Title: COMPOSITIONS AND METHODS FOR TREATING CANCER

Abstract: The instant invention provides a novel therapeutic combination and method of treating cancer, and in particular a cancer selected from the group consisting of lung cancer (including small cell and non-small cell), pancreatic cancer, colon cancer (including colorectal) and melanoma (including malignant) with MK-2206 and AZD-6244.

Strong Synergism of MK-2206 in Combination with AZD-6244 in P3K/Ras Active Cells

<table>
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<tr>
<th>Cell line</th>
<th>Cancer type</th>
<th>Combination index</th>
<th>Growth inhibition</th>
<th>P3K inhibition</th>
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<td>Colon</td>
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<td>Melanoma</td>
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*Addition of MEK inhibitor enhances MK-2206 activity in these cells*

FIG. 1
TITLE OF THE INVENTION
COMPOSITIONS AND METHODS FOR TREATING CANCER

BACKGROUND OF THE INVENTION

It is known that the PI3K/AKT/mTOR and Raf/MEK/ERK signaling pathways play pivotal roles in cellular proliferation either alone or with considerable cross-talk. Disregulation of either pathway may lead to oncogenesis and efforts are underway to identify the key signaling proteins, and inhibitors thereof, within either pathway. Importantly, current investigations suggest that dual inhibition of both pathways may provide the best therapeutic strategy for oncology patients.

The AKT/mTOR and ERK MAPK signaling pathways have been shown to cooperate in prostate cancer progression and the transition to androgen-independent disease. Kinkade et al. demonstrated that combination therapy with the mTOR inhibitor, rapamycin, and the MEK inhibitor, PD0325901, is potently anti-tumorigenic for androgen-independent prostate cancer. Kinkade et al. proposed that combination therapy targeting AKT/mTOR and ERK MAPK signaling pathways may be an effective treatment for patients with advanced prostate cancer, in particular those with hormone-refractory disease. Kinkade el al. (2008) J. CHn. Invest. 118:9, 3051-3064.

Shelton et al. have demonstrated that treatment with the PI3K inhibitor, LY294002, in combination with the MEK inhibitor, U0126, synergistically inhibit leukemogenesis. Shelton et al. (2003) Oncogene 22, 2478-2492.

Herein, we now report the synergistic effects of the combination use of the MEK inhibitor (A2D-6244) and the allosteric AKT inhibitor (MK-2206) for the treatment of cancer and, in particular, colon cancer, melanoma, pancreatic cancer and lung cancer.

SUMMARY OF THE INVENTION

In a first aspect, the instant invention relates to a method of treating cancer by administering to a patient in need thereof an effective amount of MK-2206, or a pharmaceutically acceptable salt thereof, and AZD-6244, or a pharmaceutically acceptable salt thereof.

In a second aspect, the instant invention provides a method of treating a cancer selected from the group consisting of lung cancer, pancreatic cancer, colon cancer and melanoma with AZD-6244 and MK-2206.

In a third aspect, the instant invention provides a therapeutic combination comprising AZD-6244 and MK-2206.

In a fourth aspect, the instant invention provides a combination product comprising AZD-6244 and MK-2206.
BRIEF DESCRIPTION OF THE DRAWINGS

FIGURE 1: Strong Synergism of MK-2206 in Combination with AZD-6244 in POK/Ras Active Cells. (A) Cells were simultaneously treated for 72 hours with MK-2206 and AZD-6244 at constant concentration ratios spanning IC50 dose of each agent. The evaluation of cell growth inhibitory effects of each agent alone or in combination was determined by monitoring cellular ATP concentration. The combination index (CI) values were calculated by Chou and Talalay method for drug interactions using CalcuSyn software at different fractional affected. (B) HCT-116 colon cancer cells were treated with MK-2206 and/or AZD-6244 at the indicated concentration for 24 hours. The cell lysates were analyzed by Western blot with indicated antibodies.

FIGURE 2: Strong Synergism of MK-2206 in Combination with AZD-6244 in pancreatic and lung tumor lines. Cells were simultaneously treated for 72 hours with MK-2206 and AZD-6244 at constant concentration ratios spanning IC50 dose of each agent. The CI values were calculated by Chou and Talalay method for drug interactions at different fractional affected.

FIGURE 3: MK-2206 Enhances Anti-Tumor Efficacy of AZD-6242 in vivo (A2058). A2058 cells were injected s.c. into CDI-nude mice. For PD analysis, AZD-6244 (25 mg/kg) and MK-2206 (110 mg/kg) were administered and tumors were harvested 4 hr after initial dosing for AZD-6244 and 12 hr after for MK-2206 (n=4). Inhibition of phospho-ERK for AZD-6244 and phospho-AKT for MK-2206 was determined by Western blot analysis. For efficacy, AZD-6244 was administered at 25 mg/kg p.o., b.i.d. (8 hr interval), 5 days/week for 2 weeks (on day 0 to 4 and 7 to 11). MK-2206 was dosed at 120 mpk, three times/ week for 2 weeks (on day 0, 2, 4, 7, 9 and 11). MK-2206 was dosed 2 hr after the AZD-6244 dosing. Animal body weight and physical sign were monitored during experiment. The relative tumor volume was assessed by tumor volume on different observation days / starting tumor volume. Statistical significance was evaluated using two-way repeated ANOVA test followed by Dunnett’s test or unpaired t-test.

FIGURE 4: MK-2206 Enhances Anti-Tumor Efficacy of AZD-6244 in vivo (HCT116). HCT-116 cells were injected s.c. into CDI-nude mice. After 2 weeks, when tumor size was reached to certain size, dosing of both agents was started. Dosing procedure for efficacy and PD study, and the assessment of efficacy and tolerability was conducted by the same method as described in A2058 study.

NCI-H1 975 xenografts were treated with AZD-6244, MK-2206, or a combination of both at doses and schedules as described in Example 5 below. The effect of selected doses and schedules on survival, tumor growth, tumor phosphoproteins and body weight are shown.

**FIGURE 6: MK-2206 Enhances Anti-Tumor Efficacy of AZD-6244 in vivo (AsPCI).**

AsPCI cells were implanted s.c. into flank of female nude mice. When the tumour size reached approximately 0.24 cm³ the mice were randomised into 3 treated groups of 10 mice and 1 vehicle group of 12 mice and dosing commenced as follows, AZD6244 at 25mg/kg/qd and MK-2206 at 240mg/kg/qd 3hrs after AZD6624. Tumour volume was measured every 4 days until termination of the experiment.

**12 DETAILED DESCRIPTION OF THE INVENTION**

The present inventor has found that synergistic anticancer activity can be achieved by using MK-2206 in combination with AZD-6244.

Accordingly, in a first aspect the instant invention relates to a method of treating cancer by administering to a patient in need thereof an effective amount of MK-2206, or a pharmaceutically acceptable salt thereof, and AZD-6244, or a pharmaceutically acceptable salt thereof.

The invention is especially useful in the treatment of a cancer selected from the group consisting of lung cancer (including small cell and non-small cell), pancreatic cancer, colon cancer (including colorectal) and melanoma (including malignant). However, the instant invention could prove useful in the treatment of various other cancers, such as brain cancer, cervicocerebral cancer, esophageal cancer, thyroid cancer, stomach cancer, gallbladder/bile duct cancer, liver cancer, ovarian cancer, choriocarcinoma, uterus body cancer, uterocervical cancer, renal pelvis/ureter cancer, bladder cancer, prostate cancer, penis cancer, testicule cancer, fetal cancer, Wilms' cancer, skin cancer, neuroblastoma, osteosarcoma, Ewing's tumor, soft part sarcoma, acute leukemia, chronic lymphatic leukemia, chronic myelocytic leukemia and Hodgkin's lymphoma.

Accordingly, in a second aspect the instant invention relates to a method of treating a cancer selected from the group consisting of lung cancer (including small cell and non-small cell), pancreatic cancer, colon cancer (including colorectal) and melanoma (including malignant) by administering to a patient MK-2206 and AZD-6244.

MK-2206 and AZD-6244 can each be prepared for simultaneous, separate or successive administration.

In a third aspect, the instant invention provides a therapeutic combination comprising AZD-6244, or a pharmaceutically acceptable salt thereof, and MK-2206, or a pharmaceutically acceptable salt thereof.
In a fourth aspect, the instant invention provides a combination product comprising A2D-6244, or a pharmaceutically acceptable salt thereof, and MK-2206, or a pharmaceutically acceptable salt thereof.

In one embodiment, the instant invention provides a therapeutic combination or combination product comprising AZD-6244, or a pharmaceutically acceptable salt thereof, and MK-2206, or a pharmaceutically acceptable salt thereof, for use as a medicament.

In a further embodiment, the instant invention provides a therapeutic combination or combination product comprising AZD-6244, or a pharmaceutically acceptable salt thereof, and MK-2206, or a pharmaceutically acceptable salt thereof, for use in the treatment of cancer.

In another embodiment, the instant invention provides the use of a therapeutic combination or combination product comprising AZD-6244, or a pharmaceutically acceptable salt thereof, and MK-2206, or a pharmaceutically acceptable salt thereof, in the manufacture of a medicament for use in the treatment of cancer.

Reference to embodiments set forth above is meant to include all combinations of particular and preferred groups unless stated otherwise. The meanings of the terms used in this description are described below, and the invention is described in more detail hereunder.

The term "comprising" as referred to in this description is intended to be open ended and means that any "therapeutic combination" or "combination product" comprising AZD-6244, or a pharmaceutically acceptable salt thereof, and MK-2206, or a pharmaceutically acceptable salt thereof, may also include additional, unnamed elements.

The term "simultaneous" as referred to in this description means that the pharmaceutical preparations of the invention are administered simultaneously in time.

The term "separate" as referred to in this description means that the pharmaceutical preparations of the invention are administered at different times during the course of a common treatment schedule.

The term "successive" as referred to in this description means that administration of one pharmaceutical preparation is followed by administration of the other pharmaceutical preparation; after administration of one pharmaceutical preparation, the second pharmaceutical preparation can be administered substantially immediately after the first pharmaceutical preparation, or the second pharmaceutical preparation can be administered after an effective time period after the first pharmaceutical preparation; and the effective time period is the amount of time given for realization of maximum benefit from the administration of the first pharmaceutical preparation.

The term "therapeutic combination" as referred to in this description in intended to mean any combination of the specified pharmaceutical agents that produces a therapeutic effect upon administration.

