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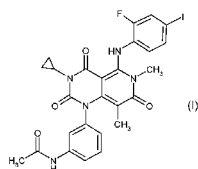
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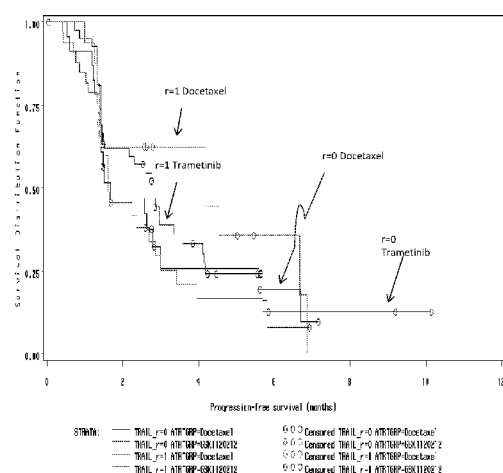
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(54) Title: METHODS OF TREATING CANCER



(57) Abstract: Methods are provided for treating cancer in a mam-
mal, such as a human, having cancer comprising determining if the
mammal has an elevated level of TRAIL and, if so, administering a
pharmaceutical composition comprising at least one MEK inhibitor
comprising a compound of Structure (I), or a pharmaceutically ac-
ceptable salt or solvate thereof.

Figure 8: TRAIL PFS



r=1, > median

**Declarations under Rule 4.17:**

- *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*
- *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*
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METHODS OF TREATING CANCER

FIELD OF THE INVENTION

The present invention relates to a method of treating cancer in a mammal. In particular, the method relates to methods comprising treating a human having an increased level of TRAIL comprising administering a MEK inhibitor. In particular, the MEK inhibitor is: N-{3-[3-cyclopropyl-5-(2-fluoro-4-iodo-phenylamino)6,8-dimethyl;-2,4,7-trioxo-3,4,6,7-tetrahydro-2H-pyrido[4,3-d]pyrimidin-1-yl]phenyl}acetamide, or a pharmaceutically acceptable salt or solvate thereof to said human.

BACKGROUND OF THE INVENTION

Effective treatment of hyperproliferative disorders, including cancer, is a continuing goal and unmet medical need in the oncology field. Generally, cancer results from the deregulation of the normal processes that control cell growth, cell division, differentiation and apoptotic cell death, among others. One such process involves kinase regulation of apoptosis and cellular signaling from growth factor receptors at the cell surface to the nucleus (Crews and Erikson, Cell, 74:215-17, 1993).

A large family of enzymes is the protein kinase enzyme family. There are about 500 different known protein kinases. Protein kinases serve to catalyze the phosphorylation of an amino acid side chain in various proteins by the transfer of the γ -phosphate of the ATP-Mg²⁺ complex to said amino acid side chain. These enzymes appear to control the majority of the signaling processes inside cells, thereby governing cell function, growth, differentiation and apoptosis through reversible phosphorylation of the hydroxyl groups of serine, threonine and tyrosine residues in proteins. Studies have shown that protein kinases regulate many cell functions, including signal transduction, transcriptional regulation, cell motility, and cell division. Several oncogenes have also been shown to encode protein kinases, suggesting that kinases play a role in oncogenesis.

For example, activation of Raf-MEK-ERK signal transduction pathway in cancer, particularly colorectal cancer, pancreatic cancer, lung cancer, breast cancer and the like, has been observed.

The ras family of oncogenes (K-ras, H-ras, and N-ras) encode for membrane proteins possessing GTPase activity. These proteins are involved in cellular signal transduction. Specific point mutations, usually within the ras codons 12, 13, or 61, can result in the activation of these protooncogenes and result in subsequent neoplasia (Bos, J. L., 1989, Can. Res. 49:4682-4689). The frequency with which ras mutations occur varies among different tumor types, although not all have been tested. Studies indicate that approximately 40-50% of colon cancers exhibit a mutation in the c-K-ras gene, with 86% of these mutations

occurring at codons 12 and 13 (Bos, J. L. et al., 1987, Nature 327: (6120)293-7, Vogelstein B. et al., 1988, N. Engl. J. Med. 319:525-532). Ras mutations result in increased cell proliferation due to decreased intrinsic GTP-ase activity of the Ras protein.

It would be useful to provide novel methods of treatment for an individual with cancer having at least one Ras protein mutation.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a schematic of the MEK114653 NSCLC Study, an open-label, multicenter phase 2 study in patients with KRAS- and non-KRAS-mutant NSCLC, randomized 2:1 to treatment with trametinib or docetaxel.

Figure 2 is a Kaplan-Meier survival plot (K-M plot) using median split of TRAIL marker, to demonstrate the potential prognostic effect on OS in the trametinib arm of the MEK114653 NSCLC Study.

Figure 3 is a K-M plot using median split of TRAIL marker, to demonstrate the potential prognostic effect on OS in the docetaxel arm of the MEK114653 NSCLC Study.

Figure 4 is a K-M plot using median split of TRAIL marker in the different arms in the NSCLC study (Trametinib vs Docetaxel).

Figure 5 is a K-M plot using the medial split of TRAIL marker in the MEK113487 study in the Trametinib+Gemcitabine treatment arm.

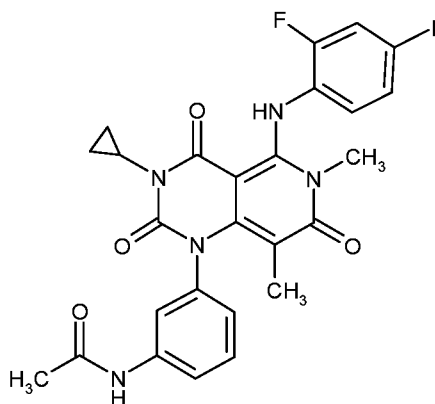
Figure 6 is a K-M plot using the medial split of TRAIL marker in the MEK113487 study in the Gemcitabine treatment arm.

