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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
26 October 2012 (26.10.2012)

WIPO | PCT

(10) International Publication Number
WO 2012/145503 A1

(51) International Patent Classification:
A61K 31/4184 (2006.01) *A61K 31/155* (2006.01)
A61K 31/4412 (2006.01) *A61P 35/00* (2006.01)

(21) International Application Number:
PCT/US2012/034239

(22) International Filing Date:
19 April 2012 (19.04.2012)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/477,741 21 April 2011 (21.04.2011) US

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

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

Published:

— *with international search report (Art. 21(3))*

WO 2012/145503 A1

(54) **Title**: PHARMACEUTICAL COMBINATIONS

(57) **Abstract**: A pharmaceutical combination comprising (a) a MEK inhibitor selected from the group consisting of 6-(4-bromo-2-fluorophenylamino)-7-fluoro-3-methyl-3H-benzoimidazole-5-carboxylic acid (2-hydroxyethoxy)-amide or (*S*)-5-fluoro-2-(2-fluoro-4-(methylthio)phenylami-*N*-(2-hydroxypropoxy)-1-methyl-6-oxo-1,6-dihydropyridine-3-carboxamide or a pharmaceutically acceptable salt thereof, and (b) at least one RAF kinase inhibitor, particularly RAF265; the uses of such combination in the treatment of proliferative diseases; and methods of treating a subject suffering from a proliferative disease comprising administering a therapeutically effective amount of such combination

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PHARMACEUTICAL COMBINATIONS

FIELD OF THE INVENTION

A pharmaceutical combination comprising (a) a MEK inhibitor selected from the group consisting of 6-(4-bromo-2-fluorophenylamino)-7-fluoro-3-methyl-3H-benzoimidazole-5-carboxylic acid (2-hydroxyethoxy)-amide or (*S*)-5-fluoro-2-(2-fluoro-4-(methylthio)phenylamino)-*N*-(2-hydroxypropoxy)-1-methyl-6-oxo-1,6-dihydropyridine-3-carboxamide or a pharmaceutically acceptable salt thereof, and (b) at least one RAF kinase inhibitor, particularly RAF265, or a pharmaceutically acceptable salt thereof; the uses of such combination in the treatment or prevention of proliferative diseases; and methods of treating a subject suffering from a proliferative disease comprising administering a therapeutically effective amount of such combination.

BACKGROUND OF THE INVENTION

The RAS/RAF/MEK signal transduction pathway is activated through initial extracellular binding and stimulation of tyrosine receptor kinases (RTKs) by their respective cognate ligands. Upon autophosphorylation of specific tyrosine residues in the cytosolic domain of RTKs, the Grb2-Sos complex translocates to the plasma membrane, and converts the inactive RAS•GDP to active RAS•GTP. The interaction between Grb2 and the activated kinases or the phosphorylated receptor associated proteins is mediated by the SH2 domain of the signaling protein that recognizes specific phosphotyrosine sequences. RAS undergoes a conformational changes upon GTP binding and causes the recruitment of RAF-1 to the cytoplasmic membrane where it is phosphorylated by serveral kinases and simultaneous disphosphorylated at key residues by protein phosphatase-2B. Activated RAF phosphorylates MEK on two serine residues in the activation loop, which results in the activation of this protein kinase. MEK then phosphorylates and activates ERK, allowing its translocation to the nucleus where it phosphorylates transcriptional factors permitting the expression of a variety of genes.

The RAS/RAF/MEK signal transduction pathway is deregulated, often through mutations that result in ectopic protein activation, in roughly 1/3 of human cancers. This deregulation in turn results in a wide array of cellular changes that are integral to the etiology and maintenance

of a cancerous phenotype including, but not limited to, the promotion of proliferation and evasion of apoptosis (Dhillon et al., Oncogene, 2007, 26: 3279-3290). Accordingly, the development of small molecule inhibitors of key members of the RAS/ RAF/ MEK signal transduction pathway has been the subject of intense effort within the pharmaceutical industry and oncology community.

Primarily, these efforts have focused on inhibitors of the RAF (ARAF, BRAF and RAF1) and MEK (MAP2K1 and MAP2K2) proteins. RAF kinase inhibitors have garnered much attention due to the high incidence of activating mutations in the BRAF protein in certain cancer types including melanoma, thyroid and colorectal cancers. Indeed, results have recently been reported from clinical trials involving the RAF kinase inhibitor PLX4032 in the treatment of BRAF-mutated malignant melanoma (Bollag et al., Nature, 2010, 467: 596-599).

MEK is a major protein in the RAS/ RAF/ MEK/ ERK pathway, which signals toward cell proliferation and survival, and frequently activated in tumors that have mutations in the RAS or RAF oncogenes or in growth receptor tyrosine kinases. Despite being only rarely mutated in cancer (Murugan et al., Cell Cycle, 2009, 8: 2122-2124; Sasaki et al., J. Thorac. Oncol., 2010, 5: 597-600), inhibitors of the MEK1 and MEK2 proteins have also been targeted for small molecule inhibition owing to their central position within the RAS/ RAF/ MEK signal transduction pathway signaling cascade (Fremin and Meloche, , J. Hematol. Oncol., 2010, 3:8). Recently a potent MEK inhibitor failed to demonstrate efficacy in clinical trials in patients with advanced NSCLC (Haura et al., Clin. Cancer Res., 2010, 16: 2450-2457). The reason for failure in this trial is not clear.

In spite of numerous treatment options for patients with cancer, there remains a need for effective and safe therapeutic agents and a need for new combination therapies that can be administered for the effective long-term treatment of cancer. It has been surprisingly discovered that the combination of an effective amount of amount of the MEK inhibitor 6-(4-bromo-2-fluorophenylamino)-7-fluoro-3-methyl-3H-benzoimidazole-5-carboxylic acid (2-hydroxyethoxy)-amide or (*S*)-5-fluoro-2-(2-fluoro-4-(methylthio)phenylamino)-*N*-(2-hydroxypropoxy)-1-methyl-6-oxo-1,6-dihydropyridine-3-carboxamide with an effective amount of a RAF kinase inhibitor results in unexpected improvement in the treatment of tumor diseases.

When administered simultaneously, sequentially or separately, the MEK inhibitor 6-(4-bromo-2-fluorophenylamino)-7-fluoro-3-methyl-3H-benzoimidazole-5-carboxylic acid (2-hydroxyethoxy)-amide or (*S*)-5-fluoro-2-(2-fluoro-4-(methylthio)phenylamino)-*N*-(2-hydroxypropoxy)-1-methyl-6-oxo-1,6-dihydropyridine-3-carboxamide and a RAF kinase inhibitor interact in a synergistic manner to strongly inhibit cell proliferation. This unexpected synergistic reaction allows reduction in the dose required for each compound, leading to a reduction in the side effects and enhancement of the long-term clinical effectively of the compounds in treatment.

SUMMARY OF THE INVENTION

The present invention relates to a pharmaceutical combination comprising: (a) a MEK inhibitor selected from the group consisting of 6-(4-bromo-2-fluorophenylamino)-7-fluoro-3-methyl-3H-benzoimidazole-5-carboxylic acid (2-hydroxyethoxy)-amide (COMPOUND A) or (*S*)-5-fluoro-2-(2-fluoro-4-(methylthio)phenylamino)-*N*-(2-hydroxypropoxy)-1-methyl-6-oxo-1,6-dihydropyridine-3-carboxamide (COMPOUND B) or a pharmaceutically acceptable salt thereof, and (b) at least one RAF kinase inhibitor or a pharmaceutically acceptable salt thereof, for simultaneous, separate or sequential administration, in particular for treating or preventing a proliferative disease.

The present invention also pertains to a combination such as a combined preparation or a pharmaceutical composition which comprises (a) a MEK inhibitor selected from the group consisting of 6-(4-bromo-2-fluorophenylamino)-7-fluoro-3-methyl-3H-benzoimidazole-5-carboxylic acid (2-hydroxyethoxy)-amide (COMPOUND A) or (*S*)-5-fluoro-2-(2-fluoro-4-(methylthio)phenylamino)-*N*-(2-hydroxypropoxy)-1-methyl-6-oxo-1,6-dihydropyridine-3-carboxamide (COMPOUND B) or a pharmaceutically acceptable salt thereof, and (b) at least one RAF kinase inhibitor or a pharmaceutically acceptable salt thereof.

The present invention particularly pertains to a combination comprising: (a) a MEK inhibitor selected from the group consisting of 6-(4-bromo-2-fluorophenylamino)-7-fluoro-3-methyl-3H-benzoimidazole-5-carboxylic acid (2-hydroxyethoxy)-amide (COMPOUND A) or (*S*)-5-fluoro-2-(2-fluoro-4-(methylthio)phenylamino)-*N*-(2-hydroxypropoxy)-1-methyl-6-oxo-1,6-dihydropyridine-3-carboxamide (COMPOUND B) or a pharmaceutically acceptable salt

thereof, and (b) at least one RAF kinase inhibitor or a pharmaceutically acceptable salt thereof, useful for treating or preventing a proliferative disease in a subject in need thereof.

The present invention also pertains to a pharmaceutical combination comprising: (a) a MEK inhibitor selected from the group consisting of 6-(4-bromo-2-fluorophenylamino)-7-fluoro-3-methyl-3H-benzoimidazole-5-carboxylic acid (2-hydroxyethoxy)-amide (COMPOUND A) or (*S*)-5-fluoro-2-(2-fluoro-4-(methylthio)phenylamino)-*N*-(2-hydroxypropoxy)-1-methyl-6-oxo-1,6-dihydropyridine-3-carboxamide (COMPOUND B) or a pharmaceutically acceptable salt thereof, and (b) at least one RAF kinase inhibitor or a pharmaceutically acceptable salt thereof, for use in the preparation of a pharmaceutical composition or medicament for the treatment or prevention of a proliferative disease in a subject in need thereof.

The present invention further pertains to the use of a MEK inhibitor selected from the group consisting of 6-(4-bromo-2-fluorophenylamino)-7-fluoro-3-methyl-3H-benzoimidazole-5-carboxylic acid (2-hydroxyethoxy)-amide (COMPOUND A) or (*S*)-5-fluoro-2-(2-fluoro-4-(methylthio)phenylamino)-*N*-(2-hydroxypropoxy)-1-methyl-6-oxo-1,6-dihydropyridine-3-carboxamide (COMPOUND B) or a pharmaceutically acceptable salt thereof, in combination with at least one RAF kinase inhibitor or a pharmaceutically acceptable salt thereof for the preparation of a pharmaceutical composition or medicament for the treatment or prevention of a proliferative disease.

The present invention relates to a method of treating a subject having a proliferative disease comprising administered to said subject a a combination comprising: (a) a MEK inhibitor selected from the group consisting of 6-(4-bromo-2-fluorophenylamino)-7-fluoro-3-methyl-3H-benzoimidazole-5-carboxylic acid (2-hydroxyethoxy)-amide (COMPOUND A) or (*S*)-5-fluoro-2-(2-fluoro-4-(methylthio)phenylamino)-*N*-(2-hydroxypropoxy)-1-methyl-6-oxo-1,6-dihydropyridine-3-carboxamide (COMPOUND B) or a pharmaceutically acceptable salt thereof, and (b) at least one RAF kinase inhibitor or a pharmaceutically acceptable salt thereof, in a quantity, which is jointly therapeutically effective against a proliferative disease.

The present invention further provides a commercial package comprising as therapeutic agents a COMBINATION OF THE INVENTION, together with instructions for simultaneous, separate or sequential administration thereof for use in the delay of progression or treatment of a proliferative disease.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is showing the pipetting scheme for the resazurin viability combination assay used in the proliferation assay with $BRAF^{V600E}$ and NRAS-mutant melanoma cell lines in combination with COMPOUND A and RAF265.

FIG. 2 is showing the pipetting scheme for the P-ERK In Cell Western combination assay used in the in the proliferation assay with $BRAF^{V600E}$ and NRAS-mutant melanoma cell lines in combination with COMPOUND A and RAF265.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to a pharmaceutical combination comprising: (a) a MEK inhibitor selected from the group consisting of 6-(4-bromo-2-fluorophenylamino)-7-fluoro-3-methyl-3H-benzoimidazole-5-carboxylic acid (2-hydroxyethoxy)-amide (COMPOUND A) or (*S*)-5-fluoro-2-(2-fluoro-4-(methylthio)phenylamino)-*N*-(2-hydroxypropoxy)-1-methyl-6-oxo-1,6-dihydropyridine-3-carboxamide (COMPOUND B) or a pharmaceutically acceptable salt thereof, and (b) at least one RAF kinase inhibitor or a pharmaceutically acceptable salt thereof, for simultaneous, separate or sequential administration, in particular for use in the treatment or prevention of a proliferative disease.

The general terms used herein are defined with the following meanings, unless explicitly stated otherwise:

The terms “comprising” and “including” are used herein in their open-ended and non-limiting sense unless otherwise noted.

The terms “a” and “an” and “the” and similar references in the context of describing the invention (especially in the context of the following claims) are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context. Where the plural form is used for compounds, salts, and the like, this is taken to mean also a single compound, salt, or the like.

