing for dosage forms wherein the pharmaceutically active components are presented in a single unit dosage form, kits including, for example, the sirolimus drug and the chloroquine (or substituted quinoline) as individual components of the kit is also envisioned. For example, one component of the kit can include a sirolimus drug and a first pharmaceutically acceptable carrier, diluent, or excipient. Another component of the kit can include chloroquine or a chloroquine derivative and a second pharmaceutically acceptable carrier, diluent, or excipient. In such embodiments, each component of the invention can be presented together in, for example, a push-through or peel-open blister pack. Other components of the kit may include implements for administering each individual active component, for example, syringes, if the active component or components are to be administered by injection.

The present invention is further defined in the following Examples. It should be understood that these examples, while indicating preferred embodiments of the invention, are

given by way of illustration only. From the above discussion and these examples, one skilled in the art can ascertain the essential characteristics of this invention, and without departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to various usages and conditions.

5

EXAMPLES

Materials and Methods

Reagents and constructs: rapamycin (sirolimus, “rapa”) and chloroquine (CQ) were purchased from Sigma-Aldrich, Inc (St. Louis, MO). The pC1-EGFP-LC3 vector was
10 contributed by Dr. Mizushima (Japan).

Cell lines, transfection, and cell counting: LN229 cells were grown in DMEM supplemented with 10% heat-inactivated fetal bovine serum (Gemini Biosciences, Woodland, CA), 10 units/ml penicillin/streptomycin, and 2 mM L-glutamine (Invitrogen Corporation).
15 LN229 cells were transfected with lipofectamine 2000 (Invitrogen) according to manufacturer protocol. Stable transfectants were selected with neomycin (Gibco), and high fluorescence clones of LN229 GFP-LC3 were selected by limiting dilution. For all *in vitro* experiments media + supplements and drugs treatments were changed daily. Cell number was assessed by using a Coulter Z2 particle analyzer and confirmed by trypan blue exclusion.

20 As depicted in FIG. 1, a combination of 5nM rapamycin and 5 μ M chloroquine resulted in a decrease in cell number. The decrease in cell number resulting from the combination of rapamycin and chloroquine was greater than the decrease in cell number observed with either rapamycin or chloroquine alone.

25 Fluorescence Microscopy: Cells were grown in sterile glass coverslips and treated in full medium as indicated above. After 48 hours of treatment cells were fixed with 4% paraformaldehyde and imaged using a Nikon upright fluorescent microscope. Images were captured at 100x and representative images were reported.

As depicted in FIG. 2, LN229 cells, when treated with increasing doses of either
30 rapamycin (top) or chloroquine (bottom) accumulated LC3-positive autophagosomes.

Electron Microscopy: Cells were fixed with 2.5% glutaraldehyde/2% formaldehyde with 0.1 M sodium cacodylate and stored at 4°C until embedding. Cells were postfixed with 2% osmium tetroxide followed by an increasing gradient dehydration step using ethanol and

propylene oxide. Cells were then embedded in LX-112 medium (Ladd) and sections were cut ultrathin (90 nm), placed on uncoated copper grids, and stained with 0.2% lead citrate and 1% uranyl acetate. Images were examined with a JEOL-1010 electron microscope (JEOL) at 80 kV. Cells were considered non-apoptotic if the integrity of the nuclear and cytoplasmic membrane were maintained. Autophagic vesicles were identified as double-membrane vesicles.

FIG. 3 depicts the electron micrographs of LN229 GFP-LC3 cells treated with either rapamycin, chloroquine, or the combination. While autophagosomes are observed when cells are treated with either rapamycin or chloroquine alone, the combination of rapamycin and chloroquine results in a greater number of autophagosomes.

What is Claimed:

1. A method of limiting the growth and proliferation of cancer cells in a patient having or suspected of having a neoplasm comprising:
co-administering to the patient a sirolimus drug together with a substituted quinoline, the
5 amounts of said sirolimus drug and substituted quinoline, in combination, being effective for achieving the limiting of growth and proliferation.
2. The method of claim 1, wherein the limiting is achieved by modulating autophagy in the cancer cells.
3. The method of claim 1 or claim 2 wherein the sirolimus drug is sirolimus.
- 10 4. The method of any one of claims 1, 2, or 3 wherein the substituted quinoline is chloroquine.
5. A method of treating cancer in a patient comprising administering to the patient a therapeutically effective amount of
a sirolimus drug; and
15 chloroquine or a substituted quinoline.
6. The method of claim 5 comprising administration of sirolimus and chloroquine.
7. The method of claim 5 comprising administration of sirolimus and a substituted quinoline.
8. The method of claim 5 comprising administration of deforolimus, everolimus,
20 temsirolimus, or zotarolimus and a substituted quinoline.
9. The method of claim 5 comprising administration of deforolimus, everolimus, temsirolimus, or zotarolimus and chloroquine.
10. The method of any one of claims 7 or 8 wherein the substituted quinoline is hydroxychloroquine, chloroquine, amodiaquine, amopyroquine, cycloquine,
25 sontoquine, primaquine, quinacrine, tebuquine, *bis* pyroquine, or pharmaceutically acceptable salt or enantiomer or prodrug thereof.