Figure 7 is a K-M plot using median split of TRAIL marker in the different arms of the MEK113487 study in pancreatic cancer (Trametinib+Gemcitabine vs Gemcitabine).

Figure 8 is a K-M plot using median split of TRAIL marker in the different arms in the NSCLC study to demonstrate the potential prognostic effect on PFS.

SUMMARY OF THE INVENTION

In one embodiment of the present invention methods are provided for treating a mammal having cancer comprising identifying a mammal with increased blood levels of TRAIL and treating said mammal with a pharmaceutical composition comprising at least one MEK inhibitor comprising a compound of Structure (I):

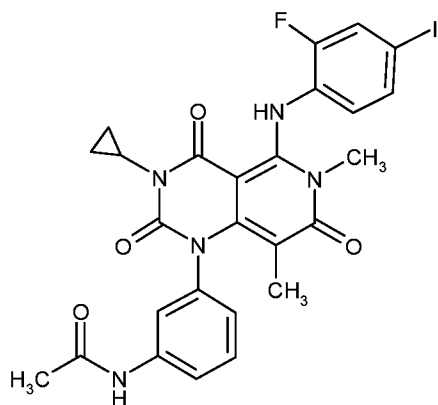


(1);

or a pharmaceutically acceptable salt or solvate thereof. In one embodiment, the mammal is human.

DETAILED DESCRIPTION OF THE INVENTION

In one embodiment of the present invention methods are provided for treating a mammal having cancer comprising identifying a mammal with increased blood levels of TRAIL and administering to said identified mammal a pharmaceutical composition comprising at least one MEK inhibitor comprising a compound of Structure (I):



(1);

or a pharmaceutically acceptable salt or solvate or thereof. In one aspect the mammal is human.

The cancer may be any cancer. In suitable embodiments of the invention, the cancer is selected from the group consisting of melanoma, pancreatic cancer, colorectal cancer, and non-small cell lung carcinoma. In further suitable embodiments of the invention, the cancer is selected from the group consisting of non-small cell lung carcinoma and pancreatic cancer. In further suitable embodiments of the invention, the cancer is non-small cell lung carcinoma. In additional and further suitable embodiments of the invention, the cancer is pancreatic cancer.

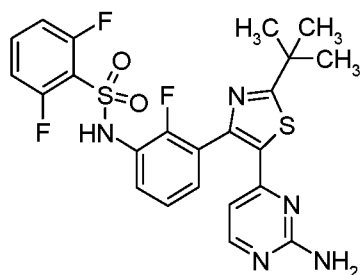
In some embodiments of the invention, the cancer is one in which an abnormal number of blast cells are present or that is diagnosed as a haematological cancer or dysplasia, such as leukemia, myeloid malignancy or myeloid dysplasia, including but not limited to, undifferentiated acute myelogenous leukemia, myeloblastic leukemia, myeloblastic leukemia, promyelocytic leukemia, myelomonocytic leukemia, monocytic leukemia, erythroleukemia and megakaryoblastic leukemia. In one aspect, the cancer is a myeloid malignancy cancer. In another aspect, the cancer is leukemia. The leukemia may be acute lymphocytic leukemia, acute non-lymphocytic leukemia, acute myeloid leukemia (AML), chronic lymphocytic leukemia, chronic myelogenous (or myeloid) leukemia (CML), and chronic myelomonocytic leukemia (CMML). In one embodiment, the human has agnogenic myeloid metaplasia and/or poor-risk myelodysplasia (MDS). In some aspects the cancer is relapsed or refractory. Patients may have received one or more treatments for leukemia prior to receiving Structure I.

In another embodiment, Structure (I), also referred to as N-{3-[3-cyclopropyl-5-(2-fluoro-4-iodo-phenylamino)-6,8-dimethyl-2,4,7-trioxo-3,4,6,7-tetrahydro-2H-pyrido[4,3-d]pyrimidin-1-yl]phenyl}acetamide, or a pharmaceutically acceptable salt or solvate thereof (hereinafter Compound A, or a pharmaceutically acceptable salt or solvate thereof) is in a sodium salt form. In another aspect, Compound A is in the form of a dimethyl sulfoxide solvate.

N-{3-[3-cyclopropyl-5-(2-fluoro-4-iodo-phenylamino)-6,8-dimethyl-2,4,7-trioxo-3,4,6,7-tetrahydro-2H-pyrido[4,3-d]pyrimidin-1-yl]phenyl}acetamide, or a pharmaceutically acceptable salt or solvate is a highly selective allosteric inhibitor of mitogen activated extracellular signal-regulated kinase 1 (MEK 1) and MEK 2. MEK proteins are a node in a certain extracellular signal-related kinase ERK pathway which is commonly hyper-activated in tumor cells. Oncogenic mutations in both B-raf and Ras signal through MEK1 and MEK2. *In vitro*, 80% of cell lines carrying activating mutations of B-Raf and 72% of Ras mutant cell lines were sensitive to N-{3-[3-cyclopropyl-5-(2-fluoro-4-iodo-phenylamino)-6,8-dimethyl-2,4,7-trioxo-3,4,6,7-tetrahydro-2H-pyrido[4,3-d]pyrimidin-1-yl]phenyl}acetamide, or a

pharmaceutically acceptable salt or solvate in cell proliferation assays, and a majority (83% of hematopoietic cancers from acute or chronic myeloid leukemia (AML or CML, respectively) origins were also very sensitive.