The term "combination" or "pharmaceutical combination", as used herein, defines either a fixed combination in one dosage unit form, a non-fixed combination or a kit of parts for the combined administration where a MEK inhibitor selected from 6-(4-bromo-2-fluorophenylamino)-7-fluoro-3-methyl-3H-benzoimidazole-5-carboxylic acid (2-hydroxyethoxy)-amide (COMPOUND A) or (*S*)-5-fluoro-2-(2-fluoro-4-(methylthio)phenylamino)-*N*-(2-hydroxypropoxy)-1-methyl-6-oxo-1,6-dihydropyridine-3-carboxamide (COMPOUND B) or a pharmaceutically acceptable salt thereof, and at least one RAF kinase inhibitor or a pharmaceutically acceptable salt thereof may be administered independently at the same time or separately within time intervals that allow that the combination partners show a cooperative, e.g., synergistic, effect.

The term "pharmaceutical composition" is defined herein to refer to a mixture or solution containing at least one therapeutic agent to be administered to a subject, e.g., a mammal or human, in order to prevent or treat a particular disease or condition affecting the mammal.

The term "pharmaceutically acceptable" is defined herein to refer to those compounds, materials, compositions and/or dosage forms, which are, within the scope of sound medical judgment, suitable for contact with the tissues a subject, e.g., a mammal or human, without excessive toxicity, irritation allergic response and other problem complications commensurate with a reasonable benefit / risk ratio.

The term "a combined preparation" is defined herein to refer to especially a "kit of parts" in the sense that the combination partners (a) and (b) as defined above can be dosed independently or by use of different fixed combinations with distinguished amounts of the combination partners (a) and (b), i.e., simultaneously or at different time points. The parts of the kit of parts can then e.g., be administered simultaneously or chronologically staggered, that is at different time points and with equal or different time intervals for any part of the kit of parts. The ratio of the total amounts of the combination partner (a) to the combination partner (b) to be administered in the combined preparation can be varied, e.g., in order to cope with the needs of a patient sub-population to be treated or the needs of the single patient.

The term "co-administration" or "combined administration" as used herein is defined to encompass the administration of the selected therapeutic agents to a single patient, and are

intended to include treatment regimens in which the agents are not necessarily administered by the same route of administration or at the same time.

The term “treating” or “treatment” as used herein comprises a treatment relieving, reducing or alleviating at least one symptom in a subject or effecting a delay of progression of a disease. For example, treatment can be the diminishment of one or several symptoms of a disorder or complete eradication of a disorder, such as cancer. Within the meaning of the present invention, the term “treat” also denotes to arrest, delay the onset (i.e., the period prior to clinical manifestation of a disease) and/or reduce the risk of developing or worsening a disease. The term “protect” is used herein to mean prevent delay or treat, or all, as appropriate, development or continuance or aggravation of a disease in a subject.

The term "prevent", "preventing" or "prevention" as used herein comprises the prevention of at least one symptom associated with or caused by the state, disease or disorder being prevented.

The term “jointly therapeutically active” or “joint therapeutic effect” means that the therapeutic agents may be given separately (in a chronologically staggered manner, especially a sequence-specific manner) in such time intervals that they prefer, in the warm-blooded animal, especially human, to be treated, still show a (preferably synergistic) interaction (joint therapeutic effect). Whether this is the case can, inter alia, be determined by following the blood levels, showing that both compounds are present in the blood of the human to be treated at least during certain time intervals.

The term “pharmaceutically effective amount” or “clinically effective amount” of a combination of therapeutic agents is an amount sufficient to provide an observable improvement over the baseline clinically observable signs and symptoms of the disorder treated with the combination.

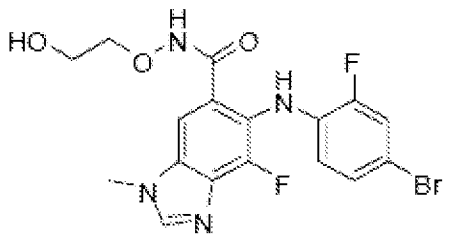
The term “subject” or “patient” as used herein includes animals, which are capable of suffering from or afflicted with a proliferative disease, particularly cancer or any disorder involving, directly or indirectly, a cancer. Examples of subjects include mammals, e.g., humans, dogs, cows, horses, pigs, sheep, goats, cats, mice, rabbits rats and transgenic non-human animals.

In the preferred embodiment, the subject is a human, e.g., a human suffering from, at risk of suffering from, or potentially capable of suffering from cancers.

The term about" or "approximately" shall have the meaning of within 10%, more preferably within 5%, of a given value or range.

Pharmaceutical combinations of the present invention include a MEK inhibitor selected from the group consisting of 6-(4-bromo-2-fluorophenylamino)-7-fluoro-3-methyl-3H-benzoimidazole-5-carboxylic acid (2-hydroxyethoxy)-amide (COMPOUND A), (*S*)-5-fluoro-2-(2-fluoro-4-(methylthio)phenylamino)-*N*-(2-hydroxypropoxy)-1-methyl-6-oxo-1,6-dihydropyridine-3-carboxamide (COMPOUND B) or a pharmaceutically acceptable salt thereof.

The MEK inhibitor compound 6-(4-bromo-2-fluorophenylamino)-7-fluoro-3-methyl-3H-benzoimidazole-5-carboxylic acid (2-hydroxyethoxy)-amide (COMPOUND A) is a compound of formula (I)



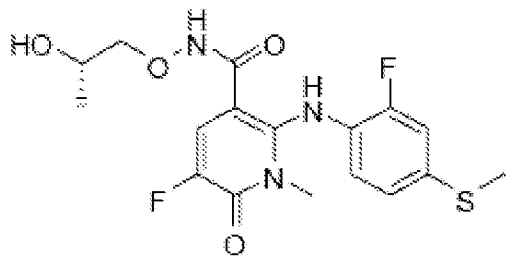
(I)

The MEK inhibitor compound 6-(4-bromo-2-fluorophenylamino)-7-fluoro-3-methyl-3H-benzoimidazole-5-carboxylic acid (2-hydroxyethoxy)-amide (COMPOUND A) is described in PCT Application No. WO 03/077914, and methods for its preparation have been described, for example, in Example 18 therein.

Except as herein disclosed, the compounds used in the present invention may possess one or more asymmetric centers and can be produced as individual (*R*)- or (*S*)-stereoisomers or as mixtures thereof as described in PCT Application No. WO03/077914. Except as otherwise indicated, the description or naming of a particular compound in the specification and claims is intended to include both individual enantiomers, diastereomeric mixtures, racemic or otherwise,

thereof. Accordingly, this invention also includes all such isomers, including diasteroemeric mixtures ad resolved enantiomers of the compounds of this invention. Diastereomieric mixtures can be separated into their individual diastereomers on the basis of their physical chemical differences by methods known to those skilled in the art, for example, by chromatography or fractional crystallization. Enantiomers can be separated by converting the enantiomer mixture into a diastereomeric mixture by reaction with an appropriate optically active compound (e.g., alcohol), separating the diastereomers and converting (e.g., hydrolyzing) the individual diastereomers to the corresponding pure enantiomers. The methods for the determination of stereochemistry and the separation of stereoisomers are well known in the art (see discussion in Chapter 4 of "Advanced organic Chemistry", 4th edition, J. March. John Wiley and Sons, New York, 1992).

The MEK inhibitor compound (*S*)-5-fluoro-2-(2-fluoro-4-(methylthio)phenylamino)-*N*-(2-hydroxypropoxy)-1-methyl-6-oxo-1,6-dihydropyridine-3-carboxamide (COMPOUND B) is a compound of formula (II)



(II)

The MEK inhibitor compound (*S*)-5-fluoro-2-(2-fluoro-4-(methylthio)phenylamino)-*N*-(2-hydroxypropoxy)-1-methyl-6-oxo-1,6-dihydropyridine-3-carboxamide (COMPOUND B) is described in Example 25-BB of PCT Application No. WO2007/044084, and methods for its preparation have been described therein.

The compounds of the invention may be administered in free form or in pharmaceutically acceptable salt form.

A "pharmaceutically acceptable salt", as used herein, unless otherwise indicated, includes salts of acidic and basic groups which may be present in the compounds of the present invention.

The compounds of the present invention that are basic in nature are capable of forming a wide variety of salts with various inorganic and organic acids. The acids that may be used to prepare pharmaceutically acceptable acid addition salts of such basic compounds of the present invention are those that form non-toxic acid addition salts, i.e., salts containing pharmaceutically acceptable anions, such as the acetate, benzoate, bromide, chloride, citrate, fumarate, hydrobromide, hydrochloride, iodide, lactate, maleate, mandelate, nitrate, oxalate, salicylate, succinate, and tartrate salts. Since a single compound of the present invention may include more than one acidic or basic moieties, the compounds of the present invention may include mono, di or tri-salts in a single compound.

In the case of an acidic moiety in a compound of the present invention, a salt may be formed by treatment of a compound of the present invention with a basic compound, particularly an inorganic base. Preferred inorganic salts are those formed with alkali and alkaline earth metals such as lithium, sodium, potassium, barium and calcium. Preferred organic base salts include, for example, ammonium, dibenzylammonium, benzylammonium, 2-hydroxyethylammonium, bis(2-hydroxyethyl)ammonium, phenylethylbenzylamine, dibenzyl-ethylenediamine, and the like salts. Other salts of acidic moieties may include, for example, those salts formed with procaine, quinine and N-methylglusoamine, plus salts formed with basic amino acids such as glycine, ornithine, histidine, phenylglycine, lysine and arginine. An especially preferred salt is a sodium or potassium salt of a compound of the present invention.

With respect to basic moieties, a salt is formed by the treatment of a compound of the present invention with an acidic compound, particularly an inorganic acid. Preferred inorganic salts of this type may include, for example, the hydrochloric, hydrobromic, sulfuric, phosphoric or the like salts. Preferred organic salts of this type, may include, for example, salts formed with acetic, succinic, citric, maleic, fumaric, D-glutamic, glycolic, benzoic, cinnamic and the like organic acids. An especially preferred salt of this type is a hydrochloride or sulfate salt of COMPOUND A of the present invention.

Additional pharmaceutically acceptable salts of COMPOUND A and COMPOUND B suitable for the present invention include the salts disclosed in PCT Application No. WO

03/077914 and PCT Application No. WO2007/044084, which are both hereby incorporated into the present application by reference.

Pharmaceutical combinations of the present invention further include at least one compound that inhibits RAF kinase, a serine/ threonine kinase that functions in the MAP kinase signaling pathway.

RAF kinase inhibitors are e.g., compounds which inhibit wild-type C-RAF at the IC_{50} of from 0.05 mmol/L to more than 4.0 mmol/L and/or mutant B-RAF^{V600E} at an IC_{50} of from 0.08 mmol/L to more than 4.0 mmol/L in the following assays:

Test for activity against the RAF kinase: Active B-RAF, C-RAF and B-RAF^{V600E} proteins of human sequence are purified from insect cells using the baculoviral expression system. RAF inhibition is tested in 96-well microplates coated with IκB-α and blocked with Superblock. The phosphorylation of IκB-α at Serine 36 is detected using a phospho- IκB-α specific antibody (Cell Signaling #9246), an anti-mous IgG alkaline phosphatase conjugated secondary antibody (Pierce #31320), and an alkaline phophatase substrate, ATTOPHOS (Promega, #S101).

The RAF kinase inhibitor may be a pan-RAF kinase inhibitor or a selective RAF kinase inhibitor. Pan-RAF kinase inhibitors include but are not limited to RAF265, sorafenib, or SB590885. In some embodiments, the RAF kinase inhibitor is a B-RAF kinase inhibitor. In some embodiments, the selective RAF kinase inhibitor is PLX4720, PLX4032, or GDC-0879. Particular examples of suitable RAF kinase inhibitors include:

Sorafenib and its tosylate salt, also known as Nexavar™ or BAY 43-9006, has been the first RAF kinase inhibitor to receive marketing approval for treatment of advanced renal cell carcinoma (RCC). Sorafenib, also known as 4-[4-[[4-chloro-3-(trifluoromethyl)phenyl] carbamoylamino]phenoxy]-N-methyl-pyridine-2-carboxamide, is a bi-aryl urea derivative of the following structure:

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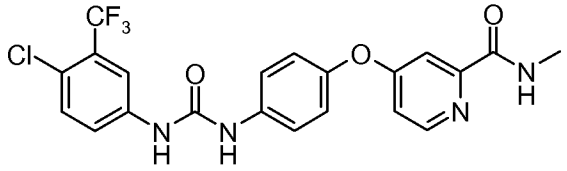
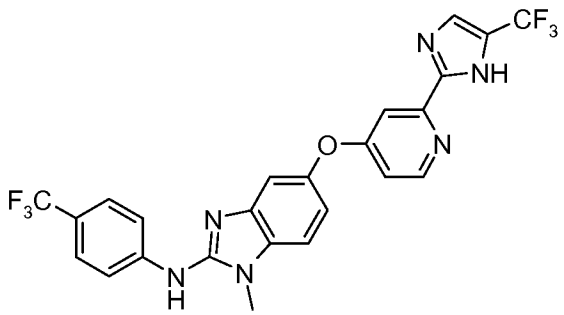


Sorafenib and methods for its preparation have been described e.g. in U.S. Patent No.7,235,576 or U.S. Patent No. 7,351,834.