The term "combination product" as referred to in this description in intended to mean any product that comprises MK-2206 and AZD-6244 and includes, but is not limited to, an
individual pharmaceutical preparation comprising both MK-2206 and AZD-6244, a kit of parts comprising pharmaceutical preparations of MK-2206 and AZD-6244 as individual or separate preparations, storage means for pharmaceutical preparations of MK-2206 and AZD-6244 as either individual or separate preparations and/or means for dispensing pharmaceutical preparations of MK-2206 and AZD-6244 as either individual or separate preparations, wherein the term "individual pharmaceutical preparation" or "individual preparations" is intended to mean a single pharmaceutical preparation which comprises both MK-2206 and AZD-6244 and wherein the term "separate preparations" is intended to mean two different pharmaceutical preparations one of which comprises MK-2206 and one of which comprises AZD-6244.

The term "cancer" as referred to in this description includes various sarcoma and carcinoma and includes solid cancer and hematopoietic cancer. The solid cancer as referred to herein includes, for example, brain cancer, cervicocerebral cancer, esophageal cancer, thyroid cancer, small cell lung cancer, non-small cell lung cancer, breast cancer, endometrial cancer, lung cancer, stomach cancer, gallbladder/bile duct cancer, liver cancer, pancreatic cancer, colon cancer, rectal cancer, ovarian cancer, choriocarcinoma, uterus body cancer, uterocervical cancer, renal pelvis/ureter cancer, bladder cancer, prostate cancer, penis cancer, testicles cancer, fetal cancer, Wilms' tumor, skin cancer, malignant melanoma, neuroblastoma, osteosarcoma, Ewing's tumor, soft part sarcoma. On the other hand, the hematopoietic cancer includes, for example, acute leukemia, chronic lymphatic leukemia, chronic myelocytic leukemia, polycythemia vera, malignant lymphoma, multiple myeloma, Hodgkin's lymphoma, non-Hodgkin's lymphoma.

The term "treatment of cancer" as referred to in this description means that an anticancer agent is administered to a cancer case so as to inhibit the growth of the cancer cells in the case. Preferably, the treatment results in cancer growth regression, or that is, it reduces the size of a detectable cancer. More preferably, the treatment results in complete disappearance of cancer.

MK-2206

Synthesis of MK-2206 is also found in WO2008/070016.
1-(4-bromophenyl)cyclobutanecarbonitrile (1-2)

TBAB (1.61 g, 0.5 mmol), dibromopropane (22.2 g, 110 mmol), and nitrile 1-1 (19.6 g, 100 mmol) were added to a stirred solution of KOH (31.17 g, 500 mmol) in a mixture of 15 mL of water and 200 mL of toluene (temperature maintained between 72 and 79 °C). The mixture was heated by steam and was stirred at 99-108 °C for 2.5 h. The mixture was cooled to 80 °C and 200 mL of heptane was added. After the resulting mixture was cooled to RT with stirring, the top clear solution was filtered, washed with water (3X30 mL) and concentrated in vacuo to give oily product 1-2.

1-(4-bromophenyl)cyclobutanecarboxamide (1-3)

H2O2 (30%, 11.3 mL, 118 mmol) was added over 3 h to a stirred mixture of nitrile 1-2 (13.88 g, -58.9 mmol) and K2CO3 (1.62 g, 11.8 mol) in 59 mL of DMSO at 40-87 °C, cooling with a water bath. The resulting mixture was cooled to 27 °C and water (100 mL) was added over 30 min. Crystalline product 1-3 formed. More water (100 mL) was added over 1 h. The resulting slurry was aged at RT for 16 h before filtration. The cake was rinsed with 100 mL of water and then with 100 mL of heptane. After drying in a vacuum oven at 50 °C, product 1-3 was obtained as a white solid.

tert-butyl [1-f4-bromophenyl]cyclobutyl]carbamate (1-4)
Pb(OAc)₄ (25.7 g, 25.7 mmol) was added to a stirred solution of amide 1-3 (12.7 g, 50 mmol) in 64 mL of t-BuOH at 57 °C to 86 °C cooling with a water bath. The resulting mixture was stirred at 65-86 °C for 0.5 h. The mixture was cooled to 26 °C and 12.7 g of Na₂CO₃ were added followed by 65 mL MTBE. After 10 min, the mixture was filtered. The cake was rinsed with 10 L of MTBE and the combined filtrate was washed with 20 mL of water and the organic layer was then washed with 3 X 10 mL of 10% KHCO₃ (caution: bubbling) dried over Na₂SO₄ and concentrated in vacuo. The resulting solid was rinsed with 8 mL of IPAc and 8 mL of heptane and dried in a vacuum oven at 40 °C to give product 1-4 as a grey solid.

tert-buty\(\gamma\)-[l-\(\alpha\)-cyanophenyl]cyclobutyl carbamate (1-5)

A stirred slurry of Pdidba₃ (101 mg; 1 mol%) and dppf (122 mg; 2 mole%) in DMF (25 mL) was sparged with nitrogen for 5 min and then warmed to 65 °C and aged for 30 min. At this temp was added the aryl bromide 1-4 (3.6 g, 11 mmol), zinc powder (51 mg; 6 mol%) and the zinc cyanide (777 mg; 0.60 equiv) rinsing with DMF (5 mL). The solution was heated to 92-95 °C and aged for 4 h. The solution was cooled to RT overnight and filtered through a pad of Solka Floe, rinsing the cake with DMF (5 mL). Water (30 mL) was added over 3.5 h at 25-33 °C, along with seed. After aging overnight at RT, the resulting crystalline solution was filtered and washed with aqueous methanol and dried overnight to yield 1-5 as a yellow solid.

tert-butyl (1-[4-phenylacetyl]phenyl)cyclobutyl carbamate (1-6)

Benzyl Grignard (19 mL, 38.5 mmol) was added to a stirred, slightly cloudy solution of nitrile 1-5 (3 g, 11 mmol) in THF (25 mL) cooled to ca. -20 °C at a rate such that the reaction temperature did not warm above -10 °C. The solution was aged for 3-4 hours keeping the reaction temperature between -10 °C and -20 °C. The stirred solution was cooled to -30 °C and added to a 15 wt% aqueous citric acid solution (60 mL) which was previously cooled to 5-10 °C, maintaining the temperature below 15 °C. The layers were separated and the aqueous layer was washed with MTBE.

The organic layers were combined, washed with half saturated brine (60 mL), and concentrated under reduced pressure. Heptane was added and the mixture was concentrated to a slurry which was filtered, washed with heptane (15 mL) and dried under nitrogen to give 1-6.

4-Amino-2-chloronicotinaldehyde (1-8)

Trifluoroacetic acid (17.4 mL, 234 mmol) was added carefully to a stirred mixture of Boc aldehyde 1-7 (20 g, 78.1 mmol) and dichloromethane (60 mL) keeping the temperature below 25 °C. The solution was warmed to 35 °C, aged overnight (vigorous off-gassing) and then cooled to room temperature. 25 mL of MTBE was added and the resulting white slurry was aged for one hour, filtered, and the filter cake rinsed with MTBE (10 mL x 2). Solid 1-8 TFA salt was dried under vacuum.

tert-butyl (1-[4-(5-chloro-3-phenyl-1,6-naphthyridin-2-yl)phenyl]cyclobutyl) carbamate (1-9)
45wt% Potassium hydroxide solution (18 mL; 5 equiv) was added dropwise over 20 minutes to a stirred mixture of chloropyridine TFA salt 1-8 (19.5 g), cyclobutylamino ketone 1-6 (26 g) and isopropanol (200 mL) keeping the temperature below 24 °C. After 1 h, water (100 mL) was added and after a further 1 h the resulting slurry was filtered, washing with 2:1 IPA/water (30 mL, then 24 mL) then with water (80 mL then 2 x 60 mL). The solid was dried under nitrogen flow to afford 1-9 as an off-white solid.

A stirred slurry of chloronaphthyridine 1-9 (1.8 g), methyl hydrazine carboxylate (0.318 g) and isopropanol (20 L) is warmed to 66 °C before becoming homogeneous. 5-6 N HCl in IPA (0.05 mL) is added and the temperature is increased to 70 °C for 16 hours and then is cooled to RT. After cooling to RT, 45wt% potassium hydroxide solution (0.52 mL) is mixed with water (5.5 mL) and added over 15 minutes. After 30 minutes, aqueous acetic acid (0.7 mL in 6 mL water) is added followed by water (2 mL). The resulting slurry is aged at RT for three hours, filtered and washed with 1:1 IPA/water (2 x 2.4 mL). The product is dried under nitrogen flow then slurried in methylene chloride at 20 °C for 4 hours, filtered and dried under nitrogen flow to afford 1-10 as an off-white solid.

A solution of aqueous concentrated HCl (12.1 M, 1.64 mL) in ethanol (2.0 mL) was added dropwise over 30 min to a stirred slurry of 1-10 (500 mg, 0.985 mol) in ethanol (1.7 mL) and water (0.2 mL) at 50°C. After 3 hours following acid addition, the mixture was seeded and aged overnight at 50°C, cooled to room temperature and filtered. Acetyl chloride (0.5 g, 7 mmol) was added over 1 h to ethanol (2 mL) at 0°C. The solution was then cooled to room temperature and aged for 30 minutes. The filter cake was washed with this solution (1 mL x 2), then with ethyl acetate (4 mL x 2) and dried, finally in a vacuum oven at 75.0°C with nitrogen sweep (50 torr) to afford MK-2206 as the bis-HCl salt.

A mono-HCl version of MK-2206 was also produced via dissolution in water. After 6 hours, the aqueous slurry turns light yellow and is filtered. Silver chloride titration of this solid reveals the presence of one equivalent of chloride.

Pharmaceutical Preparations Comprising MK-2206

Preparations of the monohydrochloride salt of MK-2206 may be prepared as a tablet involving roller compression granulation followed by milling, mixing with the other inactive ingredients, compression, and film coating.

Some of the diluents or fillers for use in this formulation are preferably swellable agents, and may include, but are not limited to, various grades of microcrystalline cellulose, such as Avicel PH101, Avicel PH 02, & Avicel PH200. If microcrystalline cellulose is added, it is preferably from about 50 to 180 microns in size, more preferably about 100. Avicel PH 101 has a
mean particle size of about 50; Avicel PH 102 has a mean particle size of about 100; and Avicel PH 200 has a mean particle size of about 190 microns. Preferably, the preferred microcrystalline cellulose is Avicel PH 102.

The edible calcium salts suitable for use herein include but are not limited to, dibasic calcium phosphate dihydrate, calcium phosphate anhydrous, and tribasic calcium phosphate; or mixtures thereof. A preferred edible calcium salt is the dibasic calcium phosphate anhydrous, which also provides good compressibility.