11. The method of any one of claims 7 or 8 wherein the substituted quinoline is mefloquine, quinine, or pharmaceutically acceptable salt or enantiomer or prodrug thereof.
12. The method of any one of the preceding claims wherein the administration is concurrent.
13. The method of any one of the preceding claims wherein the administration is sequential.
14. The method of any one of the preceding claims wherein the cancer is glioblastoma, lung cancer, or prostate cancer.
15. A pharmaceutical composition comprising a sirolimus drug; chloroquine or a chloroquine derivative; and a pharmaceutically acceptable carrier, diluent, or excipient.
16. A kit comprising:
a sirolimus drug and a first pharmaceutically acceptable carrier, diluent, or excipient; and
chloroquine or a chloroquine derivative and a second pharmaceutically acceptable carrier, diluent, or excipient.

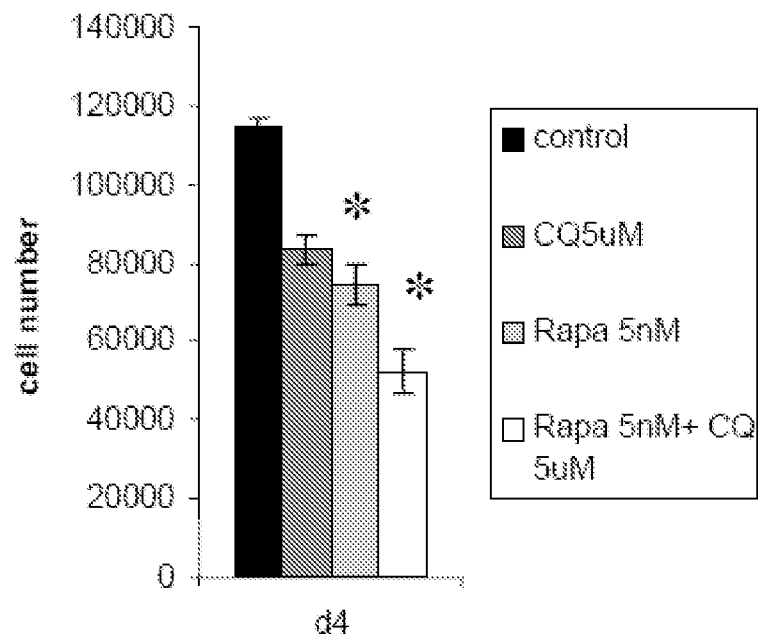


FIG. 1

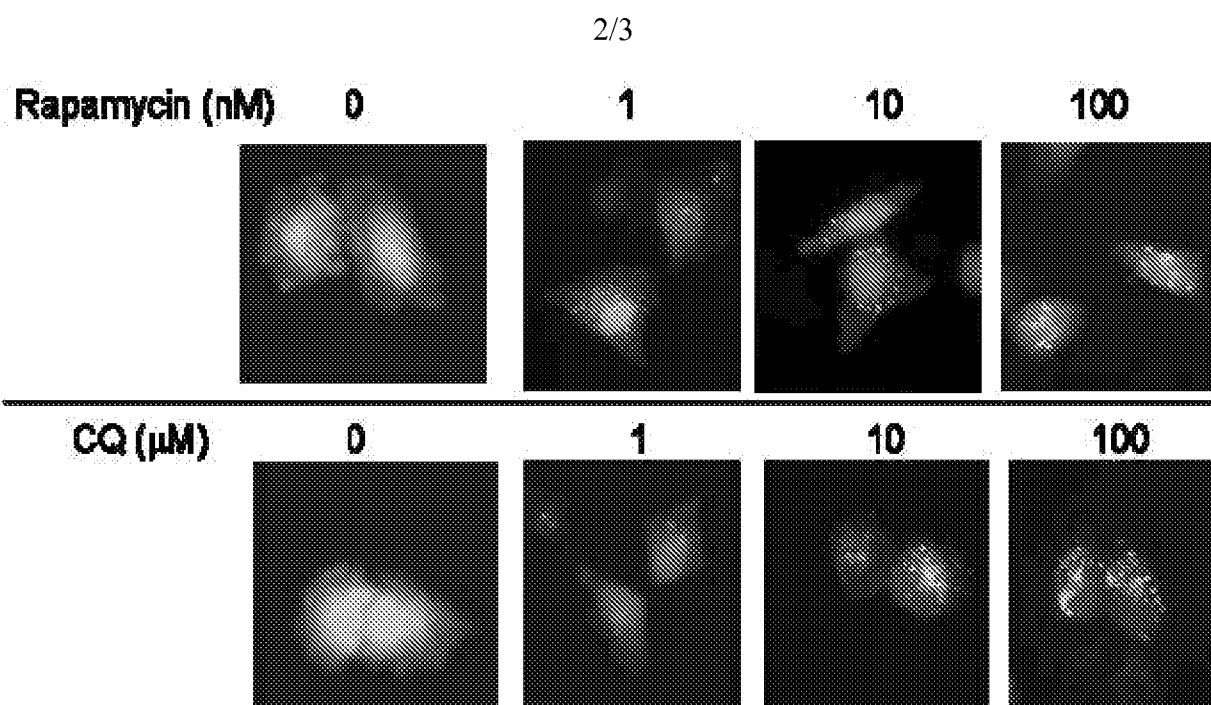


FIG. 2

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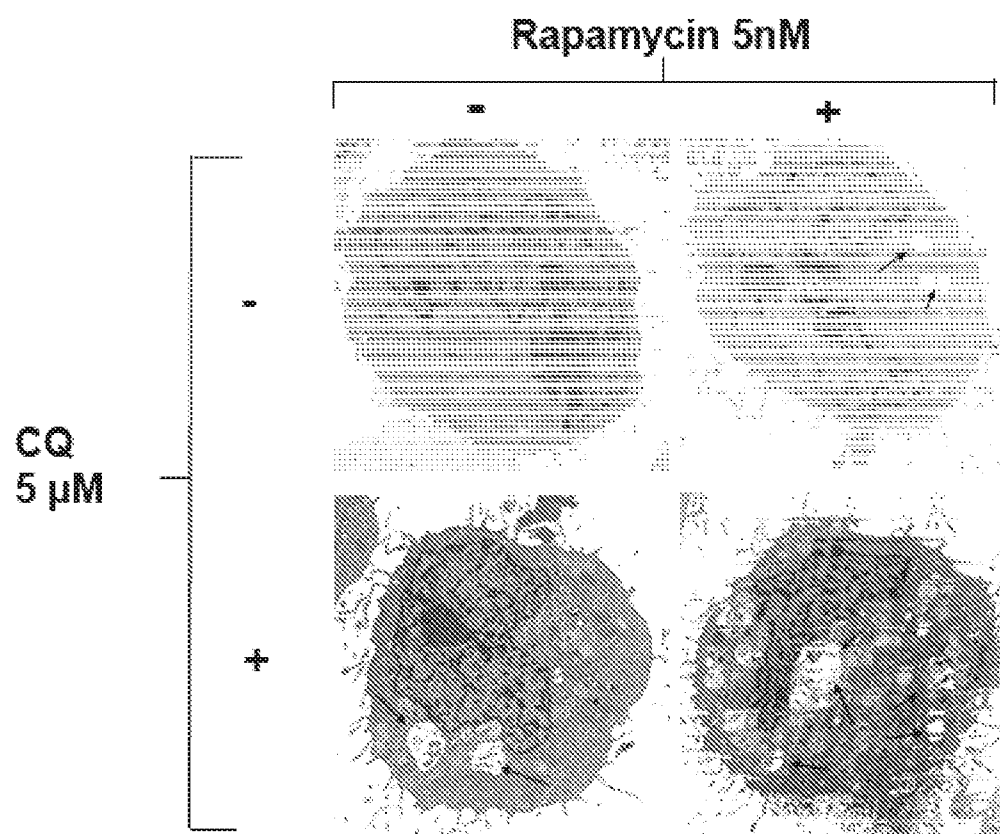


FIG. 3

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 10/33514

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A01N 43/42; A61K 31/44 (2010.01)

USPC - 514/291

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)

USPC - 514/291

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC - 514/291, 514/290, 514/279, 514/277, 514/183 (Words only)

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