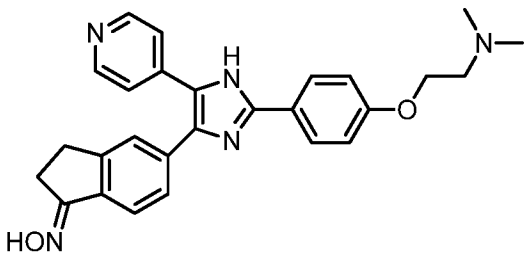
RAF265 (previously known as CHIR-265) is another orally bioavailable RAF kinase inhibitor of following structure:



RAF265 and methods for its preparation for are for instance described in WO07/030377.

Additional RAF kinase inhibitors which may be used in accordance with the present invention include for instance SB590885 (described in WO 2002/024680), XL281 (described in WO2008/120004), PLX4720, PLX4032, GDC0879 (described in WO2006/084015) and ZM336372.

SB590885



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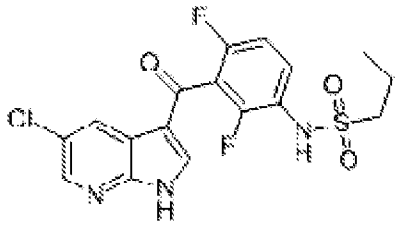
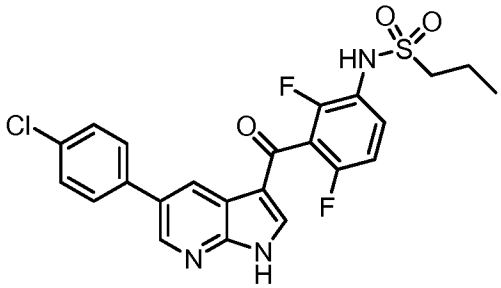
PLX4720



PLX4032



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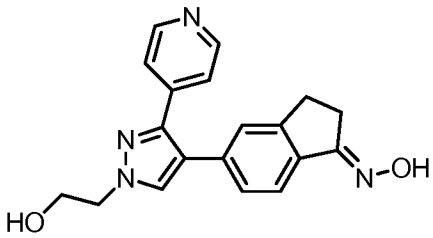

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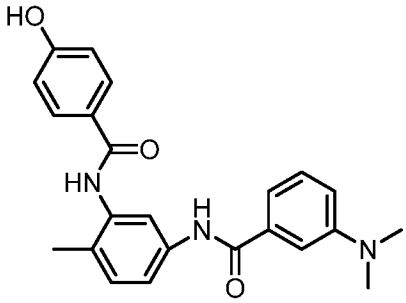

In a preferred embodiment, the pharmaceutical combination of the present invention includes at least one RAF kinase inhibitor that is RAF265.

Unless otherwise specified, or clearly indicated by the text, reference to therapeutic agents useful in the pharmaceutical combination of the present invention includes both the free base of the compounds, and all pharmaceutically acceptable salts of the compounds.

The structure of the compounds identified by code nos., generic or trade names may be taken from the actual edition of the standard compendium "The Merck Index" or from databases, e.g., Patents International (IMS World Publications). The corresponding content thereof is hereby incorporated by reference.

In each case where citations of patent applications are given above, the subject matter relating to the compounds is hereby incorporated into the present application by reference. The compounds used as therapeutic agents in the pharmaceutical combinations of the present invention can be prepared and administered as described in the cited documents, respectively. Also within the scope of this invention is the combination of two separate therapeutic agents as set forth above, i.e., a pharmaceutical combination within the scope of this invention could include three therapeutic agents or more.

A pharmaceutical combination which comprises (a) a MEK inhibitor selected from the group consisting of 6-(4-bromo-2-fluorophenylamino)-7-fluoro-3-methyl-3H-benzoimidazole-5-carboxylic acid (2-hydroxyethoxy)-amide (COMPOUND A) or (*S*)-5-fluoro-2-(2-fluoro-4-(methylthio)phenylamino)-*N*-(2-hydroxypropoxy)-1-methyl-6-oxo-1,6-dihydropyridine-3-carboxamide (COMPOUND B) or a pharmaceutically acceptable salt thereof, and (b) at least one RAF kinase inhibitor or a pharmaceutically acceptable salt thereof, will be referred to hereinafter as a COMBINATION OF THE INVENTION.

According to the present invention, the preferred combination partners are (a) a MEK inhibitor selected from the group consisting of 6-(4-bromo-2-fluorophenylamino)-7-fluoro-3-methyl-3H-benzoimidazole-5-carboxylic acid (2-hydroxyethoxy)-amide (COMPOUND A) or (*S*)-5-fluoro-2-(2-fluoro-4-(methylthio)phenylamino)-*N*-(2-hydroxypropoxy)-1-methyl-6-oxo-1,6-dihydropyridine-3-carboxamide (COMPOUND B) or a pharmaceutically acceptable salt thereof, and (B) at least one RAF kinase inhibitor which is RAF265 or a pharmaceutically acceptable salt thereof.

In another embodiment, the preferred combination partners are (a) a MEK inhibitor 6-(4-bromo-2-fluorophenylamino)-7-fluoro-3-methyl-3H-benzoimidazole-5-carboxylic acid (2-hydroxyethoxy)-amide (COMPOUND A) or a pharmaceutically acceptable salt thereof, and (B) at least one RAF kinase inhibitor which is RAF265 or a pharmaceutically acceptable salt thereof.

The present invention also pertains to a combination such as a combined preparation or a pharmaceutical composition which comprises (a) a MEK inhibitor selected from the group

consisting of 6-(4-bromo-2-fluorophenylamino)-7-fluoro-3-methyl-3H-benzoimidazole-5-carboxylic acid (2-hydroxyethoxy)-amide (COMPOUND A) or (*S*)-5-fluoro-2-(2-fluoro-4-(methylthio)phenylamino)-*N*-(2-hydroxypropoxy)-1-methyl-6-oxo-1,6-dihydropyridine-3-carboxamide (COMPOUND B) or a pharmaceutically acceptable salt thereof, and (b) at least one RAF kinase inhibitor or a pharmaceutically acceptable salt thereof.

The present invention particularly pertains to a COMBINATION OF THE INVENTION useful for treating or preventing a proliferative disease in a subject in need thereof. In this embodiment of the present invention, the COMBINATION OF THE INVENTION is used for the treatment or prevention of a proliferative disease comprising administering to the subject a combination therapy, comprising an effective amount of a MEK inhibitor compound selected from COMPOUND A or COMPOUND B and an effective amount of a RAF kinase inhibitor. Preferably, these inhibitors are administered at therapeutically effective dosages which, when combined, provide a beneficial effect. The administration may be simultaneous or sequential.

In one embodiment, the proliferative disease is cancer. The term "cancer" is used herein to mean a broad spectrum of tumors, including all solid tumors and hematological malignancies. Examples of such tumors include but are not limited to benign or malignant tumors of the brain, lung (in particular small-cell lung cancer and non-small cell lung cancer), squamous cell, bladder, gastric, pancreatic, breast, head and neck, renal, kidney, ureter, ovarian, prostate, colorectal, esophageal, testicular, gynecological (e.g., uterine sarcomas, carcinoma of the fallopian tubes, endometrial, cervix, vagina or vulva), thyroid, pancreatic, bone, skin, melanoma, uterine, ovarian, rectal, anal, colon, testicular, Hodgkin's disease, esophageal, small intestine, endocrine system (e.g., thyroid, parathyroid, or adrenal glands), sarcomas of soft tissues, urethra, penis, leukemia, lymphomas, neoplasms of the central nervous system, sarcomas, myeloma, biliary, liver, neurofibromatosis, acute myelogenous leukemia (AML), myelodysplastic syndromes (MDS), and Kaposi's sarcoma.

In a further embodiment of the present invention, the proliferative disease is melanoma, lung cancer (including non-small cell lung cancer (NSCLC)), colorectal cancer (CRC), breast cancer, kidney cancer such as e.g., renal cell carcinoma (RCC), liver cancer, endometrial cancer, acute myelogenous leukemia (AML), myelodysplastic syndromes (MDS), thyroid cancer, pancreatic cancer, neurofibromatosis or hepatocellular carcinoma.

In a further embodiment of the present invention, the proliferative disease is a solid tumor. The term "solid tumor" especially means breast cancer, ovarian cancer, colorectal cancer, and generally gastrointestinal tract, cervix cancer, lung cancer (including small-cell lung cancer and non-small cell lung cancer), head and neck cancer, bladder cancer, prostate cancer or Kaposi's sarcoma. The present combination inhibits the growth of solid tumors and also liquid tumors. Further, depending on the tumor type and particular combination used, a decrease of the tumor volume can be obtained. The COMBINATION OF THE INVENTION disclosed herein is also suited to prevent the metastatic spread of tumors and the growth or development of micrometastases. The COMBINATION OF THE INVENTION disclosed herein are suitable for the treatment of poor prognosis patients, especially such poor prognosis patients having metastatic melanoma or pancreatic cancer.

In a further embodiment, the proliferative disease is melanoma, colorectal cancer or lung cancer, particularly non-small cell lung cancer.

It will be understood that the COMBINATION OF THE INVENTION may be used solely for the treatment of a proliferative disease in accordance with the present invention.

The COMBINATION OF THE INVENTION is particularly useful for the treatment of cancers having a genetic alteration in the RAS/ RAF/ MEK signal transduction pathway such as, for example, a HRAS, KRAS, NRAS or BRAF (including $BRAF^{V600E}$) mutation or gene amplification. In one embodiment, the cancer to be treated has a KRAS mutation, e.g., KRAS mutated pancreatic cancer, colon cancer, lung cancer (e.g., NSCLC) or leukemias.

It has been found that the combination therapy comprising a MEK inhibitor compound 6-(4-bromo-2-fluorophenylamino)-7-fluoro-3-methyl-3H-benzoimidazole-5-carboxylic acid (2-hydroxyethoxy)-amide (COMPOUND A) or (*S*)-5-fluoro-2-(2-fluoro-4-(methylthio)phenylamino)-*N*-(2-hydroxypropoxy)-1-methyl-6-oxo-1,6-dihydropyridine-3-carboxamide (COMPOUND B) with a RAF kinase inhibitor, particularly RAF265, results in unexpected improvement in the treatment or prevention of proliferative diseases as compared to the monotherapy. When administered simultaneously, sequentially or separately, the MEK inhibitor compound 6-(4-bromo-2-fluorophenylamino)-7-fluoro-3-methyl-3H-benzoimidazole-5-carboxylic acid (2-hydroxyethoxy)-amide (COMPOUND A) or (*S*)-5-fluoro-2-(2-fluoro-4-

(methylthio)phenylamino)-*N*-(2-hydroxypropoxy)-1-methyl-6-oxo-1,6-dihydropyridine-3-carboxamide (COMPOUND B) and the RAF kinase inhibitor interact synergistically to inhibit cell proliferation. The COMBINATIONS OF THE INVENTION are in particular suitable for the treatment of patients with advanced cancer who have failed standard systemic therapy. This includes patients having tumor types showing resistance to monotherapy or showing resistance to combinations different from those disclosed herein.

The nature of proliferative diseases is multifactorial. Under certain circumstances, drugs with different mechanisms of action may be combined. However, just considering any combination of therapeutic agents having different mode of action does not necessarily lead to combinations with advantageous effects.

The administration of a pharmaceutical combination of the invention may result not only in a beneficial effect, *e.g.* a synergistic therapeutic effect, *e.g.* with regard to alleviating, delaying progression of or inhibiting the symptoms, but also in further surprising beneficial effects, *e.g.* fewer side-effects, an improved quality of life or a decreased morbidity, compared with a monotherapy applying only one of the pharmaceutically therapeutic agents used in the combination of the invention.

A further benefit is that lower doses of the therapeutic agents of the COMBINATION OF THE INVENTION can be used, for example, that the dosages need not only often be smaller, but are also applied less frequently, or can be used in order to diminish the incidence of side-effects observed with one of the combination partners alone. This is in accordance with the desires and requirements of the patients to be treated.

It can be shown by established test models that a COMBINATION OF THE INVENTION results in the beneficial effects described herein before. The person skilled in the art is fully enabled to select a relevant test model to prove such beneficial effects. The pharmacological activity of a COMBINATION OF THE INVENTION may, for example, be demonstrated in a clinical study or in a test procedure as essentially described hereinafter.

Suitable clinical studies are in particular, for example, open label, dose escalation studies in patients with a proliferative diseases. Such studies prove in particular the synergism of the therapeutic agents of the COMBINATION OF THE INVENTION. The beneficial effects on

proliferative diseases may be determined directly through the results of these studies which are known as such to a person skilled in the art. Such studies may be, in particular, suitable to compare the effects of a monotherapy using either therapeutic agent and a COMBINATION OF THE INVENTION. In one embodiment, the dose of a MEK inhibitor compound selected from the group consisting of COMPOUND A or COMPOUND B, is escalated until the Maximum Tolerated Dosage is reached, and at least one RAF kinase inhibitor is administered with a fixed dose. Alternatively, a MEK inhibitor compound selected from the group consisting of COMPOUND A or COMPOUND B may be administered in a fixed dose and the dose of at least one RAF kinase inhibitor may be escalated. Each patient may receive doses of a MEK inhibitor compound selected from the group consisting of COMPOUND A or COMPOUND B, either daily or intermittently. The efficacy of the treatment may be determined in such studies, *e.g.*, after 12, 18 or 24 weeks by evaluation of symptom scores every 6 weeks.