Suitable ratios for particular diluents however, are described below: For microcrystalline cellulose: Dibasic calcium phosphate, dihydrate, from about 2 to about 4:1, preferably from about 2.6-3.1 :1; For microcrystalline cellulose: Calcium phosphate, anhydrous from about 1 to about 3:1, preferably from about 1.6: 1, microcrystalline cellulose: Tribasic calcium phosphate, from about 2 to about 4:1, preferably from about 3.1:1.

A preferred disintegrating agent is sodium croscarmellose. Preferably, the sodium croscarmellose is present in an amount of about 2 to about 5% w/w.

A preferred lubricant is magnesium stearate.

An aspect of the present invention is a process for preparing a tablet formulation which comprises:

a) blending together to form an intragranular mixture of the active monohydrochloride salt of MK-2206, microcrystalline cellulose, an edible calcium salt, disintegrant, and lubricant;

b) roller compression granulation of the mixture of step (a) for the purpose of preparing granules;

c) lubricating the granulation from step (b);

D) compacting the lubricated granulates of step (c) into concave tablet; and

e) film coating tablets from step (d).

2-1

H₂SO₄ → \( \text{HOO} \cdot \text{SO₄}^+ \) \( \text{SO₄}^- \)

2-2

2-3

2-4

Pd/C, TFA
Hydrogen

2-5

2-6

AZD6244

A2D6244 Hyd-Sulfate

3-Methyl-5,6-dihydro-4,2-dioxazine (2-1)
Hydroxylamine hydrochloride (186 kg 2.68 kmol) was suspended in methanol (660 L) and heated to 50–60°C for 15-20 minutes then treated with potassium hydroxide (90 wt%, 221kg 3.54 kmol) in methanol (430 L) at 30-40°C. Ethyl acetate (229L) was added and the mixture stirred for 1 hour keeping the temperature below 30°C. The mixture was cooled to 0-5°C by addition of ice (300L), a suspension of potassium carbonate (624kg 4.51 kmol) in methanol (683L) and 1,2-dibromoethane (750kg 3.99 kmol) were added maintaining the temperature at 0-5°C. The mixture was heated to 50-55°C for 2 hours before water (460L) was added. Solvent was removed from the mixture by distillation under vacuum before further water (460L) was added and further solvent distilled. The mixture was cooled to below 30°C and ethyl acetate (621L) added. The mixture was filtered to remove precipitated salts. The aqueous phase was separated and extracted with ethyl acetate (2 x 621L). The organic extracts were combined and distilled under vacuum until 5%w/w or less residual ethyl acetate was obtained. The residue was then purified by thin layer distillation to give 2-1 (60kg, 22% yield).

Bis-(2-aminoxyethanol) sulfate (2-2)

(71kg 702.2 mol) was dissolved in methanol (200L) before sulfuric acid (50 wt%, 72kg, 367 mol) was added and the mixture heated to 60-70°C. The mixture was maintained at 60-70°C for 3 hours to achieve crystallization. If crystallization has not occurred a seed may be added. The mixture was maintained at 60-70°C of 5 hours before cooling to 0-5°C. The product was collected by filtration, washed with methanol and dried under vacuum at 40-45°C to provide 2-2 (70.8kg 80 %yield)

2,3,4-Trifluoro-5-nitrobenzoic acid (2-3)

Trifluorobenzoic acid (70kg, 398mol) in sulphuric acid (96 wt%; 194 L) and hexamethyldisiloxane (6.5kg, 40 mol) at 23°C was added to a 1:1 mixture of sulphuric acid (96 wt%) and nitric acid (98 wt%) (total 70.1kg) over 75 min. The temperature of the reaction mixture was maintained between 15°C and 25°C during the addition, The mixture was stirred for a further 5 hours and then run onto ice (700kg), keeping the temperature of the ice mixture below 0°C. Water (35 L) was used to rinse the nitration reactor into the quench reactor and the obtained mixture was stirred for 2 hours at 0°C, then isolated on a centrifuge. The resultant wet cake was washed with cold water (350 L) and the solid suspended in water (280 L) and stirred for 2 hours at 0°C. This suspension was then centrifuged and the cake was washed with cold water (210 L) and dried in a vacuum oven at 45°C for 2 days, to provide 2-3 (69.4kg, 74.3% yield).

2-Methyl 2,4-diamo-3-fluoro-5-nitrobenzoate (2-4)

2-3 (100g 0.452 mol) was dissolved in methanol (60ml) at 25-30°C. To the resulting stirred solution, at 0°C, was added chlorotrimethylsilane (98.3g 0.91mol), maintaining
the temperature between 10-20°C. On completion of the addition the mixture was heated to reflux for 5 hours. At this point 99%(area) conversion to methyl 2,3,4-trifluoro-5-nitrobenzoate was indicated by HPLC analysis. After cooling the mixture to room temperature it was diluted with N-methylpyrrolidone (NMP, 380ml) and the reaction vessel placed in an ice bath. Ammonium hydroxide solution (33 wt%, 164ml 2.7 mol) was added to the vigorously stirred mixture, keeping the temperature below 15°C. A yellow precipitate was formed during the addition. The reactor was then closed and heated to 80°C, with an internal pressure of 2.5 barg. After 5 hour the reaction mixture was cooled to 60°C and the pressure was released. The temperature was then increased to 75°C, followed by addition of ammonium hydroxide solution (33 wt%, 53ml, 1.0 mol). The mixture was then cooled to 50°C over 90 min. during which time a yellow precipitate was formed. After a further 1 hour at 50°C water (400ml) was added over 1 hour and the resultant suspension was cooled to 25°C and filtered. The filter cake was washed once with 1:1 NMP/water (540ml), once with water (540ml) and then dried in a vacuum oven at 50°C for 24 hours, to provide 2-4 (9.1g, 88% yield)

6-Amino-T-fluoro-S-methyl-SH-benzoimidazole-S-carboxylic acid methyl ester (2-5)

A mixture of 2-4 (50g, 218.18mmol), 5%Pd/C (4.4g, Type 101R/W) and trifluoroacetic acid (31.1g 272.72mmol) in methanol (400ml) was stirred under hydrogen at 10-15°C for 2 hours. The system was purged with nitrogen and HPLC analysis confirmed the reaction complete. The mixture was filtered under nitrogen pressure and washed through with methanol (12.5ml) to give a light yellow filtrate. p-Toluenesulfonic acid (45.65g, 240.0 mmol) in methanol (25ml) was added and the mixture heated to reflux temperature and concentrated by distillation. Acetonitrile (370ml) was added and solvent distilled from the mixture. Acetonitrile (200ml) was again added before further solvent distilled to leave less than 1%w/w methanol remaining. The mixture was cooled to 60°C, water (8.25ml) and diethoxymethane (50.02g 479.99mmol) were added and the mixture stirred at 60°C until HPLC analysis showed the reaction to be complete (about 1.5 hours). Di-iso-propylethylamine (112.75g 872.72mmol) was added and the mixture cooled to 10°C. The product was isolated by filtration, washed with acetonitrile and dried under vacuum at 45°C to provide 2-5 (37.98g, 78% yield).

6-(4-Bromo-2-chlorophenylamino)-7-fluoro-3-methyl-5//-benzimidazole-5-carboxylic acid sodium salt (2-6)

A mixture of Xantphos (1.296 g, 2.24 mmol) and tris(dibenzylideneacetone) dipalladium (0) (0.821 g, 0.896 mmol) in anhydrous anisole (100 mL) was stirred under nitrogen, at 50°C for 30 minutes to provide an orange-brown solution of the catalyst.
To a stirred mixture of 2-5 (10.00 g, 44.8mmol) and cesium carbonate (29.19 g, 89.61 mmol) in anhydrous anisole (200 mL) under nitrogen at 50°C was added 4-bromo-2-chloroiodobenzene (16.35 g, 51.52 mmol). The pre formed catalyst above was then added and the mixture heated to 90°C. The reaction was monitored by HPLC analysis. After 16 hours, no 2-5 remained. The mixture was filtered, then diluted with anisole (50ml) and cooled to 50-55°C. Methanol (50ml), water (4.04ml) were added followed by a methanolic solution of 30%w/w sodium methoxide (16.16g, 89.61 mmol) in methanol (10ml) and a seed. The mixture was stirred at 50-55°C for 1 hour when HPLC analysis showed the reaction complete. The product was filtered, washed with a mixture of anisole and methanol, then IMS and dried at 50°C under vacuum to give 2-6 (17.99g 94% yield).

6-r4-Bromo-2-chlorophenylamino)-2-fluoro-3-methyl-JH-benzimidazole-5-carboxylic acid (2-hydroxy-ethoxyVamide (AZD-6244))

To 2-6 (10g, 23.78 mmol) and 2-chloro-4,6-dimethoxy-1,3,5-triazine (4.91g, 27.96 mmol) in tetrahydrofuran (84ml) at 10°C under nitrogen was added a solution of N-methylmorpholine (2.94g, 29.08 mmol) in tetrahydrofuran (29ml) over 1 hour maintaining the temperature around 10°C. The mixture was stirred at 10°C until HPLC shows the reaction to be complete (about 4 hours) then cooled to 5°C. A solution of 2-2 (3.90g 15.46mmol) in water (19ml) was added and the reaction held at 5°C for 2 hours before water (42ml) and a seed were added. The mixture was heated to reflux temperature and concentrated by distillation of solvent. After cooling to 20°C, the product was collected by filtration and washed with aqueous tetrahydrofuran, 0.25% dipotassium hydrogen phosphate solution and IMS then dried at 40°C under vacuum to give AZD-6244 (10g 89% yield).

6-(4-Bromo-2-chlorophenylamino)-7-fluoro-3-methyl-iJ-benzimidazole-5-carboxylic acid (2-hydroxy-ethoxyVamide hydrogen sulphate salt (AZD-6244 Hdy-Sulfate))

AZD-6244 (10.5g, 22.94mmol) was added to a stirred solution of water (5.7ml), 5M sulphuric acid (5.5ml 27.53mmol) and tetrahydrofuran (59ml). The stirred mixture was heated to 50°C and held for 90 minutes before filtering to remove any extraneous matter. THF (297ml) was then added to the mixture maintaining the temperature above 50°C. A seed was added and the mixture was then cooled to 20°C over approximately 2 hours. The resulting slurry was filtered, washed with THF and dried 50°C under vacuum to give AZD-6244 as the hydrogen sulphate salt (12.75g 85% yield).