In another embodiment, the present invention provides methods for treating cancer comprising administering at least one Braf inhibitor with Compound A, or a pharmaceutically acceptable salt or solvate thereof. In one aspect, the Braf inhibitor is Structure (II):



(II)

or a pharmaceutically acceptable salt thereof, also referred to as *N,N*-{3-[5-(2-Amino-4-pyrimidinyl)-2-(1,1-dimethylethyl)-1,3-thiazol-4-yl]-2-fluorophenyl}-2,6-difluorobenzenesulfonamide methanesulfonate or a pharmaceutically acceptable salt thereof, (hereinafter Compound B or a pharmaceutically acceptable salt thereof).

In another aspect the mammal also has a Braf mutation. In some instances, the Braf mutation is selected from R462I, I463S, G464V, G464E, G466A, G466E, G466V, G469A, G469E, D594V, F595L, G596R, L597V, L597R, T599I, V600E, V600D, V600K, V600R, T119S, and K601E. In some instances, the BRAF mutation is detected in the same tumor cell and/or the same type of tumor cell as TRAIL expression. In other instances, the BRAF mutation is detected in a different sample from TRAIL expression; for example, the BRAF mutation is detected in a tumor cell whereas TRAIL expression is determined in a blood or plasma sample.

In another embodiment, the amount of Structure I or a pharmaceutically acceptable salt or solvate thereof administered to said human is an amount selected from 0.125mg to 10mg. In some aspects the amount of Structure I or a pharmaceutically acceptable salt or solvate thereof administered to said human is administered daily from about 1 mg/day to about 2 mg/day. The amount of BRAF inhibitor is an amount selected from 75mg to 1,000mg. In some aspects the pharmaceutical composition comprises *N,N*-{3-[5-(2-Amino-4-pyrimidinyl)-2-(1,1-dimethylethyl)-1,3-thiazol-4-yl]-2-fluorophenyl}-2,6-difluorobenzenesulfonamide methanesulfonate or a pharmaceutically acceptable salt thereof, (hereinafter Compound B or a pharmaceutically acceptable salt thereof) in an amount from about 75mg to about 1,000mg. In some aspects, the pharmaceutical composition comprising Structure I or a pharmaceutically acceptable salt or solvate thereof and the pharmaceutical composition comprising at least one Braf inhibitor are administered

separately. In another aspect, the pharmaceutical composition comprising Structure I or a pharmaceutically acceptable salt or solvate thereof is administered at the same time as the pharmaceutical composition comprising Structure II or a pharmaceutically acceptable salt or solvate thereof.

Compound A is disclosed and claimed, along with pharmaceutically acceptable salts and solvates thereof, as being useful as an inhibitor of MEK activity, particularly in treatment of cancer, in International Application No. PCT/JP2005/011082, having an International filing date of June 10, 2005; International Publication Number WO 2005/121142 and an International Publication date of December 22, 2005, the entire disclosure of which is hereby incorporated by reference, Compound A is the compound of Example 4-1. Compound A can be prepared as described in International Application No. PCT/JP2005/011082. Compound A can be prepared as described in United States Patent Publication No. US 2006/0014768, Published January 19, 2006, the entire disclosure of which is hereby incorporated by reference.

Suitably, Compound A is in the form of a dimethyl sulfoxide solvate. Suitably, Compound A is in the form of a sodium salt. Suitably, Compound A is in the form of a solvate selected from: hydrate, acetic acid, ethanol, nitromethane, chlorobenzene, 1-pentanol, isopropyl alcohol, ethylene glycol and 3-methyl-1-butanol. These solvates and salt forms can be prepared by one of skill in the art from, for example, the description in International Application No. PCT/JP2005/011082 or United States Patent Publication No. US 2006/0014768.

Compound B is disclosed and claimed, along with pharmaceutically acceptable salts thereof, as being useful as an inhibitor of BRAF activity, particularly in the treatment of cancer, in PCT patent application PCT/US09/42682. Compound B is embodied therein by Examples 58a through 58e of the application. This PCT application was published on 12 November 2009 as publication WO2009/137391, and is hereby incorporated by reference.

The compounds of the invention may contain one or more chiral atoms, or may otherwise be capable of existing as two enantiomers. Accordingly, the compounds of this invention include mixtures of enantiomers as well as purified enantiomers or enantiomerically enriched mixtures. Also, it is understood that all tautomers and mixtures of tautomers are included within the scope of Compound A, and pharmaceutically acceptable salts thereof, and Compound B, and pharmaceutically acceptable salts thereof.

The compounds of the invention may form a solvate which is understood to be a complex of variable stoichiometry formed by a solute (in this invention, Compound A or a salt thereof and/or Compound B or a salt thereof) and a solvent. Such solvents for the purpose of the invention may not interfere with the biological activity of the solute. Examples of suitable solvents include, but are not limited to, water, methanol, dimethyl sulfoxide, ethanol

and acetic acid. Suitably the solvent used is a pharmaceutically acceptable solvent. Examples of suitable pharmaceutically acceptable solvents include, without limitation, water, dimethyl sulfoxide, ethanol and acetic acid. Suitably the solvent used is water.

The pharmaceutically acceptable salts of the compounds of the invention are readily prepared by those of skill in the art.