Determining a synergistic interaction between one or more components, the optimum range for the effect and absolute dose ranges of each component for the effect may be definitively measured by administration of the components over different w/w ratio ranges and doses to patients in need of treatment. For humans, the complexity and cost of carrying out clinical studies on patients may render impractical the use of this form of testing as a primary model for synergy. However, the observation of synergy in one species can be predictive of the effect in other species and animal models exist, as described herein, to measure a synergistic effect and the results of such studies can also be used to predict effective dose and plasma concentration ratio ranges and the absolute doses and plasma concentrations required in other species by the application of pharmacokinetic/ pharmacodynamic methods. Established correlations between tumor models and effects seen in man suggest that synergy in animals may, e.g., be demonstrated in the BRAF mutant UACC-62, IGR-1, RPMI-7951, MDA-MB-435S, or WM-115 melanoma tumor models; the NRAS mutant Hs 994.T, SK-MEL-30 or MEL-JUSO melanoma tumor models; the BRAF mutant SW1417, COLO 205, LS411N, HT-29, or RKO colorectal tumor models; the KRAS mutant LoVo, SW480, COLO678, LS123, NCI-H747, HCT-15, NCT116, DLD-1, T84, LS 180, or GP2d colorectal tumor models; or the KRAS mutant Calu-6, NCI-1792, NCI-H358, NCI-H2122, NCI-H1734, NCI-H23, NCI-H1573, or NCI-H460 non-small cell lung cancer tumor models as described in the Examples below.

In a preferred embodiment of the present invention, the COMBINATION OF THE INVENTION comprises the MEK inhibitor COMPOUND A and at least one RAF kinase inhibitor that is RAF265 for use in the treatment or prevention of a proliferative disease, preferably a cancer, comprising a NRAS, KRAS or BRAF mutation. Preferably, the cancer comprising a NRAS, KRAS or BRAF mutation is melanoma, colorectal, or lung.

In one aspect, the present invention provides a synergistic combination for human administration comprising (a) a MEK inhibitor selected from the group consisting of 6-(4-bromo-2-fluorophenylamino)-7-fluoro-3-methyl-3H-benzoimidazole-5-carboxylic acid (2-hydroxyethoxy)-amide (COMPOUND A) or (*S*)-5-fluoro-2-(2-fluoro-4-(methylthio)phenylamino)-*N*-(2-hydroxypropoxy)-1-methyl-6-oxo-1,6-dihydropyridine-3-carboxamide (COMPOUND B) or a pharmaceutically acceptable salt thereof, and (b) at least one RAF kinase inhibitor, particularly RAF265, or a pharmaceutically acceptable salt thereof, in a combination range (w/w) which corresponds to the ranges observed in a tumor model, e.g., as described in the Examples below, used to identify a synergistic interaction. Suitably, the ration range in humans corresponds to a non-human range selected from between 50:1 to 1:50 parts by weight, 50:1 to 1:20, 50:1 to 1:10, 50:1 to 1:1, 20:1 to 1:50, 20:1 to 1:20, 20:1 to 1:10, 20:1 to 1:1, 10:1 to 1:50, 10:1 to 1:20, 10:1 to 1:10, 10:1 to 1:1, 1:1 to1:50, 1:1 to 1:20 and 1:1 to 1:10. More suitably, the human range corresponds to a non-human range of the order of 10:1 to 1:1, 5:1 to 1:1 or 2:1 to 1:1 parts by weight.

According to a further aspect, the present invention provides a synergistic combination for administration to humans comprising (a) a MEK inhibitor selected from the group consisting of 6-(4-bromo-2-fluorophenylamino)-7-fluoro-3-methyl-3H-benzoimidazole-5-carboxylic acid (2-hydroxyethoxy)-amide (COMPOUND A) or (*S*)-5-fluoro-2-(2-fluoro-4-(methylthio)phenylamino)-*N*-(2-hydroxypropoxy)-1-methyl-6-oxo-1,6-dihydropyridine-3-carboxamide (COMPOUND B) or a pharmaceutically acceptable salt thereof, and (b) at least one RAF kinase inhibitor, particularly RAF265, or a pharmaceutically acceptable salt thereof, where the dose range of each component corresponds to the synergistic ranges observed in a suitable tumor model, e.g., the tumor models described in the Examples below, primarily used to identify a synergistic interaction.

It is one objective of this invention to provide a pharmaceutical composition comprising a quantity, which is jointly therapeutically effective against a proliferative disease comprising the COMBINATION OF THE INVENTION. In this composition, the combination partners (a) and (b) can be either administered in a single formulation or unit dosage form, administered concurrently but separately, or administered sequentially by any suitable route. The unit dosage form may also be a fixed combination.

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

The novel pharmaceutical composition contains may contain, from about 0.1 % to about 99.9%, preferably from about 1 % to about 60 %, of the therapeutic agent(s).

Suitable pharmaceutical compositions for the combination therapy for enteral or parenteral administration are, for example, those in unit dosage forms, such as sugar-coated tablets, tablets, capsules or suppositories, or ampoules. If not indicated otherwise, these are prepared in a manner known *per se*, for example by means of various conventional mixing, comminution, direct compression, granulating, sugar-coating, dissolving, lyophilizing processes, or fabrication techniques readily apparent to those skilled in the art. It will be appreciated that the unit content of a combination partner contained in an individual dose of each dosage form need not in itself constitute an effective amount since the necessary effective amount may be reached by administration of a plurality of dosage units.

A unit dosage form containing the combination of agents or individual agents of the combination of agents may be in the form of micro-tablets enclosed inside a capsule, *e.g.* a gelatin capsule. For this, a gelatin capsule as is employed in pharmaceutical formulations can be used, such as the hard gelatin capsule known as CAPSUGEL, available from Pfizer.

The unit dosage forms of the present invention may optionally further comprise additional conventional carriers or excipients used for pharmaceuticals. Examples of such carriers include, but are not limited to, disintegrants, binders, lubricants, glidants, stabilizers, and fillers, diluents, colorants, flavours and preservatives. One of ordinary skill in the art may select one or more of the aforementioned carriers with respect to the particular desired properties of the dosage form by routine experimentation and without any undue burden. The amount of each carriers used may vary within ranges conventional in the art. The following references which are all hereby incorporated by reference disclose techniques and excipients used to formulate oral dosage forms. See *The Handbook of Pharmaceutical Excipients*, 4th edition, Rowe et al., Eds., American Pharmaceuticals Association (2003); and *Remington: the Science and Practice of Pharmacy*, 20th edition, Gennaro, Ed., Lippincott Williams & Wilkins (2003).

These optional additional conventional carriers may be incorporated into the oral dosage form either by incorporating the one or more conventional carriers into the initial mixture before or during granulation or by combining the one or more conventional carriers with granules comprising the combination of agents or individual agents of the combination of agents in the oral dosage form. In the latter embodiment, the combined mixture may be further blended, e.g., through a V-blender, and subsequently compressed or molded into a tablet, for example a monolithic tablet, encapsulated by a capsule, or filled into a sachet.

Examples of pharmaceutically acceptable disintegrants include, but are not limited to, starches; clays; celluloses; alginates; gums; cross-linked polymers, e.g., cross-linked polyvinyl pyrrolidone or crospovidone, e.g., POLYPLASDONE XL from International Specialty Products (Wayne, NJ); cross-linked sodium carboxymethylcellulose or croscarmellose sodium, e.g., AC-DI-SOL from FMC; and cross-linked calcium carboxymethylcellulose; soy polysaccharides; and guar gum. The disintegrant may be present in an amount from about 0% to about 10% by weight of the composition. In one embodiment, the disintegrant is present in an amount from about 0.1% to about 5% by weight of composition.

Examples of pharmaceutically acceptable binders include, but are not limited to, starches; celluloses and derivatives thereof, for example, microcrystalline cellulose, e.g., AVICEL PH from FMC (Philadelphia, PA), hydroxypropyl cellulose hydroxylethyl cellulose and hydroxylpropylmethyl cellulose METHOCEL from Dow Chemical Corp. (Midland, MI);

sucrose; dextrose; corn syrup; polysaccharides; and gelatin. The binder may be present in an amount from about 0% to about 50%, e.g., 2-20% by weight of the composition.

Examples of pharmaceutically acceptable lubricants and pharmaceutically acceptable glidants include, but are not limited to, colloidal silica, magnesium trisilicate, starches, talc, tribasic calcium phosphate, magnesium stearate, aluminum stearate, calcium stearate, magnesium carbonate, magnesium oxide, polyethylene glycol, powdered cellulose and microcrystalline cellulose. The lubricant may be present in an amount from about 0% to about 10% by weight of the composition. In one embodiment, the lubricant may be present in an amount from about 0.1% to about 1.5% by weight of composition. The glidant may be present in an amount from about 0.1% to about 10% by weight.

Examples of pharmaceutically acceptable fillers and pharmaceutically acceptable diluents include, but are not limited to, confectioner's sugar, compressible sugar, dextrates, dextrin, dextrose, lactose, mannitol, microcrystalline cellulose, powdered cellulose, sorbitol, sucrose and talc. The filler and/or diluent, e.g., may be present in an amount from about 0% to about 80% by weight of the composition.

In one embodiment, the present invention also pertains to a COMBINATION OF THE INVENTION for use in the preparation of a pharmaceutical composition or medicament for the treatment or prevention of a proliferative disease in a subject in need thereof.

In a further embodiment, the present invention pertains to the use of a MEK inhibitor selected from the group consisting of 6-(4-bromo-2-fluorophenylamino)-7-fluoro-3-methyl-3H-benzoimidazole-5-carboxylic acid (2-hydroxyethoxy)-amide (COMPOUND A) or (*S*)-5-fluoro-2-(2-fluoro-4-(methylthio)phenylamino)-*N*-(2-hydroxypropoxy)-1-methyl-6-oxo-1,6-dihydropyridine-3-carboxamide (COMPOUND B) or a pharmaceutically acceptable salt thereof, in combination with at least one RAF kinase inhibitor or a pharmaceutically acceptable salt thereof for the preparation of a pharmaceutical composition or medicament for the treatment or prevention of a proliferative disease in a subject in need thereof.

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

The effective dosage of each of the combination partners employed in the COMBINATION OF THE INVENTION may vary depending on the particular compound or pharmaceutical composition employed, the mode of administration, the condition being treated, and the severity of the condition being treated. Thus, the dosage regimen of the COMBINATION OF THE INVENTION is selected in accordance with a variety of factors including the route of administration and the renal and hepatic function of the patient. A clinician or physician of ordinary skill can readily determine and prescribe the effective amount of the single therapeutic agents required to alleviate, counter or arrest the progress of the condition.

The optimum ratios, individual and combined dosages, and concentrations of the combination partners (a) and (b) of the COMBINATION OF THE INVENTION that yield efficacy without toxicity are based on the kinetics of the therapeutic agents' availability to target sites, and are determined using methods known to those of skill in the art.

The effective dosage of each of the combination partners may require more frequent administration of one of the compound(s) as compared to the other compound(s) in the

combination. Therefore, to permit appropriate dosing, packaged pharmaceutical products may contain one or more dosage forms that contain the combination of compounds, and one or more dosage forms that contain one of the combination of compounds, but not the other compound(s) of the combination.

When the combination partners, which are employed in the COMBINATION OF THE INVENTION, are applied in the form as marketed as single drugs, their dosage and mode of administration can be in accordance with the information provided on the package insert of the respective marketed drug, if not mentioned herein otherwise.

The MEK inhibitor COMPOUND A may be administered to a suitable subject daily in single or divided doses at an effective dosage in the range of about 0.001 to about 100 mg per kg body weight per day, preferably about 1 to about 35 mg/kg/day, in single or divided doses. For a 70 kg human, this would amount to about 0.05 to 7 g/day, preferably about 0.05 to about 2.5 g/day.

The MEK inhibitor COMPOUND B may be administered daily to a suitable subject in single or divided doses at an effective dosage in the range of about 0.001 to about 100 mg per kg body weight per day, preferably about 1 mg/kg/day to about 35 mg/kg/day, in single or divided doses. For a 70 kg human, this would amount to about 0.07 to 2.45 g/day, preferably about 0.05 to about 1.0 g/day.

The RAF kinase inhibitor, particularly RAF265, may be administered to a suitable subject in single or divided doses at an effective dosage in the range of about 0.001 to 1000 mg and more preferred from 1.0 to 30 mg/kg body weight daily. Dosage unit compositions may contain such amounts of submultiples thereof to make up the daily dose. a total daily dose administered to a host in single or divided doses may be in amounts, for example, of from 0.001 to 1000 mg/kg body weight daily and from 1.0 to 30 mg/kg body weight daily. Dosage unit compositions may contain such amounts of submultiples thereof to make up the daily dose.

Sorafenib may be administered to a suitable subject in single or divided doses daily at an effective dose in the range of about 75 to 800 mg/day, more preferably 400 mg taken twice daily.