Pharmaceutical Preparations Comprising AZD-6244

Preparations of the hydrogen sulphate salt of AZD-6244 may be prepared using d-alpha-tocopheryl polyethylene glycol 1000 succinate as a pharmaceutical carrier. D-alpha-
tocopheryl polyethylene glycol 1000 succinate (otherwise known as Vitamin E TPGS) is a water-soluble derivative of natural source Vitamin E and has a dual nature, similar to an amphiphile, of hydrophilicity and lipophilicity. Vitamin E TPGS is obtained by esterification of crystalline d-α-tocopheryl acid succinate by polyethylene glycol (see U.S. Pharmacopeia 25 – National Formulary 20). Vitamin E TPGS is already known for it's use in pharmaceutical applications as an emulsifier, solubilizer and absorption enhancer and WO 96/36316, US 5891845 and WO 00/76482 may be cited as examples. See also "Eastman Vitamin E TPGS" Eastman Brochure, Eastman Chemical Co., Kingsport, Term. (November 2002) for further information about the use of Vitamin E TPGS in such applications.

Vitamin E TPGS preparations of the hydrogen sulphate salt of AZD-6244 are prepared by heating the carrier matrix in an oven set at 70°C for at least one hour. The hydrogen sulphate salt of AZD-6244 is then gradually added and mechanically stirred into the carrier matrix using a magnetic stir bar or a high-shear homogeniser. The system is maintained at sufficiently high temperature to keep the mixture in a molten state during stirring. Stirring is performed until a visibly homogenous mixture is obtained. The time taken for this to be achieved varies depending on the composition but is at least 10 minutes and may be up to 60 minutes. The resultant mixture is filled into hydroxypropyl methylcellulose (HPMC) capsules and allowed to cool to ambient temperature and solidify. Capsules are stored at either room temperature or under refrigerated conditions until use. Suitable per capsule proportions of AZD-6244 hydrogen sulphate salt to Vitamin E TPGS carrier matrix to are 30.25 to 119.75 mg, 60.5 to 239.5 mg, 90.75 to 359.25 mg, 30.25 to 269.75 mg and 15.12 to 134.88 mg.

The AZD-6244 Vitamin E TPGS pharmaceutical preparation is also described in PCT/GB2009/050293.

Typically the AZD-6244 hydrogen sulphate salt will be present in the Vitamin E TPGS pharmaceutical preparation in an amount within the range of from 5 to 30%, more particularly from 5 to 25%, by weight of the preparation. In a particular group of pharmaceutical preparations, the AZD-6244 hydrogen sulphate salt will be present in an amount of about 10% by weight of the final preparation. In yet a further particular group of pharmaceutical preparations, the AZD-6244 hydrogen sulphate salt will be present in an amount of about 20% by weight of the final preparation. In yet a further particular group of pharmaceutical preparations, the AZD-6244 hydrogen sulphate salt will be present in an amount of about 30% by weight of the final preparation. It is to be understood that the term 'about' when relating to the proportion of AZD-6244 hydrogen sulphate salt present in the Vitamin E TPGS pharmaceutical preparation refers to ± 2% by weight of the total preparation.

In one embodiment, the AZD-6244 hydrogen sulphate salt is dispersed within the Vitamin E TPGS and no additional solvents or additives are present.

Dosing and Routes of Administration
With regard to MK-2206 and AZD-6244, various forms of pharmaceutical preparation can be selected, and examples thereof include oral preparations such as tablets, capsules, powders, granules or liquids, or sterilized liquid parenteral preparations such as solutions or suspensions, suppositories, ointments and the like. MK-2206 and AZD-6244 are/may be available as pharmaceutically acceptable salts. MK-2206 and AZD-6244 are/may be prepared with pharmaceutically acceptable carriers or diluents.

The term "pharmaceutically acceptable salt" as referred to in this description means ordinary, pharmaceutically acceptable salt. For example, when the compound has a hydroxyl group, or an acidic group such as a carboxyl group and a tetrazolyl group, then it may form a base-addition salt at the hydroxyl group or the acidic group; or when the compound has an amino group or a basic heterocyclic group, then it may form an acid-addition salt at the amino group or the basic heterocyclic group.

The base-addition salts include, for example, alkali metal salts such as sodium salts, potassium salts; alkaline earth metal salts such as calcium salts, magnesium salts; ammonium salts; and organic amine salts such as trimethylamine salts, triethylamine salts, dicyclohexylamine salts, ethanolamine salts, diethanolamine salts, triethanolamine salts, procaine salts, N,N'-dibenzylethylenediamine salts.

The acid-addition salts include, for example, inorganic acid salts such as hydrochlorides, sulfates, nitrates, phosphates, perchlorates; organic acid salts such as maleates, fumarates, tartrates, citrates, ascorbates, trifluoroacetates; and sulfonates such as methanesulfonates, isethionates, benzenesulfonates, p-toluenesulfonates.

The term "pharmaceutically acceptable carrier or diluent" refers to excipients [e.g., fats, beeswax, semi-solid and liquid polyols, natural or hydrogenated oils, etc.]; water (e.g., distilled water, particularly distilled water for injection, etc.), physiological saline, alcohol (e.g., ethanol), glycerol, polyols, aqueous glucose solution, mannitol, plant oils, etc.); additives [e.g., extending agent, disintegrating agent, binder, lubricant, wetting agent, stabilizer, emulsifier, dispersant, preservative, sweetener, colorant, seasoning agent or aromatizer, concentrating agent, diluent, buffer substance, solvent or solubilizing agent, chemical for achieving storage effect, salt for modifying osmotic pressure, coating agent or antioxidant], and the like.

Solid preparations can be prepared in the forms of tablet, capsule, granule and powder without any additives, or prepared using appropriate carriers (additives). Examples of such carriers (additives) may include saccharides such as lactose or glucose; starch of corn, wheat or rice; fatty acids such as stearic acid; inorganic salts such as magnesium metasilicate aluminate or anhydrous calcium phosphate; synthetic polymers such as polyvinylpyrrolidone or polyalkylene glycol; alcohols such as stearyl alcohol or benzyl alcohol; synthetic cellulose derivatives such as methylcellulose, carboxymethylcellulose, ethylcellulose or hydroxypropylmethylcellulose; and other conventionally used additives such as gelatin, talc, plant oil and gum arabic.
Liquid preparations are produced in the forms of suspension, syrup, injection and drip infusion (intravenous fluid) using appropriate additives that are conventionally used in liquid preparations, such as water, alcohol or a plant-derived oil such as soybean oil, peanut oil and sesame oil.

In particular, when the preparation is administered parenterally in a form of intramuscular injection, intravenous injection or subcutaneous injection, appropriate solvent or diluent may be exemplified by distilled water for injection, an aqueous solution of lidocaine hydrochloride (for intramuscular injection), physiological saline, aqueous glucose solution, ethanol, polyethylene glycol, propylene glycol, liquid for intravenous injection (e.g., an aqueous solution of citric acid, sodium citrate and the like) or an electrolytic solution (for intravenous drip infusion and intravenous injection), or a mixed solution thereof.

Such injection may be in a form of a preliminarily dissolved solution, or in a form of powder per se or powder associated with a suitable carrier (additive) which is dissolved at the time of use. The injection liquid may contain, for example, 0.1 to 10% by weight of an active ingredient based on the total weight of each preparation.

Liquid preparations such as suspension or syrup for oral administration may contain, for example, 0.1 to 10% by weight of an active ingredient based on the total weight of each preparation.

Each preparation in the invention can be prepared by a person having ordinary skill in the art according to conventional methods or common techniques. For example, a preparation can be carried out, if the preparation is an oral preparation, for example, by mixing an appropriate amount of the compound of the invention with an appropriate amount of lactose and filling this mixture into hard gelatin capsules which are suitable for oral administration. On the other hand, preparation can be carried out, if the preparation containing the compound of the invention is an injection, for example, by mixing an appropriate amount of the compound of the invention with an appropriate amount of 0.9% physiological saline and filling this mixture in vials for injection.

The components of this invention may be administered to mammals, including humans, either alone or, in combination with pharmaceutically acceptable carriers, excipients or diluents, in a pharmaceutical composition, according to standard pharmaceutical practice. The components can be administered orally or parenterally, including the intravenous, intramuscular, intraperitoneal, subcutaneous, rectal and topical routes of administration.

Suitable dosages are known to medical practitioners and will, of course, depend upon the particular disease state, specific activity of the composition being administered, and the particular patient undergoing treatment. In some instances, to achieve the desired therapeutic amount, it can be necessary to provide for repeated administration, i.e., repeated individual administrations of a particular monitored or metered dose, where the individual administrations
axe repeated until the desired daily dose or effect is achieved. Further information about suitable dosages is provided below.

The term "administration" and variants thereof (e.g., "administering" a compound) in reference to a component of the invention means introducing the component or a prodrug of the component into the system of the animal in need of treatment. When a component of the invention or prodrug thereof is provided in combination with one or more other active agents (e.g., MK-2206), "administration" and its variants are each understood to include concurrent and sequential introduction of the component or prodrug thereof and other agents.

As used herein, the term "composition" is intended to encompass a product comprising the specified ingredients in the specified amounts, as well as any product which results, directly or indirectly, from combination of the specified ingredients in the specified amounts.

The term "therapeutically effective amount" as used herein means that amount of active compound or pharmaceutical agent that elicits the biological or medicinal response in a tissue, system, animal or human that is being sought by a researcher, veterinarian, medical doctor or other clinician.

A suitable amount of MK-2206 is administered to a patient undergoing treatment for cancer. In an embodiment, MK-2206 is administered in doses from about 30 mg - 300 mg per day. In an embodiment, MK-2206 is administered in doses from about 30 mg – 90 mg per day. In an embodiment, MK-2206 is administered in a dose of about 60 mg per day. In an embodiment, MK-2206 is administered in a dose of about 45 mg per day. In another embodiment, MK-2206 is administered in doses from about 30 - 300 mg every other day. In another embodiment, MK-2206 is administered in doses from about 30 – 60 mg every other day. In another embodiment, MK-2206 is administered in doses from about 20 – 40 mg every other day. In another embodiment, MK-2206 is administered in a dose of about 60 mg every other day. In another embodiment, MK-2206 is administered in a dose of about 45 mg every other day. In another embodiment, MK-2206 is administered in doses of about 30 mg every other day. In another embodiment, MK-2206 is administered in doses from about 30 - 200 mg once per week.