Also, contemplated herein is a method of treating cancer, for example in certain patient populations having certain biomarkers, for example elevated circulating TRAIL, using compound A, or a pharmaceutically acceptable salt or solvate thereof, or using a combination of the invention where Compound A, or a pharmaceutically acceptable salt or solvate thereof and/or Compound B or a pharmaceutically acceptable salt thereof. Further embodiments are methods of treating cancer, for example in certain patient populations having certain biomarkers, for example elevated circulating TRAIL, where Compound A or a pharmaceutically acceptable salt or solvate thereof, and/or Compound B or a pharmaceutically acceptable salt thereof, or both, are administered as pro-drugs. Pharmaceutically acceptable pro-drugs of the compounds of the invention are readily prepared by those of skill in the art.

The term "treating" and grammatical variations thereof as used herein, refers to therapeutic therapy. In reference to a particular condition, treating means: (1) to ameliorate or prevent the condition of one or more of the biological manifestations of the condition, (2) to interfere with (a) one or more points in the biological cascade that leads to or is responsible for the condition or (b) one or more of the biological manifestations of the condition, (3) to alleviate one or more of the symptoms, effects or side effects associated with the condition or treatment thereof, or (4) to slow the progression of the condition or one or more of the biological manifestations of the condition.

For example, treating and/or treatment can be measured, quantified, or otherwise determined in a number of ways known to one of skill in the art, including RECIST (Response Evaluation Criteria in Solid Tumors) criteria version 1.0 and/or RECIST criteria version 1.1. RECIST criteria version 1.1 is described in Eisenhauer, E.A. et al., "New Response Evaluation Criteria in Solid Tumours: Revised RECIST guideline," EUROPEAN JOURNAL OF CANCER (2009), 45, 228- 247, which is incorporated by reference in its entirety herein. Methods of evaluating non-solid cancers of the blood and specific deviations from RECIST for certain cancers such as mesothelioma are also well known in the art.

For example, treating can be measured, quantified, or otherwise determined by a change in overall survival (OS), progression free survival (PFS), or both, in patients that were given a composition comprising Compound A or B or both relative to untreated patients.

"Prevention" is understood to refer to the prophylactic administration of a drug to substantially diminish the likelihood or severity of a condition or biological manifestation thereof, or to delay the onset of such condition or biological manifestation thereof. Prophylactic therapy is appropriate, for example, when a subject is considered at high risk for developing cancer, such as when a subject has a strong family history of cancer or when a subject has been exposed to a carcinogen. The skilled artisan will appreciate that "prevention" is not an absolute term.

By the term "combination" and grammatical variations thereof, as used herein is meant either simultaneous administration or any manner of separate sequential administration of a therapeutically effective amount of Compound A, or a pharmaceutically acceptable salt or solvate thereof, and Compound B or a pharmaceutically acceptable salt thereof. Preferably, if the administration is not simultaneous, the compounds are administered in a close time proximity to each other. Furthermore, it does not matter if the compounds are administered in the same dosage form, e.g. one compound may be administered topically and the other compound may be administered orally. Suitably, both compounds are administered orally.

By the term "combination kit" as used herein is meant the pharmaceutical composition or compositions that are used to administer Compound A, or a pharmaceutically acceptable salt or solvate thereof, and Compound B, or a pharmaceutically acceptable salt thereof, according to the invention. When both compounds are administered simultaneously, the combination kit can contain Compound A, or a pharmaceutically acceptable salt or solvate thereof, and Compound B, or a pharmaceutically acceptable salt thereof, in a single pharmaceutical composition, such as a tablet, or in separate pharmaceutical compositions. When the compounds are not administered simultaneously, the combination kit will contain Compound A, or a pharmaceutically acceptable salt or solvate thereof, and Compound B, or a pharmaceutically acceptable salt thereof, in separate pharmaceutical compositions. The combination kit can comprise Compound A, or a pharmaceutically acceptable salt or solvate thereof, and Compound B, or a pharmaceutically acceptable salt thereof, in separate pharmaceutical compositions in a single package or in separate pharmaceutical compositions in separate packages.

In one aspect there is provided a combination kit comprising the components:

Compound A, or a pharmaceutically acceptable salt or solvate thereof, in association with a pharmaceutically acceptable carrier; and

Compound B, or a pharmaceutically acceptable salt thereof, in association with a pharmaceutically acceptable carrier.

In one embodiment of the invention the combination kit comprises the following components:

Compound A, or a pharmaceutically acceptable salt or solvate thereof, in association with a pharmaceutically acceptable carrier; and

Compound B, or a pharmaceutically acceptable salt thereof, in association with a pharmaceutically acceptable carrier,

wherein the components are provided in a form which is suitable for sequential, separate and/or simultaneous administration.

In one embodiment the combination kit comprises:

a first container comprising Compound A, or a pharmaceutically acceptable salt or solvate thereof, in association with a pharmaceutically acceptable carrier; and

a second container comprising Compound B, or a pharmaceutically acceptable salt thereof, in association with a pharmaceutically acceptable carrier, and a container means for containing said first and second containers.

The "combination kit" can also be provided by instruction, such as dosage and administration instructions. Such dosage and administration instructions can be of the kind that is provided to a doctor, for example by a drug product label, or they can be of the kind that is provided by a doctor, such as instructions to a patient.

As used herein the term "Compound A²" means ---Compound A, or a pharmaceutically acceptable salt or solvate thereof---.

As used herein the term "Compound B²" means ---Compound B, or a pharmaceutically acceptable salt thereof---.

Suitably the combinations of this invention are administered within a "specified period".

By the term "specified period" and grammatical variations thereof, as used herein is meant the interval of time between the administration of one of Compound A² and Compound B² and the other of Compound A² and Compound B². Unless otherwise defined, the specified period can include simultaneous administration. Unless otherwise defined the specified period refers to administration of Compound A² and Compound B² during a single day.