The optimal dosage of each combination partner for treatment of a proliferative disease can be determined empirically for each individual using known methods and will depend upon a

variety of factors, including, though not limited to, the degree of advancement of the disease; the age, body weight, general health, gender and diet of the individual; the time and route of administration; and other medications the individual is taking. Optimal dosages may be established using routine testing and procedures that are well known in the art.

The amount of each combination partner that may be combined with the carrier materials to produce a single dosage form will vary depending upon the individual treated and the particular mode of administration. In some embodiments the unit dosage forms containing the combination of agents as described herein will contain the amounts of each agent of the combination that are typically administered when the agents are administered alone.

Frequency of dosage may vary depending on the compound used and the particular condition to be treated or prevented. In general, the use of the minimum dosage that is sufficient to provide effective therapy is preferred. Patients may generally be monitored for therapeutic effectiveness using assays suitable for the condition being treated or prevented, which will be familiar to those of ordinary skill in the art.

The present invention relates to a method of treating a subject having a proliferative disease comprising administered to said subject a COMBINATION OF THE INVENTION in a quantity, which is jointly therapeutically effective against a proliferative disease. In particular, the proliferative disease to be treated with a COMBINATION OF THE INVENTION is a melanoma, colorectal cancer or lung cancer, particularly non-small cell lung cancer. Furthermore, the treatment can comprise surgery or radiotherapy.

The present invention further relates to the COMBINATION OF THE INVENTION for use in the treatment of a proliferative disease, particularly cancer.

The present invention further provides a commercial package comprising as therapeutic agents COMBINATION OF THE INVENTION, together with instructions for simultaneous, separate or sequential administration thereof for use in the delay of progression or treatment of a proliferative disease in a subject in need thereof.

The following Examples illustrate the invention described above; they are not, however, intended to limit the scope of the invention in any way. The beneficial effects of the

pharmaceutical combination of the present invention can also be determined by other test models known as such to the person skilled in the pertinent art.

EXAMPLE 1 – MELANOMA BRAF AND NRAS MUTANTS

Methods and Materials

B-RAF^{V600E} and NRAS mutant human melanoma cell lines used are obtained from the Novartis Oncology Cell Line Encyclopedia, cultured at 37°C in a 5% CO_2 incubator in appropriate mediums as set forth below:

Cell		Culture media
B-Raf mutant	A375, RPMI-7951, IGR-1, IGR-39	DMEM high glucose, 10% FCS, 1% penicillin/streptomycin, 4mM L-Glutamine and 1mM Sodium Pyruvate
	UACC-62	RPMI 1640, 10% FCS, 1% Penicillin/Streptomycin, 200 mM L-Glutamine, 1mM Sodium Pyruvate, and 10 mM Hepes
	MDA-MB-435S, Colo-800	RPMI 1640, 10% FCS, 1% Penicillin/Streptomycin, 200 mM L-Glutamine, 1mM Sodium Pyruvate, and 10 mM Hepes
	WM-115	EMEM, 10% FCS, 1% penicillin/streptomycin, 4mM L-Glutamine and 1mM Sodium Pyruvate
N-Ras mutant	Hs 944.T, IPC-298, MEL-JUSO, SK-MEL-30	RPMI 1640, 10% FCS, 1% Penicillin/Streptomycin , 200 mM L-Glutamine, 1mM Sodium Pyruvate, and 10 mM Hepes
	SK-MEL-2	EMEM, 10% FCS, 1% penicillin/streptomycin, 4mM L-Glutamine and 1mM Sodium Pyruvate

The components used for the culture medium are obtained as follows: RPMI 1640 (Gibco, Cat. No 61870), 10% FCS (Amimed, Cat. No. 2-01F16-1), 1% Penicillin/Streptomycin (Gibco, Cat. No. 15070-063), 200 mM L-Glutamine (Gibco, Cat. No. 25030), DMEM high glucose (Gibco, Cat No 41865); EMEM (Amimed, Cat No 1-31S01), Sodium Pyruvate (Gibco, Cat. No 11360039), and Hepes (Gibco, Cat No 15630-056).

For maintenance in cell culture and for experiments, the cells are counted with a CASY® cell counter (Schärfe System GmbH, Reutlingen, Germany).

The MEK inhibitor 6-(4-bromo-2-fluorophenylamino)-7-fluoro-3-methyl-3H-benzoimidazole-5-carboxylic acid (2-hydroxyethoxy)-amide (COMPOUND A) and the RAF kinase inhibitor RAF265 are prepared into 10 mM stock solutions with 100% DMSO. From this stock, 2-fold (combination) or 5-fold (single treatment) serial dilutions are prepared in DMSO, followed by a 1:100 dilution in the culture medium. This allows for an equal DMSO concentration of 0.1% for all compound dilutions. Compound-containing medium is then added directly onto the cell cultures to obtain final concentrations ranging from 10 μM to 0.1 nM. 0.1% DMSO is used as a vehicle control.

Resazurin Viabiity Assay

Single Agent Treatment: Cell treatment with single compounds and resazurin assay are performed as described previously. The MEK inhibitor 6-(4-bromo-2-fluorophenylamino)-7-fluoro-3-methyl-3H-benzoimidazole-5-carboxylic acid (2-hydroxyethoxy)-amide (COMPOUND A) and the RAF kinase inhibitor RAF265 are prepared into 10 mM stock solutions with 100% DMSO. From this stock, 2-fold (combination) or 5-fold (single treatment) serial dilutions are prepared in DMSO, followed by a 1:100 dilution in the culture medium. This allows for an equal DMSO concentration of 0.1% for all compound dilutions. Compound-containing medium is then added directly onto the cell cultures to obtain final concentrations ranging from 10 μM to 0.1 nM. 0.1% DMSO is used as a vehicle control. Relative, absolute IC_{50} values and maximal inhibition are calculated by Excel Fit software. Relative IC_{50} values are calculated by setting upper and lower curve plateau from the experimental values. Absolute IC_{50} values are calculated by setting upper plateau at 100 % of control and lower value at 0% of control.

Combination Treatment: Cells are seeded onto three parallel 96-well plates at a density of 1,000 cells / well in 90 μl of appropriate medium for DMSO control or for single treatments (columns B, K and L, FIG. 1), or in 80 μl for combination treatments (columns C to J, FIG. 1). Column A is used as the background control without cells. At 24 hours following the cell seeding, cells are treated with 10 μl of the prepared diluted compounds, either alone or in combination with 10 μl of the combination partner. At 24 hours after cell seeding, a day 0 plate is measured to assess the starting cell number. At 72 hours after starting treatment, 10 μl of 130

μg/ml resazurin solution is directly added onto the cells and plates are read 4 hours later using a fluorescence plate reader Synergy HT from Biotek, plus Biostack, sensitivity 40 (software KC4 3.8 rev18). The proprietary Chalice ™ Analyzer software from CombinatorX is used to analyze the combination effects.

P-ERK in Cell Western Assay

Single Agent Treatment: Cell treatment with single compounds and P-Erk in-cell Western assay are performed as described previously. The MEK inhibitor 6-(4-bromo-2-fluorophenylamino)-7-fluoro-3-methyl-3H-benzoimidazole-5-carboxylic acid (2-hydroxyethoxy)-amide (COMPOUND A) and the RAF kinase inhibitor RAF265 are prepared into 10 mM stock solutions with 100% DMSO. From this stock, 2-fold (combination) or 5-fold (single treatment) serial dilutions are prepared in DMSO, followed by a 1:100 dilution in the culture medium. This allows for an equal DMSO concentration of 0.1% for all compound dilutions. Compound-containing medium is then added directly onto the cell cultures to obtain final concentrations ranging from 10 μM to 0.1 nM. 0.1% DMSO is used as a vehicle control.

Combination Treatment: Cell lines are seeded on three parallel plates at a density of 15,000 cells / well in 180 μl of appropriate medium for controls or single treatments (columns A, B, K and L, FIG. 2), or in 160 μl of medium for combination treatments (columns C to J, in FIG. 2). Black 96-well plates suited for fluorescence measurements are used (Greiner BioOne). Two different controls are performed on each plate: a positive control with 5 μM of MEK inhibitor Compound X ("Compound Control") (set as maximum inhibition) and a DMSO control without compound treatment (set as zero inhibition). A background control that excludes the primary antibody is performed in a separate plate. At 24 hours after seeding, cells are treated with 20 μl of the prepared diluted compounds, either alone or in combination with 20 μl of the combo partner, as depicted in FIG. 2. At 24 hours after starting treatment, cells are lyzed and P-Erk in-cell western is performed.

Combination Effect Analysis

Chalice™ analyzer software is used to analyze the combination between COMPOUND A and RAF265 based on several mathematical models including the Loewe additivity model

described in Lehár et al, Nat Biotechnol. (July 2009), 27(4): 69:66. The results are interpreted based on Best Combination Index at ED_{50} and based on Synergy Score from excess inhibition 2-D data analysis. The terms "Best Combination Index at ED_{50}" or "Best C.I. (at 50% inhibition)" are technical terms referring to the calculated combination index derived from isobologram graphs at the drug dosage required to achieve 50% inhibition in the defined cell-line model, which corresponds to the lowest value on the isobologram. The terms do not refer to the selection of the best single combination index value from several conducted experiments.

Interpretation of values for combination index and synergy score are provided below:

Combination Index (CI)	Synergy Score
CI = 1 → Dose additive	S ~ 0 → Dose additive across surface, or less
CI < 0.5 → "real" synergy (2x dose shift)	S > 2 σ_S → Real synergy detected
CI < 0.3 → "useful" synergy (3x shift)	S > 1 → Usually getting interesting (depends on dose matrix design)
CI < 0.1 → "strong" synergy (10x shift)	

A. Evaluation of Combination Effect in BRAF-mutant melanoma cell lines

Method:

To evaluate effects of dual pathway inhibition by both COMPOUND A and RAF265 in $BRAF^{V600E}$-mutant human melanoma cell lines, viability and P-Erk assay are performed after combining two agents pursuant to the method described above. Concentrations used for combination experiments are determined using single agent IC_{50} values. For IGR-1, MDA-MB-435S, WM-115 and IGR-39 tumor cell models, the concentrations used for combination experiments are determined from viability assays. The concentration ranges that are used in the assay are shown below:

Cell line	Mutation	Assay	Concentration (nM)

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			COMPD A	RAF265
A375	$BRAF^{V600E}$, CDKN2A	Viability	3 – 334	12 - 1536
		P-Erk	1 – 80	6 - 800
UACC-62	$BRAF^{V600E}$, CDKN2A, PTEN	Viability	2 – 277	4 - 483
		P-Erk	1 – 80	6 - 800
IGR-1	$BRAF^{V600E}$, CDKN2A	Viability	22 – 2845	54 - 6966
		P-Erk	1 – 80	6 - 800
RPMI-7951	$BRAF^{V600E}$, CDKN2A, PTEN, TP53	Viability	15 – 1933	31 - 3930
		P-Erk	1 – 80	6 - 800
MDA-MB-435S	$BRAF^{V600E}$, CDKN2A, TP53	Viability	3 – 330	7 - 865
		P-Erk	1 – 80	6 - 800
WM-115	$BRAF^{V600E}$, PTEN, CDKN2A	Viability	11 - 1424	6 - 735
		P-Erk	0.1 - 1250	0.1 - 6250
IGR-39	$BRAF^{V600E}$, PTEN, TP53	Viability	78 – 10,000	22 - 2773
		P-Erk	6 - 800	6 - 800
Colo-800	$BRAF^{V600E}$, TP53, CDKN2A	Viability	5 - 570	3 - 390
		P-Erk	0.5 - 61	8 - 1037

Cell culture viability/proliferation 72 hour assays are done in triplicates in a 96-well format. Cellular in cell Western P-Erk 24 h assays are done in triplicates in a 96-well format. Combination effects for viability and P-Erk inhibition are calculated using Chalice software. C.I., combination index values are derived from isobolograms at 50% inhibition. Best C.I. at ED_{50} corresponds to the lowest value on the isobologram.

Synergy score is derived from Excess Inhibition 2D matrix calculated according to Loewe additivity model in Chalice software. Effect description is qualitative description of the type of combination effect observed, based on the synergy score and the Best C.I. at ED_{50}. Percentage of maximum cell death with combination is calculated against single agent, if cell death is detected with a single agent, or against pretreatment control, if single agent does not induce cell death.

Results from the combination viability and P-Erk In-Cell Western assay are analyzed using Chalice software in accordance with the process set forth in Lehár et al, Nat Biotechnol. (July 2009), 27(4): 69:66. The Chalice analyses results are presented in a series of graphical and 2-D tabular representations, including isobolograms, inhibition matrices and excess inhibition matrices. Inhibition matrices are also produced using the data after subtraction of Day 0 values in viability assays. In Day 0-subtracted matrices, values >100% indicate cell death.