A suitable amount of AZD-6244 is administered to a patient undergoing treatment for cancer. In an embodiment, AZD-6244 is administered in doses from about 50 mg - 180 mg per day. In an embodiment, AZD-6244 is administered in doses from about 50 mg - 160 mg per day. In an embodiment, AZD-6244 is administered in doses from about 70 mg - 160 mg per day. In an embodiment, AZD-6244 is administered in a dose of about 150 mg per day. In another embodiment, AZD-6244 is administered in doses from about 25 - 90 mg twice daily. In another embodiment, AZD-6244 is administered in doses from about 25 - 80 mg twice daily. In another
embodiment, AZD-6244 is administered in doses from about 35 - 80 mg twice daily. In another
embodiment, AZD-6244 is administered in a dose of about 75 mg twice daily.

The therapeutic combination comprising MK-2206 and AZD-6244 is administered to a human patient, in accord with known methods, such as intravenous
administration as a bolus or by continuous infusion over a period of time, by intramuscular,
intraperitoneal, intracerobrospinal, subcutaneous, intra-articular, intrasynovial, intrathecal, oral,
topical, or inhalation routes. MK-2206 and AZD-6244 are preferably administered orally.

In a combination therapy regimen, the compositions of MK-2206 and AZD-6244 are administered in a therapeutically effective or synergistic amount. As used herein, a
therapeutically effective amount is such that co-administration of MK-2206 and AZD-6244, or
administration of a composition of MK-2206 and AZD-6244, results in reduction or inhibition of
the targeting disease or condition. A therapeutically synergistic amount is that amount of MK-
2206 and AZD-6244 necessary to synergistically or significantly reduce or eliminate conditions
or symptoms associated with a particular disease.

In a broad embodiment, the treatment of the present invention involves the
combined administration of MK-2206 and AZD-6244. The combined administration includes co
administration, using separate formulations or a single pharmaceutical formulation, and
consecutive administration in either order, wherein preferably there is a time period while both
(or all) active agents simultaneously exert their biological activities. Preparation and dosing
schedules for such chemotherapeutic agents may be used according to manufacturers’
instructions or as determined empirically by the skilled practitioner. Preparation and dosing
schedules for chemotherapy are also described in Chemotherapy Service Ed., M. C. Perry,
Williams & Wilkins, Baltimore, Md. (1992). MK-2206 may precede, or follow administration
of AZD-6244 or may be given simultaneously therewith. The clinical dosing of therapeutic
combination of the present invention are likely to be limited by the extent of adverse reactions.

Depending on the type and severity of the disease, about 60 mg of MK-2206 is an
initial candidate dosage for administration to the patient, whether, for example, by one or more
separate administrations, or by continuous infusion. Depending on the type and severity of the
disease, about 45 mg of MK-2206 is an initial candidate dosage for administration to the patient,
whether, for example, by one or more separate administrations, or by continuous infusion. A
typical every other day dosage might range from about 30 mg to about 60 mg or more,
depending on the factors mentioned above. For repeated administrations over several days or
longer, depending on the condition, the treatment is sustained until a desired suppression of
disease symptoms occurs. However, other dosage regimens may be useful.

Depending on the type and severity of the disease, about 150 mg of AZD-6244 is
an initial candidate dosage for administration to the patient, whether, for example, by one or
more separate administrations. A typical daily dosage might range from about 50 mg to about
180 mg, depending on the factors mentioned above. For repeated administrations over several
days or longer, depending on the condition, the treatment is sustained until a desired suppression of disease symptoms occurs. Other dosage regimens may be useful, but doses higher than 150 mg/day may not be tolerated by the patient.

In some aspects, the chemotherapy regimen involves the traditional high-dose intermittent administration. In some other aspects, the chemotherapeutic agents are administered using smaller and more frequent doses without scheduled breaks ("metronomic chemotherapy"). The progress of the therapy of the invention is easily monitored by conventional techniques and assays.

Sample dosing regimens for the combination are as follows:

<table>
<thead>
<tr>
<th>Sample regimen</th>
<th>MK-2206</th>
<th>AZD-6244</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>30 mg - 60 mg</td>
<td>75 mg</td>
</tr>
<tr>
<td></td>
<td><em>Every other day (qOD)</em></td>
<td><em>BID</em></td>
</tr>
<tr>
<td>2</td>
<td>30 mg - 60 mg</td>
<td>75 mg</td>
</tr>
<tr>
<td></td>
<td><em>Every other day (qOD)</em></td>
<td><em>Every day, qd</em></td>
</tr>
<tr>
<td>3</td>
<td>30 - 90 mg</td>
<td>75 mg</td>
</tr>
<tr>
<td></td>
<td><em>Three times per week, qd</em></td>
<td><em>Every day, qd</em></td>
</tr>
<tr>
<td>4</td>
<td>30 mg - 300 mg</td>
<td>75 mg</td>
</tr>
<tr>
<td></td>
<td><em>Once per week, qd</em></td>
<td><em>Every day, qd</em></td>
</tr>
<tr>
<td>5</td>
<td>30 mg - 90 mg</td>
<td>75 mg</td>
</tr>
<tr>
<td></td>
<td><em>Three times per week, qd</em></td>
<td><em>Every day, BID</em></td>
</tr>
<tr>
<td>6</td>
<td>30 mg - 300 mg</td>
<td>75 mg</td>
</tr>
<tr>
<td></td>
<td><em>Once per week, qd</em></td>
<td><em>Every day, BID</em></td>
</tr>
<tr>
<td>7</td>
<td>20 mg - 40 mg</td>
<td>75 mg</td>
</tr>
<tr>
<td></td>
<td><em>Every other day (qOD)</em></td>
<td><em>BID</em></td>
</tr>
<tr>
<td>8</td>
<td>30 mg</td>
<td>75 mg</td>
</tr>
<tr>
<td></td>
<td><em>Every other day (qOD)</em></td>
<td><em>BID</em></td>
</tr>
<tr>
<td>9</td>
<td>30 mg - 200 mg</td>
<td>75 mg</td>
</tr>
<tr>
<td></td>
<td><em>Once per week, qd</em></td>
<td><em>Every day, qd</em></td>
</tr>
<tr>
<td>10</td>
<td>30 mg - 200 mg</td>
<td>75 mg</td>
</tr>
<tr>
<td></td>
<td><em>Once per week, qd</em></td>
<td><em>Every day, BID</em></td>
</tr>
</tbody>
</table>

Additional indications

In addition to the treatment of lung cancer (including small cell and non-small cell), pancreatic cancer, colon cancer (including colorectal) and melanoma (including malignant), MK-2206 and AZD-6244 in combination may also be useful for the treatment of the following cancers: **Cardiac**: sarcoma (angiosarcoma, fibrosarcoma, rhabdomyosarcoma,
liposarcoma), myxoma, rhabdomyoma, fibroma, lipoma and teratoma; Lung: bronchogenic carcinoma (squamous cell, undifferentiated small cell, undifferentiated large cell, adenocarcinoma), alveolar (bronchiolar) carcinoma, bronchial adenoma, sarcoma, lymphoma, chondromatous hamartoma, mesothelioma; Gastrointestinal: esophagus (squamous cell carcinoma, adenocarcinoma, leiomyosarcoma, lymphoma), stomach (carcinoma, lymphoma, leiomyosarcoma), pancreas (ductal adenocarcinoma, insulinoma, glucagonoma, gastrinoma, carcinoid tumors, vipoma), small bowel (adenocarcinoma, lymphoma, carcinoid tumors, Karposi's sarcoma, leiomyoma, hemangioma, lipoma, neurofibroma, fibroma), large bowel (adenocarcinoma, tubular adenoma, villous adenoma, hamartoma, leiomyoma), colon, colorectal, rectal; Genitourinary tract: kidney (adenocarcinoma, Wilm's tumor [nephroblastoma], lymphoma, leukemia), bladder and urethra (squamous cell carcinoma, transitional cell carcinoma, adenocarcinoma), prostate (adenocarcinoma, sarcoma), testis (seminoma, teratoma, embryonal carcinoma, teratocarcinoma, choriocarcinoma, sarcoma, interstitial cell carcinoma, fibroma, fibroadenoma, adenomatoid tumors, lipoma); Liver: hepatoma (hepatocellular carcinoma), cholangiocarcinoma, hepatoblastoma, angiosarcoma, hepatocellular adenoma, hemangioma; Bone: osteogenic sarcoma (osteosarcoma), fibrosarcoma, malignant fibrous histiocytoma, chondrosarcoma, Ewing's sarcoma, malignant lymphoma (reticulum cell sarcoma), multiple myeloma, malignant giant cell tumor chordoma, osteochonfrroma (osteocartilaginous exostoses), benign chondroma, chondroblastoma, chondromyxofibroma, osteoid osteoma and giant cell tumors; Nervous system: skull (osteoma, hemangioma, granuloma, xanthoma, osteitis deformans), meninges (meningioma, meningiosarcoma, gliomatosis), brain (astrocytoma, medulloblastoma, glioma, ependymoma, germinoma [pinealoma], glioblastoma multiform, oligodendrogloma, schwannoma, retinoblastoma, congenital tumors), spinal cord neurofibroma, menigioma, glioma, sarcoma); Gynecological: uterus (endometrial carcinoma), cervix (cervical carcinoma, pre-tumor cervical dysplasia), ovaries (ovarian carcinoma [serous cystadenocarcinoma, mucinous cystadenocarcinoma, unclassified carcinoma], granulosa-theal cell tumors, Sertoli-Leydig cell tumors, dysgerminoma, malignant teratoma), vulva (squamous cell carcinoma, intraepithelial carcinoma, adenocarcinoma, fibrosarcoma, melanoma), vagina (clear cell carcinoma, squamous cell carcinoma, botryoid sarcoma (embryonal rhabdomyosarcoma), fallopian tubes (carcinoma); Hematologic: blood (myeloid leukemia [acute and chronic], acute lymphoblastic leukemia, chronic lymphocytic leukemia, myeloproliferative diseases, multiple myeloma, myelodysplasia syndrome), Hodgkin's disease, non-Hodgkin's lymphoma [malignant lymphoma]; Skin: malignant melanoma, basal cell carcinoma, squamous cell carcinoma, Karposi's sarcoma, moles dysplastic nevi, lipoma, angioma, dermatofibroma,; and Adrenal glands: neuroblastoma. Thus, the term "cancerous cell" as provided herein, includes a cell afflicted by any one of the above-identified conditions.

Exemplifying the invention is the use of the MK-2206 and AZD-6244 combination described above in the preparation of a medicament for the treatment and/or
prevention of lung cancer (including small cell and non-small cell), pancreatic cancer, colon cancer (including colorectal) and melanoma (including malignant).