USPTO WEST (PGPB, USPT, USOC, EPAB, JPAB); Google

Sirolimus, substituted quinoline, chloroquine, neoplasm, cancer, pharmaceutically acceptable, carrier, deforolimus, everolimus, emsirolimus and zotarolimus

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2006/0264384 A1 (JOHANSEN, et al.) 23 November 2006 (23.11.2006), especially, para [0007], [0008], [0016], [0122]	1, 3-7, 10, 11, 15, 16
Y		2, 8, 9
Y	MATTHEW, et al. Role Of Autophagy In Cancer. Nature Review: Cancer. [online, Author Manuscript]; 01 December 2007, Vol. 7, No. 12, Pg 961-967; document retrieved on 11 June 2010, [retrieved from http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2866167/?tool=pubmed]; especially, pg 2, para 4; pg 4, para 2; pg 8, para 1.	2
Y	TOURNEAU, et al. mTORC1 Inhibitors: Is Temsirolimus In Renal Cancer Telling Us How They Really Work? British Journal of Cancer. 14 October 2008, Vol. 99, No.8, Pg 1197-1203; especially, pg 1198, col 1, para 2.	8, 9

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

* 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

12 June 2010 (12.06.2010)

Date of mailing of the international search report

07 JUL 2010

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450

Facsimile No. 571-273-3201

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PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 10/33514

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claims Nos.: 12-14
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.