Results:

A summary of main parameters calculated by Chalice in all $BRAF^{V600E}$ mutant human melanoma cell lines is shown as follows:

Cell line	Mutation	Assay	Best C.I. at ED_{50}	Synergy Score	Effect Description	Max. Cell Death (%)
A375	$BRAF^{V600E}$, CDKN2A	Viability	0.747	1.74	Additive	0
		P-Erk	1.00	0.633	Additive	
UACC-62	$BRAF^{V600E}$, CDKN2A, PTEN	Viability	0.584	2.67	Additive/Synergy	35
		P-Erk	Not Calculated	1.5	Additive	
IGR-1	$BRAF^{V600E}$, CDKN2A	Viability	0.144	4.49	Synergy	9
		P-Erk	0.472	2.15	Additive/Synergy	
RPMI-	$BRAF^{V600E}$,	Viability	0.301	3.24	Synergy	16

7951	CDKN2A, PTEN, TP53	P-Erk	0.311	2	Additive/Synergy	
MDA-MB-435S	$BRAF^{V600E}$, CDKN2A, TP53	Viability	0.506	4.77	Additive/Synergy	21
		P-Erk	1.29	1.99	Additive	
WM-115	$BRAF^{V600E}$, PTEN, CDKN2A	Viability	0.515	4.70	Synergy	19
		P-Erk	0.524	5.42	Synergy	
IGR-39	$BRAF^{V600E}$, PTEN, TP53	Viability	0.768	1.2	Additive	0
		P-Erk	1.20	0.563	Additive	
Colo-800	$BRAF^{V600E}$, TP53, CDKN2A	Viability	1.20	2.06	Additive	26
		P-Erk	2.35	0.353	Additive	

The results generally indicate synergy or additivity for the combination of COMPOUND A and RAF265 in both viability and P-Erk assays. COMPOUND A-insensitive lines, such as IGR-1, RPMI-7951 and MDA-MB-435S, and a medium sensitive line WM-115 show a trend for synergy. IGR-39, which is an insensitive line, showed additivity. COMPOUND A-sensitive lines, such as A375, UACC-62, and Colo-800, show a trend for additive anti-proliferative effects. Cell death (up to 35%) is induced by the combination in some cell lines, independently of their sensitivity to COMPOUND A. In conclusion, the results indicate that combination of COMPOUND A and RAF265 result in additive to synergistic effects on BRAF mutant cancer cell viability. Importantly, greatest synergy is observed in lines resistant to COMPOUND A single agent, suggesting that melanoma response in xenograft vivo models and in patients may improve with this combination.

B. Evaluation of Combination Effect in NRAS mutant melanoma cell lines

Method:

The combination of COMPOUND A and RAF265 compounds is tested in NRAS-mutant human melanoma cell lines in viability and P-Erk assays pursuant to the method described

above. Concentration ranges for combination experiments are determined using single agent IC_{50} values. The concentration ranges used in the assay are shown below:

Cell line	Mutation	Assay	Concentration (nM)	
			COMPOUND A	RAF265
IPC-298	NRAS, TP53, CDKN2A	Viability	1 – 89	3 - 336
		P-Erk	1 – 118	23 - 2906
SK-MEL-30	TP53, CDKN2A	Viability	2 – 186	26 - 3282
		P-Erk	1 – 78	24 - 3045
SK-MEL-2	TP53	Viability	5 – 574	2 - 294
		P-Erk	0.5 – 42	10 - 1271
MEL-JUSO	NRAS, HRAS, CDKN2A	Viability	10 – 1276	5 - 608
		P-Erk	0.01 – 488	0.1 – 10,000
Hs 944.T	NRAS, PTEN, CDKN2A	Viability	28 - 3533	9 - 1123
		P-Erk	1 - 68	45 - 5811

Cell culture viability/proliferation 72 hour assays are done in triplicates in a 96-well format. Cellular in cell Western P-Erk 24 h assays are done in triplicates in a 96-well format. Combination effects for viability and P-Erk inhibition are calculated using Chalice software. Compound combinations are assessed by combination index analysis derived from isobologram graphs at 50% inhibition, and are reported as Best Combination Index (C.I.) at ED_{50}. The term "Best Combination Index (C.I.) at ED_{50}" or "Best C.I. at ED_{50}" is a technical term referring to the calculated combination index derived from isobologram graphs at the drug dosage required to achieve 50% inhibition in the defined cell-line model, which corresponds to the lowest value on the isobologram. The term does not refer to the selection of the best single combination index value from several conducted experiments. Synergy score is derived from Excess Inhibition 2D matrix is calculated according to Loewe additivity model in Chalice software. Effect description is a qualitative description of the type of combination effect observed, based on the synergy score and best C.I. Percentage of maximum cell death with combination is calculated against single agent, if cell death is detected with a single agent, or against pretreatment control, if single agent does not induce cell death.

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Results:

A summary of main parameters calculated by Chalice in all NRAS mutant human melanoma cell lines is shown as follows:

Cell line	Mutation	Assay	Best C.I. at ED_{50}	Synergy Score	Effect Description	Max. Cell Death (%)
Hs 944.T	NRAS, PTEN, CDKN2A	Viability	1.04	4.76	Additive/Synergy	16
		P-Erk	1.44	0.375	Additive	
SK-MEL-2	NRAS, TP53	Viability	0.767	2.06	Additive	28
		P-Erk	Not Calculated	5.95	Inconclusive	
IPC-298	NRAS, TP53, CDKN2A	Viability	1.12	1.16	Additive	6
		P-Erk	0.734	1.78	Additive	
SK-MEL-30	NRAS, TP53, CDKN2A	Viability	0.567	3.07	Additive/Synergy	14
		P-Erk	0.603	0.288	Additive	
MEL-JUSO	NRAS, HRAS, CDKN2A	Viability	1.08	1.59	Additive	5
		P-Erk	0.143	6.77	Synergy	

The results generally indicate additive effects for COMPOUND A and RAF265 in both viability and P-Erk assays in several cell lines. Using synergy score parameter, a synergy is detected in viability of two cell lines (Hs944.T and SK-MEL-30), suggesting effect boosts effect without dose shifts. Synergy score indicates a synergy in P-Erk assay in two lines (SK-MEL-2 and MEL-JUSO). Unlike in the BRAF mutant panel, this NRAS mutant line panel is showing no correlation of synergy with insensitivity of cell lines to COMPOUND A single agent. It is known that in the RAS mutant background, RAF kinase inhibitors are not effective in P-Erk inhibition and that even pathway activation may occur, depending on a cell line which may

provide an explanation for the results. The combination induces variable level of cell death with a maximal 28% in COMPOUND A-sensitive IPC-298 line. In conclusion, combination of COMPOUND A and RAF265 in NRAS mutant background leads to at least additive and sometimes synergistic effects in cell lines.

EXAMPLE 2 – COLORECTAL CANCER - BRAF and KRAS MUTANTS

Methods and Materials

The following BRAF and KRAS mutant human colorectal cancer cell lines are obtained from the American Type Culture Collection (ATCC), cultured at 37°C in a 5% CO_2 incubator for 72 hours, and maintained in their respective culture medium as specified by ATCC:

Cell-line Name	BRAF	KRAS	NRAS	PIK3CA	TP53	PTEN	CDKN2A	Doubling time	COMPOUND A	RAF265
								[hrs]	IC50 [nM]	IC50 [nM]
SW1417	mut	wt	wt	wt	mut	wt	wt	63.1	53.9	560
COLO 205	mut	wt	wt	wt	mut	wt	wt	23.4	34.4	918
LS411N	mut	wt	wt	wt	mut	wt	wt	28.7	54.7	864
HT-29	mut	wt	wt	mut	mut	wt	wt	27.6	84.0	2190
HT-29*	mut	wt	wt	mut	mut	wt	wt	27.6	66.5	1280
RKO	mut	wt	wt	mut	wt	wt	wt	21.0	>2700	680
OUMS-23	mut	nd	nd	wt	nd	mut	nd	40.8	>2700	>5400
SW620	wt	mut	wt	wt	mut	wt	wt	24.8	26.9	5240
LoVo	wt	mut	wt	wt	wt	wt	mut	27.0	148	109
SW480	wt	mut	wt	amp	mut	nd	wt	30.5	2030	2160
SW837	wt	mut	wt	wt	mut	wt	wt	50.9	557	928
COLO-678	wt	mut	wt	wt	wt	wt	mut	37.2	1710	4730
LS123	wt	mut	wt	wt	mut	wt	mut	88.6	2280	2420
NCI-H747	wt	mut	wt	amp	mut	wt	wt	42.6	153	2460
HCT-15	wt	mut	wt	mut	mut	wt	wt	22.6	>2700	483
HCT 116	wt	mut	wt	mut	wt	wt	mut	22.5	881	1370
HCT 116*	wt	mut	wt	mut	wt	wt	mut	22.1	735	920
DLD-1	wt	mut	wt	mut	mut	wt	wt	25.2	>2700	617
T84**	wt	mut	wt	mut	mut	wt	wt	43.5	1280	1840

SW948**	wt	mut	wt	mut	wt	wt	wt	52.5	89.8	1400
LS 180	wt	mut	wt	mut	nd	nd	nd	29.2	143	3630
GP2d	wt	mut	wt	mut	wt	wt	wt	32.0	756	2620
HuTu 80	wt	wt	wt	wt	wt	wt	wt	24.5	>2700	1730
CW-2	wt	wt	wt	wt	wt	wt	wt	31.1	>2700	2200
SW48	wt	wt	wt	wt	wt	wt	wt	36.0	78.4	4750
NCI-H716	wt	wt	wt	wt	mut	wt	wt	50.9	>2700	1260
C2BBe1	wt	wt	wt	wt	mut	wt	wt	32.6	1580	4150
SNU-C1	wt	wt	wt	wt	mut	wt	wt	27.7	15.1	3290
KM12	wt	wt	wt	wt	mut	mut	wt	40.9	339	793
						Median		31.1	756	1840
						Mean		36.2	1160	2180

"amp" – amplified, "nd" - no data

The MEK inhibitor 6-(4-bromo-2-fluorophenylamino)-7-fluoro-3-methyl-3H-benzoimidazole-5-carboxylic acid (2-hydroxyethoxy)-amide (COMPOUND A) and the RAF kinase inhibitor RAF265 are used to prepare stock solutions in DMSO/water (90:10) at 2,000-fold the desired final concentration. Both compounds are serially diluted 3-fold to achieve concentrations ranging from 27-fold to $1/27^{th}$ of the cellular IC_{50} for the primary target estimated based on cell-based target modulation assays. The highest and lowest final concentrations are 2700 and 3.7 nM for COMPOUND A, and 5400 and 7.4 nM for RAF265, respectively. Compound dilutions of the single agents encompassing 9-fold to $1/9^{th}$ of the desired final concentration are tested individually or are combined in all possible permutations in a checkerboard fashion. In addition, the highest (27-fold) and lowest ($1/27^{th}$) concentrations of each compound are combined individually to arrive at an extended 5x5 grid layout. The final compound dilution master plates are stored at 4°C.

For the assessment of combination effects, cells are seeded into 384-well plates at 500 cells/well and incubated overnight. The contents of the compound master plates are pre-diluted 1:200 (1 μL compound solution to 200 μL RPMI-160 cell culture medium containing 10% fetal calf serum) before transferring 5 μL of this pre-dilution to the cell plates containing 20 μL cell culture medium, to achieve the targeted final compound concentrations as well as a vehicle (DMSO) concentration of 0.09%.

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Effects of single agents as well as their checkerboard combinations on cell viability are assessed by quantification of cellular ATP levels (CellTiterGlo, Promega) using 25 μL reagent/well and n=2 replicate plates per condition. The number/viability of cells at time of compound addition are assessed and are used to estimate the population doubling time of a particular cell line. Single agent IC_{50}s are calculated using standard four-parametric curve fitting (XLFit, model 205). Potential synergistic interactions between compound combinations are assessed using the Excess Inhibition 2D matrix according to the Loewe additivity model and are reported as Synergy Score. In addition, compound combinations are assessed by combination index analysis derived from isobologram graphs at 50% inhibition, and are reported as Best Combination Index (C.I.) at 50% inhibition, which corresponds to the lowest value on the isobologram. The terms "Best Combination Index (C.I.) at 50% inhibition" or "Best C.I. (at 50% inhibition)" are technical terms referring to the calculated combination index derived from isobologram graphs at the drug dosage required to achieve 50% inhibition in the defined cell-line model, which corresponds to the lowest value on the isobologram. The terms do not refer to the selection of the best single combination index value from several conducted experiments. All synergy calculations are performed using CHALICE software as described in Lehár et al, Nat Biotechnol. (July 2009), 27(4): 69:66. Compounds are also combined with themselves to determine the effect of assay noise on the synergy assessment parameters of the expected dose-additive interaction.

Pairwise multiple comparisons of result groups are performed by one-way ANOVA (Holm-Sidak method, overall significance level of 0.05) using SigmaPlot V11 (Systat Software Inc).