Exemplifying the invention is the use of the MK-2206 and AZD-6244 combination described above in the preparation of a medicament for the treatment and/or prevention of lung cancer (including small cell and non-small cell), pancreatic cancer, colon cancer (including colorectal), stomach cancer (gastric) and melanoma (including malignant).

Additional anti-cancer agents

The MK-2206 and AZD-6244 combination of the instant invention is also useful in combination with additional therapeutic, chemotherapeutic and anti-cancer agents. Further combinations of MK-2206 and AZD-6244 with therapeutic, chemotherapeutic and anti-cancer agents are within the scope of the invention. Examples of such agents can be found in Cancer Principles and Practice of Oncology by V.T. Devita and S. Hellman (editors), 6th edition (February 15, 2001), Lippincott Williams & Wilkins Publishers. A person of ordinary skill in the art would be able to discern which combinations of agents would be useful based on the particular characteristics of the drugs and the cancer involved. Such additional agents include the following: estrogen receptor modulators, androgen receptor modulators, retinoid receptor modulators, cytotoxic/cytostatic agents, antiproliferative agents, prenyl-protein transferase inhibitors, HMG-CoA reductase inhibitors and other angiogenesis inhibitors, HIV protease inhibitors, reverse transcriptase inhibitors, inhibitors of cell proliferation and survival signaling, bisphosphonates, aromatase inhibitors, siRNA therapeutics, γ-secretase inhibitors, agents that interfere with receptor tyrosine kinases (RTKs) and agents that interfere with cell cycle checkpoints. MK-2206 and AZD-6244 may be particularly useful when co-administered with radiation therapy.

MK-2206 and AZD-6244 may also be useful for treating cancer in further combination with the following therapeutic agents: abarelix (Plenaxis depot®); aldesleukin (Prokine®); Aldesleukin (Proluekin®); Alemtuzumabb (Campath®); alitretinoin (Panretin®); allopurinol (Zyloprim®); altretamine (Hexalen®); amifostine (Ethylol®); anastrozole (Arimidex®); arsenic trioxide (Trisenox®); asparaginase (Elspar®); azacitidine (Vidaza®); bevacuzimab (Avastin®); bexarotene capsules (Targetretin®); bexarotene gel (Targetretin®); bleomycin (Blenoxane®); bortezomib (Velcade®); busulfan intravenous (Busulfex®); busulfan oral (Myleran®); calusterone (Methosarb®); capecitabine (Xeloda®); carboptatin (Paraplatin®); carmustine (BCNU®, BiCNU®); carmustine (Gliadel®); carmustine with Polifeprosan 20 Implant (Gliadel Wafer®); celecoxib (Celebrex®); cetuximab (Erbitux®); chlorambucil (Leukeran®); cisplatin (Platinol®); cladribine (Leustatin®, 2-CdA®); clofarabine (Clolar®); cyclophosphamide (Cytoxan®, Neosar®); cyclophosphamide (Cytoxan Injection®); cyclophosphamide (Cytoxan Tablet®); cytarbaine (Cytosar-U®); cytarbaine liposomal (DepoCyt®); dacarbazine (DTIC-Dome®); dactinomycin, actinomycin D (Cosmegen®);
Darbepoetin alfa (Aranesp®); daunorubicin liposomal (DanuoXome®); daunorubicin, daunomycin (Daunorubicin®); daunorubicin, daunomycin (Cerubidine®); Denileukin difitox (Ontak®); dexrazoxane (Zincard®); docetaxel (Taxotere®); doxorubicin (Adriamycin PFS®); doxorubicin (Adriamycin®, Rubex®); doxorubicin (Adriamycin PFS Injection®); doxorubicin liposomal (Doxil®); dromostanolone propionate (Dromostanolone ®); dromostanolone propionate (Masterone Injection®); Elliott's B Solution (Elliott's B Solution®); epirubicin (Ellence®); Epoeitin alfa (epogen®); erlotinib (Tarceva®); estramustine (Emcyt®); etoposide phosphate (Etopophos®); etoposide, VP-16 (Vepesid®); exemestane (Aromasin®); Filgrastim (Neupogen®); flouxuridine (intraarterial) (FUDR®); fludarabine (Fludara®); fluorouracil, 5-FU (Adrucil®); Mvestrant (Faslodex®); gefitinib (Iressa®); gemcitabine (Gemzar®); gentuzumab ozogamicin (Mylotarg®); goserein acetate (Zoladex Implant®); goserein acetate (Zoladex®); histrelin acetate (Histrelin implant®); hydroxyurea (Hydrea®); Ibritumomab Tiuxetan (Zevalin®); idarubicin (Idamycin®); ifosfamide (IFEX®); imatinib mesylate (Gleevec®); interferon alfa 2a (Roferon A®); Interferon alfa-2b (Intron A®); irinotecan (Camptosar®); lenalidomide (Revlimid®); letrozole ( Femara®); leucovorin (Wellcovorin®, Leucovorin®); Leuprolide Acetate (Eliargid®); levamisole (Ergamisol®); lomustine, CCNU (CeeBU®); meclorethamine, nitrogen mustard (Mustargen®); megestrol acetate (Megace®); melphalan, L-PAM (Alkeran®); mercaptopurine, 6-MP (Purinethol®); mesna (Mesnex®); mesna (Mesnex tabs®); methotrexate (Methotrexate®); methoxsalen (Uvadex®); mitomycin C (Mutamycin®); mitotane (Lysodren®); mitoxantrone (Novantrone®); nandrolone phenpropionate (Durabolin-50®); nelarabine (Arranon®); Nofetumomab (Verluma®); Oprelvekin (Neumega®); oxaliplatin (Eloxatin®); palicitaxel (Paxene®); paclitaxel (Taxol®); paclitaxel protein-bound particles (Abraxane®); palifermin (Kepivance®); pamidronate (Aredia®); pegademase (Adagen (Pegademase Bovine®)); pegaspargase (Oncaspar®); Pegfilgrastim (Neulasta®); pemetrexed disodium (Alinta®); pentostatin (Nipent®); pipobroman (Vercyte®); plicamycin, mithramycin (Mithracin®); porfimer sodium (Photofrin®); procarbazine (Matulane®); quinacrine (Atabrine®); Rasburicase (Elitek®); Rituximab (Rituxan®); sargramostim (Leukine®); Sargramostim (Prokine®); sorafenib (Nexavar®); streptozocin (Zanosar®); sunitinib maleate (Sutent®); talc (Sclerosol®); tamoxifen (Nolvadex®); temozolomide (Temodar®); teniposide, VM-26 (Vumon®); testolactone (Teslac®); thioguanine, 6-TG (Thioguanine®); thiopeta (Thioplex®); topotecan (Hycamtin®); toremifene (Fareston®); Tositumomab (Bexxar®); Tositumomab/I-131 tositumomab (Bexxar®); Trastuzumab (Herceptin®); tretnoin, ATRA (Vesanoid®); Uracil Mustard (Uracil Mustard Capsules®); valrubcin (Valstar®); vinblastine (Velban®); vincristine (Oncovin®); vinorelbine (Navelbine®); and zoledronate (Zometa®).

MK-2206 and AZD-6244 may also be useful for treating cancer in further combination with sorafenib.

MK-2206 and AZD-6244 may also be useful for treating cancer in further combination with the following c-MET inhibitors selected from ARQl 97, PF-2341066, PF-
MK-2206 and AZD-6244 may also be useful for treating cancer in further
combination with a JAK-Stat inhibitor.

AU patents, publications and pending patent applications identified are hereby
incorporated by reference.

EXAMPLE 1

STRONG SYNERGISM OF MK-2206 IN COMBINATION WITH AZD-6244 IN PI3K RAS
ACTIVE CELLS

1.1. Compounds
MK-2206 and AZD-6244 were dissolved in dimethyl sulfoxide (DMSO) (SIGMA, #D2650) to
make 10 mM stock solution and stored at -20 °C.

1.2. Cell lines
Human colon cancer cell line (HCT-116, HCT-15, and HT-29) and human melanoma cell line
(A2058) were obtained from the American Type Culture Collection (ATCC). All cell lines were
cultured in RPMI-1640 containing heat-inactivated 10% fetal bovine serum (FBS) in a
humidified incubator at 37 °C in 5% CO₂.

13. In vitro combination of MK-2206 with AZD-6244
All cell lines (total 4 cell lines) were seeded at 2,000 cells/well in RPMI 1640 supplemented with
10% heat-inactivated FBS in 96-well tissue culture plates (CORNING #3340) and incubated at
37°C in 5% CO₂ overnight. Then MK-2206 or AZD-6244 was added as a dilution series in
RPMI with final DMSO concentration of 0.1%. The plates were then incubated at 37 °C in 5% 
CO₂ for 72 hours. The number of viable cells was measured using CellTiter-Glo cell viability
assay (Promega #G7571). The luminiscence signal was read using a ARVO-SX multimode plate
reader (Perkin-Elmer). Percent inhibition of cell growth was calculated relative to the vehicle
treated control. Concentration of the compound that inhibits 50% of control cell growth (IC50)
was interpolated using nonlinear regression and the equation;

\[ Y = \text{Bottom} + \frac{(\text{Top} - \text{Bottom})}{(1 + 10^\left(-\left(\text{LogED50} - X\right) \times \text{Hill slope}\right))} \]

X is the logarithm of concentration, Y is the response. Y starts at Bottom and goes to Top with a
sigmoid shape.

A 2- or 3-fold serial dilution series of concentrated MK-2206/ AZD-6244 combinations was
prepared, in which the concentration ratio of the two agents was fixed and equal to IC₅₀ ratio of
MK-2206 and AZD-6244 determined in single agent titrations described above. Corresponding
single agent dilution series of concentrated MK-2206 and AZD-6244 were also prepared. The
three dilution series were then tested in the proliferation/viability assay as described above. The
data were analyzed using CalcuSyn software (BIOSOFT) that calculates combination index (CI)
for each combination of MK-2206/ AZD-6244 as described below. Combination index values were generated by inserting the interpolated values into the mutually exclusive equation derived by Chou and Talalay.