Suitably, if the compounds are administered within a "specified period" and not administered simultaneously, they are both administered within about 24 hours of each other – in this case, the specified period will be about 24 hours; suitably they will both be administered within about 12 hours of each other – in this case, the specified period will be about 12 hours; suitably they will both be administered within about 11 hours of each other – in this case, the specified period will be about 11 hours; suitably they will both be administered within about 10 hours of each other – in this case, the specified period will be about 10 hours; suitably they will both be administered within about 9 hours of each other – in this case, the specified period will be about 9 hours; suitably they will both be

administered within about 8 hours of each other – in this case, the specified period will be about 8 hours; suitably they will both be administered within about 7 hours of each other – in this case, the specified period will be about 7 hours; suitably they will both be administered within about 6 hours of each other – in this case, the specified period will be about 6 hours; suitably they will both be administered within about 5 hours of each other – in this case, the specified period will be about 5 hours; suitably they will both be administered within about 4 hours of each other – in this case, the specified period will be about 4 hours; suitably they will both be administered within about 3 hours of each other – in this case, the specified period will be about 3 hours; suitably they will be administered within about 2 hours of each other – in this case, the specified period will be about 2 hours; suitably they will both be administered within about 1 hour of each other – in this case, the specified period will be about 1 hour. As used herein, the administration of Compound A² and Compound B² in less than about 45 minutes apart is considered simultaneous administration.

Suitably, when the combination of the invention is administered for a “specified period”, the compounds will be co-administered for a “duration of time”.

By the term “duration of time” and grammatical variations thereof, as used herein is meant that both compounds of the invention are administered for an indicated number of consecutive days. Unless otherwise defined, the number of consecutive days does not have to commence with the start of treatment or terminate with the end of treatment, it is only required that the number of consecutive days occur at some point during the course of treatment.

Regarding “specified period” administration:

Suitably, both compounds will be administered within a specified period for at least one day – in this case, the duration of time will be at least one day; suitably, during the course to treatment, both compounds will be administered within a specified period for at least 3 consecutive days – in this case, the duration of time will be at least 3 days; suitably, during the course to treatment, both compounds will be administered within a specified period for at least 5 consecutive days – in this case, the duration of time will be at least 5 days; suitably, during the course to treatment, both compounds will be administered within a specified period for at least 7 consecutive days – in this case, the duration of time will be at least 7 days; suitably, during the course to treatment, both compounds will be administered within a specified period for at least 14 consecutive days – in this case, the duration of time will be at least 14 days; suitably, during the course to treatment, both compounds will be administered within a specified period for at least 30 consecutive days – in this case, the duration of time will be at least 30 days.

Suitably, if the compounds are not administered during a “specified period”, they are administered sequentially. By the term “sequential administration”, and derivatives thereof, as

used herein is meant that one of Compound A² and Compound B² is administered once a day for two or more consecutive days and the other of Compound A² and Compound B² is subsequently administered once a day for two or more consecutive days. Also, contemplated herein is a drug holiday utilized between the sequential administration of one of Compound A² and Compound B² and the other of Compound A² and Compound B². As used herein, a drug holiday is a period of days after the sequential administration of one of Compound A² and Compound B² and before the administration of the other of Compound A² and Compound B² where neither Compound A² nor Compound B² is administered. Suitably the drug holiday will be a period of days selected from: 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 11 days, 12 days, 13 days and 14 days. Regarding sequential administration:

Suitably, one of Compound A² and Compound B² is administered for from 2 to 30 consecutive days, followed by an optional drug holiday, followed by administration of the other of Compound A² and Compound B² for from 2 to 30 consecutive days. Suitably, one of Compound A² and Compound B² is administered for from 2 to 21 consecutive days, followed by an optional drug holiday, followed by administration of the other of Compound A² and Compound B² for from 2 to 21 consecutive days. Suitably, one of Compound A² and Compound B² is administered for from 2 to 14 consecutive days, followed by a drug holiday of from 1 to 14 days, followed by administration of the other of Compound A² and Compound B² for from 2 to 14 consecutive days. Suitably, one of Compound A² and Compound B² is administered for from 3 to 7 consecutive days, followed by a drug holiday of from 3 to 10 days, followed by administration of the other of Compound A² and Compound B² for from 3 to 7 consecutive days.

Suitably, Compound B² will be administered first in the sequence, followed by an optional drug holiday, followed by administration of Compound A². Suitably, Compound B² is administered for from 3 to 21 consecutive days, followed by an optional drug holiday, followed by administration of Compound A² for from 3 to 21 consecutive days. Suitably, Compound B² is administered for from 3 to 21 consecutive days, followed by a drug holiday of from 1 to 14 days, followed by administration of Compound A² for from 3 to 21 consecutive days. Suitably, Compound B² is administered for from 3 to 21 consecutive days, followed by a drug holiday of from 3 to 14 days, followed by administration of Compound A² for from 3 to 21 consecutive days. Suitably, Compound B² is administered for 21 consecutive days, followed by an optional drug holiday, followed by administration of Compound A² for 14 consecutive days. Suitably, Compound B² is administered for 14 consecutive days, followed by a drug holiday of from 1 to 14 days, followed by administration of Compound A² for 14 consecutive days. Suitably, Compound B² is administered for 7 consecutive days, followed by a drug holiday of from 3 to 10 days, followed by administration

of Compound A² for 7 consecutive days. Suitably, Compound B² is administered for 3 consecutive days, followed by a drug holiday of from 3 to 14 days, followed by administration of Compound A² for 7 consecutive days. Suitably, Compound B² is administered for 3 consecutive days, followed by a drug holiday of from 3 to 10 days, followed by administration of Compound A² for 3 consecutive days.