Results:

In this study, the following results demonstrating the growth inhibition effects of COMPOUND A and RAF265, alone and in combination, are obtained:

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Cell-line Name	COMPOUND A	RAF265	Synergy Score	Best CI (at 50% inhibition)	Effect Description
	IC50 [nM]				
SW1417	53.9	560	4.28	0.46	Synergy
COLO 205	34.4	918	2.29	0.65	Additive/Synergy
LS411N	54.7	864	4.50	0.47	Synergy
HT-29	84	2190	2.64	0.64	Additive/Synergy
HT-29*	66.5	1280	2.60	0.46	Additive/Synergy
RKO	>2700	680	4.36	0.29	Synergy
OUMS-23	>2700	>5400	0.30	nc	Additive
SW620	26.9	5240	0.65	0.70	Additive
LoVo	148	109	3.49	1.28	Additive/Synergy
SW480	2030	2160	3.39	0.18	Synergy
SW837	557	928	1.13	0.99	Additive
COLO-678	1710	4730	2.63	0.14	Synergy
LS123	2280	2420	1.79	0.19	Additive/Synergy
NCI-H747	153	2460	2.77	0.29	Additive/Synergy
HCT-15	>2700	483	2.73	0.41	Additive/Synergy
HCT 116	881	1370	7.48	0.24	Synergy
HCT 116*	735	920	8.40	0.18	Synergy
DLD-1	>2700	617	2.72	0.43	Additive/Synergy
T84**	1280	1840	3.39	0.29	Additive/Synergy
SW948**	89.8	1400	0.65	1.39	Additive
LS 180	143	3630	2.26	0.31	Synergy
GP2d	756	2620	3.90	0.16	Synergy
HuTu 80	>2700	1730	3.39	0.33	Synergy
CW-2	>2700	2200	2.04	0.53	Additive/Synergy
SW48	78.4	4750	5.11	0.35	Synergy
NCI-H716	>2700	1260	2.70	0.50	Additive/Synergy
C2BBe1	1580	4150	3.52	0.14	Synergy
SNU-C1	15.1	3290	1.21	0.74	Additive
KM12	339	793	4.30	0.49	Synergy
Median	756	1840	2.73	0.42	
Mean	1160	2180	2.95	0.48	

* = Independent repeat experiment are performed at end. These values are excluded from calculation of median and mean.

** = Duplicate data points and dose response display some variability.

In summary, COMPOUND A inhibits cell growth with a median IC_{50} of 756 nM (range: 15 nM to > 27000 nM, average 1160 nM), potently inhibiting 4 out of 6 cell lines reported to carry a $BRAF^{V600E}$ mutation with a median IC_{50} of 54 nM (range: 34 nM to 84 nM, average 57 nM). RAF265 inhibits cell growth with a median IC_{50} of 1840 nM (rnage: 110 to > 5400 nM, average 2180 nM), displaying activity in a broader set of cell lines.

Potential synergistic interactions between compound combinations are assessed using a Synergy Score (Lehár et al, Nat Biotechnol. (July 2009), 27(4): 69:66) relative to the Loewe additivity model. Calculations are performed using the CHALICE software, and the following results are obtained:

Cell-line Name	COMPOUND A + RAF265		COMPOUND A + COMPOUND A		RAF265 + RAF265	
	Mean	SD	Mean	SD	Mean	SD
SW1417	4.28	0.23	0.67	0.22	0.73	0.21
COLO 205	2.29	0.11	0.41	0.20	0.43	0.17
LS411N	4.50	0.13	0.40	0.11	0.76	0.18
HT-29	2.64	0.17	0.88	0.37	0.40	0.16
HT-29*	2.60	0.17	0.31	0.17	0.63	0.37
RKO	4.36	0.22	0.11	0.26	0.61	0.35
OUMS-23	0.30	0.15	0.09	0.17	0.80	0.29
SW620	0.65	0.18	0.49	0.13	0.50	0.23
LoVo	3.49	0.34	0.65	0.41	0.19	0.35
SW480	3.39	0.17	0.12	0.26	0.70	0.12
SW837	1.13	0.32	0.41	0.45	0.93	0.51
COLO-678	2.63	0.30	0.53	0.32	0.62	0.26
LS123	1.79	0.30	0.62	0.50	0.85	0.31
NCI-H747	2.77	0.30	0.28	0.30	0.68	0.30
HCT-15	2.73	0.21	0.20	0.13	0.33	0.18
HCT 116	7.48	0.15	0.38	0.20	0.74	0.22
HCT 116*	8.40	0.12	0.25	0.19	0.46	0.24
DLD-1	2.72	0.14	0.20	0.10	0.62	0.17
T84**	3.39	0.85	0.94	0.83	0.33	0.51
SW948**	0.65	0.49	0.49	0.59	0.54	0.51
LS 180	2.26	0.34	0.38	0.29	0.36	0.20
GP2d	3.90	0.25	0.19	0.25	0.95	0.22
HuTu 80	3.39	0.21	0.17	0.17	0.49	0.19
CW-2	2.04	0.30	0.21	0.28	0.48	0.30
SW48	5.11	0.32	0.25	0.55	0.61	0.21

NCI-H716	2.70	0.33	0.72	0.34	0.82	0.34
C2BBe1	3.52	0.42	0.60	0.32	0.97	0.39
SNU-C1	1.21	0.31	1.25	0.28	0.98	0.40
KM12	4.30	0.40	0.49	0.50	2.23	0.45
Median	2.73	Na	0.413	na	0.623	na
Mean	2.95	Na	0.449	na	0.691	na

* = Independent repeat experiments are performed at end. These values are excluded from calculation of median and mean.

** Duplicate data points and dose response display some variability.

Potential synergistic interactions between compound combinations are further assessed by Best Combination Index (C.I.) at 50% inhibition. Calculations are performed using CHALICE software and are reported as Best Combination Index (CI) at 50% inhibition, which corresponds to the lowest value on the isobologram. Interpretation of values for combination index and synergy score are provided below:

Combination Index (CI)	**Synergy Score**
CI = 1 → Dose additive	S ~ 0 → Dose additive across surface, or less
CI < 0.5 → "real" synergy (2x dose shift)	S > 2 σ_S → Real synergy detected
CI < 0.3 → "useful" synergy (3x shift)	S > 1 → Usually getting interesting (depends on dose matrix design)
CI < 0.1 → "strong" synergy (10x shift)	

The combination of RAF265 and COMPOUND A produces synergy scores with a median of 2.73 (range: 0.302 to 8.40) across all 27 cell lines tested, compared with median synergy scores of 0.413 (range: 0.086 to 1.25) and 0.623 (range: 0.187 to 2.23) when one agent is combined with the same agent (e.g., COMPOUND A combined with COMPOUND A). The synergy scores for RAF265 combined with COMPOUND A are higher than the corresponding single agent combinations in 18 out of the 27 cell lines assessed.

The median synergy scores for cell line groups with mutations in BRAF (n=6), KRAS (n=7), KRAS/PIK3CA (n=7) or wild-type (wt) for BRAF and KRAS (n=7) are 3.46, 2.63, 2.73

and 3.39, respectively, with no statistically significant difference between the different mutation groups. In contrast, within a given mutation group, the median synergy score for the COMPOUND A/RAF265 combinations are significantly higher than the corresponding self-with-self combinations. The highest synergy score is reproducibly observed in HCT 116 cells (7.48 and 8.40 in two independent experiment), which are wild-type (wt) for BRAF, NRAS, TP53, PTEN while mutant (mut) for KRAS, PIK3CA and CDKN2A. The lowest concentrations at which synergies occur are 11 nM for COMPOUND A, and 67 nM for RAF265. In the majority of cell lines, signs of synergy start at concentrations of 33 nM and 200 nM for COMPOUND A and RAF265, respectively. For KRAS wild-type (wt) cell lines, the lowest concentration for synergies occurs generally at the next dose level, i.e 100 and 600 nM for COMPOUND A and RAF265, respectively.

In summary, COMPOUND A and RAF265 interact synergistically in the majority of KRAS mutant colorectal cancer cells. Furthermore, COMPOUND A and RAF265 also elicit some synergy in cell lines that display reduced sensitivity to the single agents, e.g. SW480 COLO-678, HuTu 80 and C2BBe1.

Synergistic combinations in the majority of colorectal cell lines tested require concentrations of 33 nM for COMPOUND A, and 200 nM for RAF265. Considering the very high plasma protein binding of RAF265 (99.9%) and the presence of 10% FBS in the viability assay as potential explanation for the difference between the estimated human exposure and the efficacious concentrations in the cellular assay, at least some of the synergy occurs at concentrations that match estimated plasma free exposures for these compounds in humans. These results suggest combination potential for COMPOUND A and RAF265 in colorectal cancers with different mutation patterns, including tumors that display reduced sensitivity to one or both of the single agents.

Furthermore, the results suggest that dual node inhibition within the RAS/RAF/MEK/ERK pathway may translate into synergistic inhibition of tumor cell viability. Under the presumption that pathway activation sensitizes cells to pharmacological inhibition of RAF and MEK, it is interesting to note that synergies are also seen in cell lines wild-type (wt) for KRAS and BRAF. One possible explanation for the partial sensitivity of the cell lines wild-type

(wt) for KRAS and BRAF is that mechanisms other than mutations in KRAS and BRAF, such as aberrant activation of growth factor receptors or other upstream components, are responsible for elevated RAS/RAF/MEK/ERK pathway activity in these cells.

EXAMPLE 3 – NON-SMALL CELL LUNG CANCER - KRAS MUTANTS

Methods and Materials

All KRAS-mutant human non-small cell lung cancer cell lines used are obtained from the American Type Culture Collection (ATCC). Cells are cultured in the ATCC supplier recommended media (RPMI 1640 (catalog number 30-2001), DMEM (catalog number 30-2002) and EMEM (catalog number 30-2003) and FBS (Invitrogen catalog number 10099-14)). The cells lines used in this study as follows: Calu-6 (bearing KRAS mutation), NCI-H1792 (bearing KRAS mutation), NCI-H358 (bearing KRAS mutation), NCI-H441 (bearing KRAS mutation and amplification of the MET gene), SW1573 (bearing KRAS mutation and activating mutation of the PIK3C-alpha), NCI-H2122 (bearing KRAS mutation and STK11 mutation), NCI-H1734 (bearing KRAS mutation and STK11 mutation), NCI-H23 (bearing KRAS mutation and STK11 mutation), NCI-H1573 (bearing KRAS mutation, STK11 mutation, and amplification of the MET gene), and NCI-H460 (bearing KRAS mutation, STK11 mutation and activating mutation of the PIK3C-alpha).

The MEK inhibitor 6-(4-bromo-2-fluorophenylamino)-7-fluoro-3-methyl-3H-benzoimidazole-5-carboxylic acid (2-hydroxyethoxy)-amide (COMPOUND A) and the RAF kinase inhibitor RAF265 are suspended in 100% DMSO (Cellgro catalog number 25-290-CQC) to final concentrations of 10mM, and subsequently are diluted with media to working solutions covering a range of concentrations 10-fold above the concentrations to be assayed on cells. COMPOUND A concentrations (nM) prior to the addition to cells are 0, 37, 111, 333, 1000, 3000, 9000 and 27000 and RAF265 concentrations are 0, 137, 412, 1235, 3704, 11111, 33333 and 100000. Cells are plated at seeding densities of 2000 cells/well in 96-well assay plate (Costar catalog number 3904). In all cases cells are plated in 80µl of vendor recommended media and FBS concentrations. Approximately 12-24 hours following plating, 10µl of each of the compound dilutions described above and 10µl of media are added to wells to a final volume

100µl such that all pair-wise combinations as well as the single agents are represented. Triplicate wells for all compound concentrations are plated in all cases. Cells are cultured for 72 hrs at 37°C in a 5% CO_2 incubator following compound addition after which the effects of the various compound treatments on cell proliferation are determined using the CellTiter-Glo luminescent cell viability assay (Promega catalog number G755B) and Victor4 plate reader (Perkin Elmer). IC_{50} values are determined using a fitted curve generated using XLfit add-in version 4.3.2 model 203 (build 11) which uses the following formula $Y=(A+((B-A)/1+(((10^C/x)^D))))$. Synergy scores and CI_{50} calculations are determined as described in Lehár et al, Nat Biotechnol. (July 2009), 27(4): 69:66.

The cell lines are tested with single agents COMPOUND A and RAF265 and their combinations in a checkerboard experiment design. Combination effects are calculated using Chalice software. In addition, compound combination effects are assessed by combination index analysis derived from isobologram graphs at 50% inhibition, and are reported as Best Combination Index (C.I.) at 50% inhibition, which corresponds to the lowest value on the isobologram. The terms "Best Combination Index (C.I.) at 50% inhibition" or "Best C.I. (at 50% inhibition)" are technical terms referring to the calculated combination index derived from isobologram graphs at the drug dosage required to achieve 50% inhibition in the defined cell-line model, which corresponds to the lowest value on the isobologram. The terms do not refer to the selection of the best single combination index value from several conducted experiments. Synergy score is derived from Excess Inhibition 2D matrix calculated according to Loewe additivity model in Chalice software. Interpretation of best C.I. and synergy score values are provided in Example 2 herein. Effect description is a qualitative description of the type of combination effect observed, based on the synergy score and best C.I.