### 1.4. Combination index

Synergistic interaction between agents was analyzed by the median effect method described by Chou and Talalay (Adv. Enzyme Regul. 22: 27-55, 1984). The fixed ratio experimental design was used to compare of various MK-2206/ AZD-6244 combinations and corresponding single agent treatments. The combination index was calculated by the following equation based on doses that had equivalent effects in tumor cell proliferation/ viability assay;

\[
CI = \frac{(Dl/Dlc)}{+ (D2/D2c)}
\]

where D1 and D2 represent doses for each drug alone that effect x % of cells and Die \(a\) and \(\epsilon\)2c are the concentrations of combined drugs that effect the same percentage of cells. D1 and D2 are known from the composition of the combination and Die \(a\) and \(\epsilon\)2c can be calculated from the equation;

\[
Dc = D_{m} \cdot \frac{[f_{a}]/(1-fa)]^{1/m}}
\]

where \(D_{m}\) is the concentration of drugs giving 50% effect, \(f_{a}\) is the fraction affected, and \(m\) is the slope from the median effect plot of log (fa/fu) where fu is the fraction unaffected versus logD. A CI equal to 1 signifies an additive effect, less than 1 a synergistic effect, and greater than 1 an antagonistic effect.

### 1.5. Western blot analysis

Cells were grown to -70% confluence, and treated with DMSO alone or AZD-6244 (3, 30, 300, and 3000 nM) in the presence or absence of 3 uM of MK-2206 for 24 hours. Cells were lysed in cell lysis buffer [20 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1% Triton-X100, containing protease inhibitor cocktails (Roche #04906837001) and phosphatase inhibitor cocktails (Calbiochem #524625)]. The concentration of soluble proteins was determined by Dc protein assay kit (Pierce), and proteins were resolved on SDS-PAGE, transferred to nitrocellulose filter, and probed with following antibodies; phospho-Akt (S473), phospho-Erk (T202/Y204), phospho-S6 (S235/236), and phospho-4E-BP1 (T70). All antibodies were obtained from Cell Signaling Technologies. The protein-antibody complexes were detected by using an enhanced chemiluminescence kit (GE healthcare).
EXAMPLE 2
STRONG SYNERGISM OF MK-2206 IN COMBINATION WITH AZD-6244 IN PANCREATIC AND LUNG TUMOR LINES

2.1. Cell lines
Human pancreatic cancer cell line (AsPC-1, BxPC-3, and MIA-Pa-Ca2) and human lung cancer cell line (Calu-6 and NCI-H460) were obtained from the American Type Culture Collection (ATCC). All cell lines were cultured in RPMI-1640 containing heat-inactivated 10% fetal bovine serum (FBS) in a humidified incubator at 37 °C in 5% CO₂.

2.2. In vitro combination of MK-2206 with AZD-6244
All cell lines (total 5 cell lines) were seeded at 2,000 cells/well in RPMI 1640 supplemented with 10% heat-inactivated FBS in 96-well tissue culture plates (CORNING #3340) and incubated at 37°C in 5% CO₂ overnight. MK-2206 and/or AZD-6244 were added as a dilution series in RPMI with final DMSO concentration of 0.1%. The plates were then incubated at 37 °C in 5% CO₂ for 72 hours. The number of viable cells was measured using CellTiter-Glo cell viability assay (Promega #G7571). The CI values were calculated by the method as described in 1.4.

EXAMPLE 3
MK-2206 ENHANCES ANTI-TUMOR EFFICACY OF AZD-6244 IN VIVO (A2058)
A2058 cancer cell was suspended in 50% Matrigel and 50% PBS, and were subcutaneously transplanted into side flank of female CD1-nude mice by using needle and syringe (A2058; 1 x10⁷ cells /100 μL) under isoflurane anesthesia. After 2 weeks, when tumor size was reached to certain size, dosing of both agents was started. Mice were randomized according to tumor volumes and distributed into treatment groups with approximately equivalent ranges of tumor volumes between treatment groups. AZD-6244 was dissolved in 0.5%MCl+0.1%Tween80 with leq of HCL. The hydrochloride salt of MK-2206 was dissolved in 30% captisol. Tumor tissues were immediately frozen in liquid nitrogen and used for PD analysis. Expression of phospho-ERK (T202/204) for AZD-6244 and phospho-AKT (T308) for MK-2206 in tumor was examined by Western blot analysis. For efficacy, tumor volume was measured with digital caliper on the day 0, 4, 7, 10, 14 and 17 and tumor volume (mm³) was calculated as length x (width)² x 0.5. Measurement of body weight and gross observation were performed. Statistical analysis was performed using repeated measure ANOVA followed by Dunnett.

EXAMPLE 4
MK-2206 ENHANCES ANTI-TUMOR EFFICACY OF AZD-6244 IN VIVO (HCT16)
Anti-tumor efficacy and PD analysis was performed as the same condition as the study with A2058 mouse xenograft model.
EXAMPLE 5

MK-2206 ENHANCES ANTI-TUMOR EFFICACY OF AZD-6244 IN VIVO (NCI-H1 1975)

AZD-6244 and MK-2206 were evaluated, alone and combined, in the subcutaneous NCI-H1 975 human non-small cell lung cancer (NSCLC) xenograft model established in female nude mice. Xenografts were initiated by subcutaneously implanting $1 \times 10^7$ NCI-H1 975 tumor cells (0.2 mL cell suspension in 50% Matrigel), into the right flank of each test mouse. Treatments began nine days later, on Day 1 (D1) of the study, in a control group (n = 10) and eleven treatment groups (n = 15/group) with group mean tumor volumes of -139 mm$^3$. The control group received vehicle 1 (0.5% hydroxypropylmethylcellulose : 0.1% Tween® 80®), orally (p.o.), daily for twenty-two days (qd x22) and vehicle 2 (30% Captisol), p.o., weekly for four weeks (qwk x4). Oral AZD-6244 at 25 mg/kg was dosed qd x22. Oral MRK-2206 at 240 mg/kg was dosed on Days 1, 3, 5, 8, 10, 12, 15, 17, 19, and 22 (Days 1..22). Oral MK-2206 was also administered at 240, 360, 480, or 720 mg/kg, p.o., qwk x4. AZD-6244 and MK-2206 were also combined on these same schedules. Data is shown in Figure 5 for the 240 qod and 480 qw doses of MK-2206. Due to treatment related (TR) deaths, MK-2206 at 720 mg/kg was decreased to 600 mg/kg after one dose (720/600). Test animals in each group were euthanized when their tumors reached an endpoint volume of 2000 mm$^3$ or on D61, whichever came first, and the time to endpoint (TTE) was calculated for each mouse using a 900 mm$^3$ endpoint for analysis. Treatment response was determined from tumor growth delay (TGD), defined as the increase in the median TTE in treated versus control mice. Response was also evaluated based on regression responses and logrank significance of differences in survival. Tolerability of treatment was assessed by body weight (BW) measurements and by frequent observation for signs of toxicity. Blood and tissue samples were collected from a "sampling only" group of five test animals that received one oral dose of vehicle 1 and vehicle 2 on D8 and from five animals in each of the eleven treatment groups.

EXAMPLE 6

MK-2206 ENHANCES ANTI-TUMOUR EFFICACY OF AZD-6244 IN VIVO (AsPC-I)

To establish the in vivo efficacy of combination therapy of AZD-6244 and MK-2206 in AsPC-I human pancreatic tumour xenograft model, experiments were conducted in female athymic mice (Swiss nu/nu genotype, ≥ 6 weeks of age). AsPC-I human pancreatic tumour xenografts were established in mice by injecting $3 \times 10^6$ cells (100µl volume containing 50% Matrigel®) subcutaneously in the dorsal flank. Tumour volumes were assessed using bilateral Vernier calliper measurement at least twice weekly and calculated using the formula (length x width) x $\sqrt{2}$ (length x width) x ($\pi$/6), where length was taken to be the longest diameter across the tumour and width the corresponding perpendicular. When the mean tumour volume reached approximately 0.25 cm$^3$, the mice were randomised into 4 treatment groups (10-12 mice/group) with equivalent distribution of tumour volumes across treatment groups. Following randomisation, mice were
treated with drag vehicles for AZD-6244 (0.5%MC+0.1 %Tween80) or MK-2206 (30% captisol), AZD-6244, MK-2206 or a combination of AZD-6244 plus MK-2206. Tumour growth inhibition was assessed by comparison of the differences in tumour volume between control and treated groups on Day 21 of the study (equivalent to 14 days dosing period). The effects of combination treatment were assessed by comparing any effect on tumour growth in the group of animals receiving AZD-6244 plus MK-2206 with tumour growth in the groups where animals received single agent therapy alone. Statistical analysis was performed using Student's t test (1 tailed) for control vs. treated groups and using a two way ANOVA for monotherapies vs. combination group.
WHAT IS CLAIMED IS:

I. A method of treating cancer by administering to a patient in need thereof an effective amount of MK-2206, or a pharmaceutically acceptable salt thereof, and AZD-6244, or a pharmaceutically acceptable salt thereof.

2. The method according to Claim 1, wherein the cancer is selected from the group consisting of lung cancer (including small cell and non-small cell), pancreatic cancer, colon cancer (including colorectal) and melanoma (including malignant).

3. The method of Claim 1 or Claim 2 wherein MK-2206 is administered in a dose between 30 mg and 90 mg per day.

4. The method of Claim 1 or Claim 2 wherein MK-2206 is administered at a dose of about 60 mg per day.

5. The method of Claim 1 or Claim 2 wherein MK-2206 is administered at a dose of about 45 mg per day.

6. The method of Claim 1 or Claim 2 wherein MK-2206 is administered at a dose of about 60 mg every other day.

7. The method of Claim 1 or Claim 2 wherein MK-2206 is administered at a dose of about 45 mg every other day.

8. The method of Claim 1 or Claim 2 wherein MK-2206 is administered at a dose of about 30 mg every other day.

9. The method of Claim 1 or Claim 2 wherein MK-2206 is administered at a dose of about 30 - 200 mg once per week.

10. The method of Claim 1 or Claim 2 wherein AZD-6244 is administered in a dose between 50 mg and 180 mg per day.

11. The method of Claim 1 or Claim 2 wherein AZD-6244 is administered at a dose of about 150 mg per day.

12. A therapeutic combination comprising:
MK-2206, or a pharmaceutically acceptable salt thereof; and
13. A combination product comprising:
- MK-2206, or a pharmaceutically acceptable salt thereof; and
- AZD-6244, or a pharmaceutically acceptable salt thereof.

14. A combination product according to Claim 13, wherein the combination product is an individual pharmaceutical preparation comprising:
- MK-2206, or a pharmaceutically acceptable salt thereof; and
- AZD-6244, or a pharmaceutically acceptable salt thereof;
in association with a pharmaceutically acceptable carrier or diluent.

15. A combination product according to Claim 14, wherein the pharmaceutical preparation comprises the hydrogen sulphate salt of AZD-6244.