It is understood that a “specified period” administration and a “sequential” administration can be followed by repeat dosing or can be followed by an alternate dosing protocol, and a drug holiday may precede the repeat dosing or alternate dosing protocol.

Suitably, the amount of Compound A² administered as part of the combination according to the present invention will be an amount selected from about 0.125mg to about 10mg; suitably, the amount will be selected from about 0.25mg to about 9mg; suitably, the amount will be selected from about 0.25mg to about 8mg; suitably, the amount will be selected from about 0.5mg to about 8mg; suitably, the amount will be selected from about 0.5mg to about 7mg; suitably, the amount will be selected from about 1mg to about 7mg; suitably, the amount will be about 5mg. Accordingly, the amount of Compound A administered as part of the combination according to the present invention will be an amount selected from about 0.125mg to about 10 mg. For example, the amount of Compound A² administered as part of the combination according to the present invention can be 0.125mg, 0.25mg, 0.5mg, 0.75mg, 1mg, 1.5mg, 2mg, 2.5mg, 3mg, 3.5mg, 4mg, 4.5mg, 5mg, 5.5mg, 6mg, 6.5mg, 7mg, 7.5mg, 8mg, 8.5mg, 9mg, 9.5mg, 10mg.

Suitably, the amount of Compound B² administered as part of the combination according to the present invention will be an amount selected from about 75mg to about 1,000mg; suitably, the amount will be selected from about 100mg to about 900mg; suitably, the amount will be selected from about 150mg to about 850mg; suitably, the amount will be selected from about 200mg to about 800mg; suitably, the amount will be selected from about 250mg to about 750mg; suitably, the amount will be selected from about 300mg to about 600mg; suitably, the amount will be about 450mg. Accordingly, the amount of Compound B² administered as part of the combination according to the present invention will be an amount selected from about 75mg to about 1,000mg. For example, the amount of Compound B² administered as part of the combination according to the present invention can be 75mg, 100 mg, 125mg, 150 mg, 175mg, 200mg, 225mg, 250mg, 275mg, 300mg, 325mg, 350mg, 375mg, 400mg, 425mg, 450mg, 475mg, 500mg, 525mg, 550mg, 575mg, 600mg, 625mg, 650mg, 675mg, 700mg, 725mg, 750mg, 775mg, 800mg, 825mg, 850mg, 875mg, 900mg, 925mg, 950mg, 975mg or 1,000mg.

As used herein, all amounts specified for Compound A² and Compound B² are indicated as the administered amount of free or unsalted and unsolvated compound per dose.

The method of the present invention may also be employed with other therapeutic methods of cancer treatment.

While it is possible that, for use in therapy, therapeutically effective amounts of the combinations of the present invention may be administered as the raw chemical, it is preferable to present the combinations as a pharmaceutical composition or compositions. Accordingly, the invention further provides pharmaceutical compositions, which include Compound A² and/or Compound B², and one or more pharmaceutically acceptable carriers. The combinations of the present invention are as described above. The carrier(s) must be acceptable in the sense of being compatible with the other ingredients of the formulation, capable of pharmaceutical formulation, and not deleterious to the recipient thereof. In accordance with another aspect of the invention there is also provided a process for the preparation of a pharmaceutical formulation including admixing Compound A² and/or Compound B² with one or more pharmaceutically acceptable carriers. As indicated above, such elements of the pharmaceutical combination utilized may be presented in separate pharmaceutical compositions or formulated together in one pharmaceutical formulation.

Pharmaceutical formulations may be presented in unit dose forms containing a predetermined amount of active ingredient per unit dose. As is known to those skilled in the art, the amount of active ingredient per dose will depend on the condition being treated, the route of administration and the age, weight and condition of the patient. Preferred unit dosage formulations are those containing a daily dose or sub-dose, or an appropriate fraction thereof, of an active ingredient. Furthermore, such pharmaceutical formulations may be prepared by any of the methods well known in the pharmacy art.

Compound A² and Compound B² may be administered by any appropriate route. Suitable routes include oral, rectal, nasal, topical (including buccal and sublingual), vaginal, and parenteral (including subcutaneous, intramuscular, intravenous, intradermal, intrathecal, and epidural). It will be appreciated that the preferred route may vary with, for example, the condition of the recipient of the combination and the cancer to be treated. It will also be appreciated that each of the agents administered may be administered by the same or different routes and that Compound A² and Compound B² may be compounded together in a pharmaceutical composition/formulation.

The compounds or combinations of the current invention are incorporated into convenient dosage forms such as capsules, tablets, or injectable preparations. Solid or liquid pharmaceutical carriers are employed. Solid carriers include, starch, lactose, calcium sulfate dihydrate, terra alba, sucrose, talc, gelatin, agar, pectin, acacia, magnesium stearate, and stearic acid. Liquid carriers include syrup, peanut oil, olive oil, saline, and water. Similarly, the carrier may include a prolonged release material, such as glyceryl monostearate or glyceryl distearate, alone or with a wax. The amount of solid carrier varies

widely but, preferably, will be from about 25 mg to about 1 g per dosage unit. When a liquid carrier is used, the preparation will suitably be in the form of a syrup, elixir, emulsion, soft gelatin capsule, sterile injectable liquid such as an ampoule, or an aqueous or nonaqueous liquid suspension.