Results:

The following table summarizes the results that are obtained using the above method:

Case 54627A

Cell line	Mutation	Assay	CPD. A IC_{50} (nM)	RAF265 IC_{50} (nM)	Best C.I.	Synergy Score	Effect Description
Calu-6	KRAS	Viability	>2700	372	0.392	7.56	Synergy
NCI-H1792	KRAS	Viability	>2700	421	0.294	7.54	Synergy
NCI-H358	KRAS	Viability	1600	>10000	0.043	7.09	Synergy
NCI-H441	KRAS, MET^{AMP}	Viability	>2700	>10000	0.805	1.24	Additive
SW 1573	KRAS, PIK3Cα	Viability	>2700	>10000	1.1	2.14	Additive
NCI-H2122	KRAS, STK11	Viability	430	556	0.329	6.85	Synergy
NCI-H1734	KRAS, STK11	Viability	>2700	223	0.424	5.36	Synergy
NCI-H23	KRAS, STK11	Viability	>2700	1790	0.242	5.2	Synergy
NCI-H1573	KRAS, STK11, MET^{AMP}	Viability	>2700	>10000	0.255	2.14	Additive/Synergy
NCI-H460	KRAS, STK11, PIK3Cα	Viability	>2700	1660	0.127	3.6	Additive/Synergy

IC_{50} values (nM) for COMPOUND A and RAF265 are shown in columns 4 and 5, respectively. The synergy score calculation is shown in column 7 and a qualitative description of the type of combination effect observed, based on the synergy score and Best C.I. at 50% inhibition, is shown above.

As shown by the data above, the combination of RAF265 and COMPOUND A results in the strongly synergistic inhibition of proliferation in six of ten KRAS-mutant cell lines. These six cell lines are evenly split between STK11 wild-type lines and those harboring loss-of-function mutations, or deletions in this gene (Shackelford and Shaw, Nat. Rev. Cancer, 2009, 9: 563-575). Interestingly, cell lines containing either activating mutations in the PIK3Cα or amplification of the MET gene in addition to the lesion in KRAS are far less sensitive to the combination as judged by synergy score calculations. NCI-H1573 and NCI-H460 cell lines demonstrate robust synergy when analyzed using CI_{50} values.

In further study of the percent inhibition of growth observed for single agent and combinations of COMPOUND A and RAF265, it is observed that the robust synergy of the combination may be restrictive based upon concentration of RAF265 concentration. Data indicates that for all cell lines that harbored only mutations in KRAS, many combinations of COMPOUND A and RAF265 result in growth suppression in excess of 75%, with 90% inhibition of growth being frequently achieved. In contrast as single agents COMPOUND A and RAF265 only rarely achieve inhibition in excess of 60%, even at the highest doses (2.7μM and 10μM, respectively). However, in these same cell lines, at concentrations that roughly

approximate clinically relevant doses (up to approximately 0.3 μM of COMPOUND A and 0.36 μM of RAF265 in the described study), maximal inhibition of growth observed is usually closer to 70–80%.

The strongest synergies and the majority of the overall combination effect occur at drug concentrations that may be difficult to achieve clinically in the NCI-H358, NCI-H1792, Calu-6, NCI-H23, NCI-H1734 and NCI-H2122 tumor models. However, it should be noted that good combination activity is still observed at the upper end of clinically relevant doses that are converted often ineffective single agent effects into concentrations that created robust inhibition of growth.

The NCI-H460, SW1573, NCI-H441 and NCI-H1573 tumor models which have activating lesions in oncogenes in addition to KRAS are largely insensitive to the combination tested. Some good combination activity is observed for NCI-H460 but less robust than other tested tumor models. In NCI-H460, the good combination effects are observed in a narrow range of RAF265 concentrations (0.12 – 1.1 μM), and the maximal inhibition observed did not reach 80% at even the highest drug concentrations.

Case 54627A

CLAIMS:

1. A pharmaceutical combination comprising:

(a) a MEK inhibitor selected from the group consisting of 6-(4-bromo-2-fluorophenylamino)-7-fluoro-3-methyl-3H-benzoimidazole-5-carboxylic acid (2-hydroxyethoxy)-amide or (*S*)-5-fluoro-2-(2-fluoro-4-(methylthio)phenylamino)-*N*-(2-hydroxypropoxy)-1-methyl-6-oxo-1,6-dihydropyridine-3-carboxamide or a pharmaceutically acceptable salt thereof, and

(b) at least one RAF kinase inhibitor or a pharmaceutically acceptable salt thereof,

for simultaneous, separate or sequential administration.

2. A pharmaceutical combination according to claim 1, wherein the MEK inhibitor is 6-(4-bromo-2-fluorophenylamino)-7-fluoro-3-methyl-3H-benzoimidazole-5-carboxylic acid (2-hydroxyethoxy)-amide.

3. A pharmaceutical combination according to any one of claims 1 to 2, wherein the RAF kinase inhibitor is selected from Sorafenib or its tosylate salt, RAF265, SB590885, SL281, PLX4072, PLX4032, GDC0879 and ZM336372 and a pharmaceutically acceptable salt thereof.

4. A pharmaceutical combination according to claim 1 for use in the treatment of a proliferative disease in a subject in need thereof.

5. A pharmaceutical combination according to claim 1 for use in the preparation of a medicament for the treatment of a proliferative disease.

6. A pharmaceutical combination according to claim 5, wherein the proliferative disease is a cancer.

7. A pharmaceutical combination according to claim 6, wherein the proliferative disease is melanoma, lung cancer (including non-small-cell lung cancer (NSCLC)), colorectal cancer (CRC), breast cancer, kidney cancer, renal cell carcinoma (RCC), liver cancer, acute myelogenous leukemia (AML), myelodysplastic syndromes (MDS), thyroid cancer, pancreatic cancer, neurofibromatosis or hepatocellular carcinoma.

8. Use of the combination according to claim 1 for the manufacture of a medicament for the treatment of a proliferative disease.

9. Use according to claim 8, wherein the RAF kinase inhibitor is selected from Sorafenib or its tosylate salt, RAF265, SB590885, SL281, PLX4072, PLX4032, GDC0879 and ZM336372 and a pharmaceutically acceptable salt thereof.

10. A method for treating a proliferative disease, comprising the simultaneous, separate or sequential administration of a therapeutically effective amount of a MEK inhibitor selected from 6-(4-bromo-2-fluorophenylamino)-7-fluoro-3-methyl-3H-benzoimidazole-5-carboxylic acid (2-hydroxyethoxy)-amide or (*S*)-5-fluoro-2-(2-fluoro-4-(methylthio)phenylamino)-*N*-(2-hydroxypropoxy)-1-methyl-6-oxo-1,6-dihydropyridine-3-carboxamide or a pharmaceutically acceptable salt thereof, in combination with at least one RAF kinase inhibitor or a pharmaceutically acceptable salt thereof, to a patient having a proliferative disease.

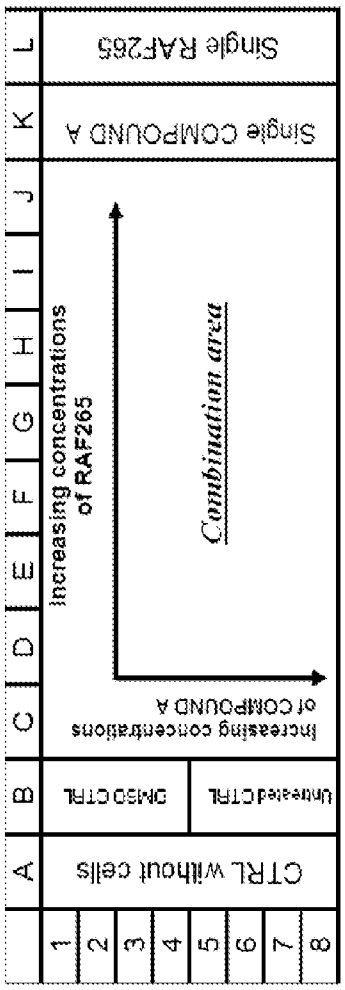
11. A method according to claim 10, wherein the proliferative disease is melanoma, lung cancer, colorectal cancer (CRC), breast cancer, kidney cancer, renal cell carcinoma (RCC), liver cancer, acute myelogenous leukemia (AML), myelodysplastic syndromes (MDS), non-small-cell lung cancer (NSCLC), thyroid cancer, pancreatic cancer, neurofibromatosis or hepatocellular carcinoma.

12. A combined preparation comprising (a) a MEK inhibitor selected from the group consisting of 6-(4-bromo-2-fluorophenylamino)-7-fluoro-3-methyl-3H-benzoimidazole-5-carboxylic acid (2-hydroxyethoxy)-amide or (*S*)-5-fluoro-2-(2-fluoro-4-(methylthio)phenylamino)-*N*-(2-hydroxypropoxy)-1-methyl-6-oxo-1,6-dihydropyridine-3-carboxamide or a pharmaceutically acceptable salt thereof, and (b) at least one RAF kinase inhibitor or a pharmaceutically acceptable salt thereof.

13. A combined preparation according to claim 12, wherein the MEK inhibitor and RAF kinase inhibitor are provided in synergistically effective amounts for the treatment of a proliferative disease.

14. A synergistic combination comprising for administration to humans comprising (a) a MEK inhibitor compound 6-(4-bromo-2-fluorophenylamino)-7-fluoro-3-methyl-3H-benzoimidazole-5-carboxylic acid (2-hydroxyethoxy)-amide (COMPOUND A) or a pharmaceutically acceptable salt thereof, and (b) at least one RAF kinase inhibitor or a pharmaceutically acceptable salt thereof in a dosage which corresponds to a synergistic combination at ED_{50} in a tumor model selected from the group consisting of UACC-62, IGR-1, RPMI-7951, MDA-MB-435S, or WM-115 melanoma tumor models; Hs 994.T, SK-MEL-30 or MEL-JUSO melanoma tumor models; SW1417, COLO 205, LS411N, HT-29, or RKO colorectal tumor models; LoVo, SW480, COLO678, LS123, NCI-H747, HCT-15, NCT116, DLD-1, T84, LS 180, or GP2d colorectal tumor models; or Calu-6, NCI-1792, NCI-H358, NCI-H2122, NCI-H1734, NCI-H23, NCI-H1573, or NCI-H460 non-small cell lung cancer tumor models.

FIG. 1


A
B
C
D
E
F
G
H
I
J
K
L
1
2
3
4
5
6
7
8
CTRL without cells
Untreated CTRL
DMSO CTRL
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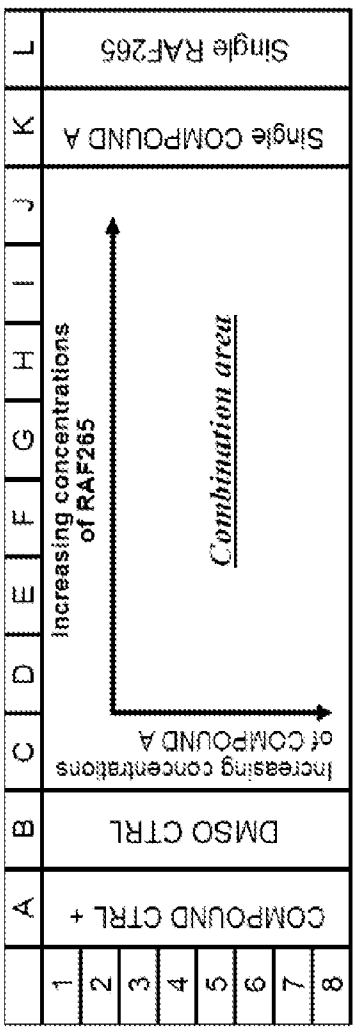
Increasing concentrations of RAF265
Combination area
Single COMPOUND A
Single RAF265

FIG. 2


A
B
C
D
E
F
G
H
I
J
K
L
1
2
3
4
5
6
7
8
COMPOUND CTRL +
DMSO CTRL
Increasing concentrations of COMPOUND A
Increasing concentrations of RAF265
Combination area
Single COMPOUND A
Single RAF265

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2012/034239

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K31/4184 A61K31/4412 A61K31/155 A61P35/00
ADD.

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)
A61K

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2007/071951 A1 (ASTRAZENECA AB [SE]; ASTRAZENECA UK LTD [GB]; ROBERTS RONALD JOHN [GB]) 28 June 2007 (2007-06-28) claims 1,17 page 3, line 1 - line 8 page 12, line 8 - line 23 page 14, line 11 - line 12 -----	1-14
X	WO 2007/076245 A2 (ARRAY BIOPHARMA INC [US]; ASTRAZENECA AB [SE]; SQUIRE CHRISTOPHER JOHN) 5 July 2007 (2007-07-05) claims 1,14 page 1, paragraph 0004 page 9, paragraph 0048 page 10, paragraph 0051 page 12, paragraph 0060 -----	1-14

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
11 June 2012	15/06/2012

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Baurand, Petra

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2012/034239

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2009/018238 A1 (ARDEA BIOSCIENCES INC [US]; MINER JEFFREY N [US]; CHAPMAN MARK S [US];) 5 February 2009 (2009-02-05) claims 1,2 page 84, last paragraph to page 86, last but one paragraph page 71, "Treatment of hyperproliferative disorders" page 50, "Raf protein kinase inhibitors" -----	1-14
Y	WO 2008/120004 A1 (ASTRAZENECA AB [SE]; ASTRAZENECA UK LTD [GB]; ADJEI ALEX [US]; DREW LI) 9 October 2008 (2008-10-09) claims 1,7,9,10 -----	1-14

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Information on patent family members

International application No
PCT/US2012/034239

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WO 2008120004 A1	09-10-2008	NONE	