16. A combination product according to Claim 15, wherein the hydrogen sulphate salt of AZD-6244 is in association with a d-alpha-tocopheryl polyethylene glycol 1000 succinate carrier matrix.

17. A combination product according to Claim 16, wherein the AZD-6244 hydrogen sulphate salt is present in the pharmaceutical preparation in an amount within the range of from 5 to 30% by weight of the preparation.

18. A combination product according to Claim 13, wherein said combination product is a kit of parts comprising the following components:
- a first pharmaceutical preparation comprising MK-2206, or a pharmaceutically acceptable salt thereof, in association with a pharmaceutically acceptable carrier or diluent; and
- a second pharmaceutical preparation comprising AZD-6244, or a pharmaceutically acceptable salt thereof, in association with a pharmaceutically acceptable carrier or diluent;
wherein the components are provided in a form which is suitable for sequential, separate and/or simultaneous administration.

19. A combination product according to Claim 18, wherein the second pharmaceutical preparation comprises the hydrogen sulphate salt of AZD-6244.
20. A combination product according to Claim 19, wherein the hydrogen sulphate salt of AZD-6244 is in association with a d-alpha-tocopheryl polyethylene glycol 1000 succinate carrier matrix.

21. A combination product according to Claim 20, wherein the AZD-6244 hydrogen sulphate salt is present in the second pharmaceutical preparation in an amount within the range of from 5 to 30% by weight of the second pharmaceutical preparation.

22. A therapeutic combination according to Claim 12 or a combination product according to any one of claims 13 to 21, for use as a medicament.

23. A therapeutic combination according to Claim 10 or a combination product according to any one of claims 13 to 21, for use in the treatment of cancer.

24. A therapeutic combination or combination product according to Claim 23 wherein the cancer is selected from the group consisting of lung cancer (including small cell and non-small cell), pancreatic cancer, colon cancer (including colorectal) and melanoma (including malignant).

25. Use of a therapeutic combination according to Claim 12 or a combination product according to any one of claims 13 to 21 in the manufacture of a medicament for use in the treatment of cancer.

26. A use according to Claim 25 wherein the cancer is selected from the group consisting of lung cancer (including small cell and non-small cell), pancreatic cancer, colon cancer (including colorectal) and melanoma (including malignant).
### Strong Synergism of MK-2206 in Combination with AZD-6244 in PI3K/Ras Active Cells

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Cancer type</th>
<th>Combination index for each fractional inhibition</th>
<th>Known defects in PI3K, PTEN or Ras</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>ED50</td>
<td>ED75</td>
</tr>
<tr>
<td>HCT116</td>
<td>Colon</td>
<td>0.20</td>
<td>0.14</td>
</tr>
<tr>
<td>HCT15</td>
<td>Colon</td>
<td>0.12</td>
<td>0.12</td>
</tr>
<tr>
<td>HT29</td>
<td>Colon</td>
<td>0.68</td>
<td>0.73</td>
</tr>
<tr>
<td>A2058</td>
<td>Melanoma</td>
<td>0.35</td>
<td>0.19</td>
</tr>
</tbody>
</table>

- Addition of MEK inhibitor enhances MK-2206 activity in these cells

**FIG. 1**

**HCT-116**

MEK inhibitor (nM)

<table>
<thead>
<tr>
<th>MEK inhibitor (nM)</th>
<th>0</th>
<th>3</th>
<th>30</th>
<th>300</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phospho-AKT (S473)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Phospho-ERK (T202/Y204)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Phospho-S6 ribosomal protein (S235/236)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Phospho-4E-BP1 (T70)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

Harvest at 24 hr after treatment

% inhibition

- 0 50 55 64 45 83 72

3 μM MK2206
Strong Synergism of MK–2206 in Combination with AZD–6244 in Pancreatic and Lung Tumor Lines

Methods: Cells were incubated with multiple concentrations of compounds for 72 hr. The effect analyses and calculation of the combination indices were performed with the method of Chou and Talay using the CalcuSyn commercial software package.

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>Cancer Type</th>
<th>Known defects in each</th>
<th>Fractional Inhibition</th>
<th>Combination index</th>
<th>Known defects in each</th>
<th>Fractional Inhibition</th>
<th>Combination index</th>
</tr>
</thead>
<tbody>
<tr>
<td>AsPC-1</td>
<td>Pancreas</td>
<td></td>
<td>0.18</td>
<td>0.09</td>
<td>WT</td>
<td>0.15</td>
<td>WT</td>
</tr>
<tr>
<td>BxPC-3</td>
<td>Pancreas</td>
<td></td>
<td>0.11</td>
<td>0.09</td>
<td>WT</td>
<td>0.15</td>
<td>WT</td>
</tr>
<tr>
<td>MiaPaCa2</td>
<td>Pancreas</td>
<td></td>
<td>0.42</td>
<td>0.25</td>
<td>WT</td>
<td>0.58</td>
<td>WT</td>
</tr>
<tr>
<td>Calu-6</td>
<td>Lung</td>
<td></td>
<td>0.25</td>
<td>0.2</td>
<td>WT</td>
<td>0.18</td>
<td>WT</td>
</tr>
<tr>
<td>NCI-H690</td>
<td>Lung</td>
<td></td>
<td>0.04</td>
<td>0.03</td>
<td>E545K</td>
<td>0.02</td>
<td>E545K</td>
</tr>
</tbody>
</table>

Cl<0.1: Very strong synergism
0.1–0.3: Strong synergism
0.3–0.7: Synergy
0.7–0.9: Moderate to slight synergism

- Combination of MK–2206 with MEKi had broad spectrum of anti-tumor efficacy in vitro against other tumor cell types
MK-2206 enhances anti-tumor efficacy of AZD-6244 in vivo (A2058)

**Efficacy: CD1-nude mouse model (n=5)**
- MEKi (AZD6244), PO, 50 mpk, bid x 5 days/wk x 2wk
- MK2206 PO, 120 mpk, QOD/wk x 2wk

- **A2058 model**  
  (Melanoma, BRAF/PTEN mutation)

- **PD:**
  - MK2206 110 mpk single PO, 12h
  - MEKi 25 mpk single PO, 4h

<table>
<thead>
<tr>
<th>Animal #</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>0.5% MC</td>
<td>MEKi</td>
<td>25 mpk, 4 hr</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A2058</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pERK (T202/Y204)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pAKT (T308)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Body weight change**

- Control
- MEKi 50 mpk, bid
- MK-2206 120 mpk
- MEKi + MK-2206

**FIG. 3**
MK-2206 enhances anti-tumor efficacy of AZD-6244 in vivo (HCT116)

Efficacy: CD1-nude mouse model (n=5)
• MEKi (AZD6244), PO, 50 mpd, bid x 5 days/wk x 2wk
• MK2206 PO, 120 mpd, QOD/wk x 2wk

PD: (n=4)
• MK2206 110 mpk single PO, 12h
• MEKi 25mpk single PO, 4h

HCT-116

Animal #
Vehicle 0.5% MC MEKi 25 mpk, 4 hr

1 2 3 4 5 6 7 8

HCT116

(Colorectal cancer, K-ras/PI3K MT)

Relative tumor volume of HCT116
(mean TV at day0 = 0.21cm^3=1)

Days after 1st administration

Control MK2206
MEKi MEKi + MK-2206

Body weight change

Relative body weight change (BW at day1 = 32.4g = 1)

control MEKi 50 mpd, bid
MK-2206 120 mpd MEKi+ MK-2206

FIG. 4
MK-2206 enhances anti-tumor efficacy of AZD-6244 in Xenograft Mouse Model (NCI-H1975)

**Survival (N=15)**

- Vehicle 1 + Vehicle 2
- AZD6244 25mpk
- MK-2206 240mpk qod
- MK-2206 480mpk qw
- MK-2206 240mpk qod + AZD6244 25mpk
- MK-2206 480mpk qw + AZD6244 25mpk

**Pharmacodynamic Markers (N=5)**
- Day 22, 12.5 hours post-dose

- pAKT
- pERK

![Graph showing survival and pharmacodynamic markers](image-url)
MK-2206 enhances anti-tumor efficacy of AZD-6244 in Xenograft Mouse Model (NCI-H1975)

**Tumor Efficacy (N=15)**

- Vehicle 1 + Vehicle 2
- AZD6244 25mpk
- MK-2206 240mpk qod
- MK-2206 480mpk qw
- MK-2206 240mpk qod + AZD6244 25mpk
- MK-2206 480mpk qw + AZD6244 25mpk

**Body weight change**

- Vehicle 1 + Vehicle 2
- AZD6244 25mpk
- MK-2206 240mpk qod
- MK-2206 480mpk qw
- MK-2206 240mpk qod + AZD6244
- MK-2206 480mpk qw + AZD6244

**FIG. 5-1**
MK-2206 enhances anti-tumor efficacy of AZD-6244 in vivo (AsPC1)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Tumour growth inhibition (%)</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle control</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>AZD6244 25 mg/kg qd</td>
<td>31.8</td>
<td>P=0.076</td>
</tr>
<tr>
<td>MK-2206 240 mg/kg qod</td>
<td>22.8</td>
<td>P=0.166</td>
</tr>
<tr>
<td>AZD6244 25 mg/kg qd MK-2206 240 mg/kg qod</td>
<td>69.1</td>
<td>P=0.0007</td>
</tr>
</tbody>
</table>

FIG. 6
INTERNATIONAL SEARCH REPORT

International application No
PCT/US 10/35562

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 39/395 (2010.01)
USPC - 424/155.1

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
USPC - 424/155.1 (see search terms below)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC - 514/210.21; 514/265.1; 514/27; 514/34; 514/109 (see search terms below)

Electronic data base consulted during the international search (name of database and, where practicable, search terms used)
USPTO-WEST - PGPB,USPT,USOC,EPAB,JPAB keywords: combination cancer therapy, MEK inhibitor, ARRY-142886, AZD6244, AKT inhibitors, treating cancer, method, administering, patient, lung cancer, pancreatic, colorectal, composition, carrier, manufacturing, medicament, simultaneously, sequentially, MK-2206, allosteric Akt inhibitor. INTERNET search - Goo

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category* Citation of document, with indication, where appropriate, of the relevant passages Relevant to claim No


Date of the actual completion of the international search 29 June 2010 (29.06.2010)

Date of mailing of the international search report

19 JUL 2010

Name and mailing address of the ISA/US
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P.O. Box 1450, Alexandria, Virginia 22313-1450

Facsimile No. 571-273-3201

Form PCT/ISA7210 (second sheet) (July 2009)