For instance, for oral administration in the form of a tablet or capsule, the active drug component can be combined with an oral, non-toxic pharmaceutically acceptable inert carrier such as ethanol, glycerol, water and the like. Powders are prepared by comminuting the compound to a suitable fine size and mixing with a similarly comminuted pharmaceutical carrier such as an edible carbohydrate, as, for example, starch or mannitol. Flavoring, preservative, dispersing and coloring agent can also be present.

It should be understood that in addition to the ingredients mentioned above, the formulations may include other agents conventional in the art having regard to the type of formulation in question, for example those suitable for oral administration may include flavoring agents.

As indicated, therapeutically effective amounts of the combinations of the invention (Compound A² in combination with Compound B²) are administered to a human. Typically, the therapeutically effective amount of the administered agents of the present invention will depend upon a number of factors including, for example, the age and weight of the subject, the precise condition requiring treatment, the severity of the condition, the nature of the formulation, and the route of administration. Ultimately, the therapeutically effective amount will be at the discretion of the attendant physician.

The combinations of the present invention are tested for efficacy, advantageous and synergistic properties according to known procedures. Suitably, the combinations of the invention are tested for efficacy, advantageous and synergistic properties generally according to the following combination cell proliferation assays. Cells are plated in 384-well plates at 500 cells/well in culture media appropriate for each cell type, supplemented with 10% FBS and 1% penicillin/streptomycin, and incubated overnight at 37°C, 5% CO₂. Cells are treated in a grid manner with dilution of Compound A² (20 dilutions, including no compound, of 2-fold dilutions starting from 1-20 µM depending of compound) from left to right on 384-well plate and also treated with Compound B² (20 dilutions, including no compound, of 2-fold dilutions starting from 1-20 µM depending of compound) from top to bottom on 384-well plate and incubated as above for a further 72 hours. In some instances compounds are added in a staggered manner and incubation time can be extended up to 7 days. Cell growth is measured using CellTiter-Glo® reagent according to the manufacturer's protocol and signals are read on a PerkinElmer EnVision™ reader set for luminescence mode with a 0.5-second read. Data are analyzed as described below.

Results are expressed as a percentage of the $t=0$ value and plotted against compound(s) concentration. The $t=0$ value is normalized to 100% and represents the number of cells present at the time of compound addition. The cellular response is determined for each compound and/or compound combination using a 4- or 6-parameter curve fit of cell viability against concentration using the IDBS XLfit plug-in for Microsoft Excel software and determining the concentration required for 50% inhibition of cell growth (IC_{50}). Background correction is made by subtraction of values from wells containing no cells. For each drug combination a Combination Index (CI), Excess Over Highest Single Agent (EOHSA) and Excess Over Bliss (EOBliss) are calculated according to known methods such as described in Chou and Talalay (1984) *Advances in Enzyme Regulation*, 22, 37 to 55; and Berenbaum, MC (1981) *Adv. Cancer Research*, 35, 269-335.

Because each of the inhibitors and combinations thereof are active in the above assays they exhibit advantageous therapeutic utility in treating cancer.

Examples of cancers that are suitable for treatment with the MEK inhibitor, the BRAF inhibitor, or both include, but are not limited to, both primary and metastatic forms of head and neck, breast, lung, colon, ovary, and prostate cancers. Suitably the cancer is selected from: brain (gliomas), glioblastomas, astrocytomas, glioblastoma multiforme, Bannayan-Zonana syndrome, Cowden disease, Lhermitte-Duclos disease, breast, inflammatory breast cancer, Wilm's tumor, Ewing's sarcoma, Rhabdomyosarcoma, ependymoma, medulloblastoma, colon, head and neck, kidney, lung, liver, melanoma, ovarian, pancreatic, prostate, sarcoma, osteosarcoma, giant cell tumor of bone, thyroid, lymphoblastic T cell leukemia, Chronic myelogenous leukemia, Chronic lymphocytic leukemia, Hairy-cell leukemia, acute lymphoblastic leukemia, acute myelogenous leukemia, AML, Chronic neutrophilic leukemia, Acute lymphoblastic T cell leukemia, plasmacytoma, Immunoblastic large cell leukemia, Mantle cell leukemia, Multiple myeloma Megakaryoblastic leukemia, multiple myeloma, acute megakaryocytic leukemia, promyelocytic leukemia, Erythroleukemia, malignant lymphoma, hodgkins lymphoma, non-hodgkins lymphoma, lymphoblastic T cell lymphoma, Burkitt's lymphoma, follicular lymphoma, neuroblastoma, bladder cancer, urothelial cancer, lung cancer, vulval cancer, cervical cancer, endometrial cancer, renal cancer, mesothelioma, esophageal cancer, salivary gland cancer, hepatocellular cancer, gastric cancer, nasopharyngeal cancer, buccal cancer, cancer of the mouth, GIST (gastrointestinal stromal tumor) and testicular cancer.

Additionally, examples of a cancer to be treated include Barret's adenocarcinoma; billiary tract carcinomas; breast cancer; cervical cancer; cholangiocarcinoma; central nervous system tumors including primary CNS tumors such as glioblastomas, astrocytomas (e.g., glioblastoma multiforme) and ependymomas, and secondary CNS tumors (i.e., metastases to the central nervous system of tumors originating outside of the central

nervous system); colorectal cancer including large intestinal colon carcinoma; gastric cancer; carcinoma of the head and neck including squamous cell carcinoma of the head and neck; hematologic cancers including leukemias and lymphomas such as acute lymphoblastic leukemia, acute myelogenous leukemia (AML), myelodysplastic syndromes, chronic myelogenous leukemia, Hodgkin's lymphoma, non-Hodgkin's lymphoma, megakaryoblastic leukemia, multiple myeloma and erythroleukemia; hepatocellular carcinoma; lung cancer including small cell lung cancer and non-small cell lung cancer; ovarian cancer; endometrial cancer; pancreatic cancer; pituitary adenoma; prostate cancer; renal cancer; sarcoma; skin cancers including melanomas; and thyroid cancers.

Suitably, the present invention relates to a method for treating or lessening the severity of a cancer selected from: brain (gliomas), glioblastomas, astrocytomas, glioblastoma multiforme, Bannayan-Zonana syndrome, Cowden disease, Lhermitte-Duclos disease, breast, colon, head and neck, kidney, lung, liver, melanoma, ovarian, pancreatic, prostate, sarcoma and thyroid.

Suitably, the present invention relates to a method for treating or lessening the severity of a cancer selected from ovarian, breast, pancreatic and prostate.

Suitably the present invention relates to a method for treating or lessening the severity of pre-cancerous syndromes in a mammal, including a human, wherein the pre-cancerous syndrome is selected from: cervical intraepithelial neoplasia, monoclonal gammopathy of unknown significance (MGUS), myelodysplastic syndrome, aplastic anemia, cervical lesions, skin nevi (pre-melanoma), prostatic intraepithelial (intraductal) neoplasia (PIN), Ductal Carcinoma in situ (DCIS), colon polyps and severe hepatitis or cirrhosis.

As used herein, the terms "cancer," "neoplasm," and "tumor," are used interchangeably and in either the singular or plural form, refer to cells that have undergone a malignant transformation that makes them pathological to the host organism. Primary cancer cells (that is, cells obtained from near the site of malignant transformation) can be readily distinguished from non-cancerous cells by well-established techniques, particularly histological examination. The definition of a cancer cell, as used herein, includes not only a primary cancer cell, but any cell derived from a cancer cell ancestor. This includes metastasized cancer cells, and *in vitro* cultures and cell lines derived from cancer cells. When referring to a type of cancer that normally manifests as a solid tumor, a "clinically detectable" tumor is one that is detectable on the basis of tumor mass; e.g., by procedures such as CAT scan, MR imaging, X-ray, ultrasound or palpation, and/or which is detectable because of the expression of one or more cancer-specific antigens in a sample obtainable from a patient. Tumors may be hematopoietic tumor, for example, tumors of blood cells or the like, meaning liquid tumors. Specific examples of clinical conditions based on such a

tumor include leukemia such as chronic myelocytic leukemia or acute myelocytic leukemia; myeloma such as multiple myeloma; lymphoma and the like.

Typically, any anti-neoplastic agent that has activity versus a susceptible tumor being treated may be co-administered in the treatment of cancer in the present 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. Typical anti-neoplastic agents useful in the present invention include, but are not limited to, anti-microtubule agents such as diterpenoids and vinca alkaloids; platinum coordination complexes; alkylating agents such as nitrogen mustards, oxazaphosphorines, alkylsulfonates, nitrosoureas, and triazenes; antibiotic agents such as anthracyclins, actinomycins and bleomycins; topoisomerase II inhibitors such as epipodophyllotoxins; antimetabolites such as purine and pyrimidine analogues and anti-folate compounds; topoisomerase I inhibitors such as camptothecins; hormones and hormonal analogues; signal transduction pathway inhibitors; receptor tyrosine kinase inhibitors; serine-threonine kinase inhibitors; non-receptor tyrosine kinase inhibitors; angiogenesis inhibitors, immunotherapeutic agents; proapoptotic agents; and cell cycle signaling inhibitors.

The present invention also provides methods for treating cancer comprising administering Compound A or pharmaceutically acceptable salt thereof with or without a Braf inhibitor, including, but not limited to, Compound B or a pharmaceutically acceptable salt or solvate thereof and another anti-neoplastic agent.

Examples of a further active ingredient or ingredients (anti-neoplastic agent) for use in combination or co-administered with Compound A or pharmaceutically acceptable salt thereof are chemotherapeutic agents.

Anti-microtubule or anti-mitotic agents are phase specific agents active against the microtubules of tumor cells during M or the mitosis phase of the cell cycle. Examples of anti-microtubule agents include, but are not limited to, diterpenoids and vinca alkaloids.

Diterpenoids, which are derived from natural sources, are phase specific anti-cancer agents that operate at the G₂/M phases of the cell cycle. It is believed that the diterpenoids stabilize the β -tubulin subunit of the microtubules, by binding with this protein. Disassembly of the protein appears then to be inhibited with mitosis being arrested and cell death following. Examples of diterpenoids include, but are not limited to, paclitaxel and its analog docetaxel.

Paclitaxel, 5 β ,20-epoxy-1,2 α ,4,7 β ,10 β ,13 α -hexa-hydroxytax-11-en-9-one 4,10-diacetate 2-benzoate 13-ester with (2R,3S)-N-benzoyl-3-phenylisoserine; is a natural diterpene product isolated from the Pacific yew tree *Taxus brevifolia* and is commercially available as an injectable solution TAXOL®. It is a member of the taxane family of terpenes. It was first isolated in 1971 by Wani et al. J. Am. Chem. Soc., 93:2325. 1971), who characterized its structure by chemical and X-ray crystallographic methods. One mechanism for its activity relates to paclitaxel's capacity to bind tubulin, thereby inhibiting cancer cell growth. Schiff et al., Proc. Natl. Acad. Sci. USA, 77:1561-1565 (1980); Schiff et al., Nature, 277:665-667 (1979); Kumar, J. Biol. Chem, 256: 10435-10441 (1981). For a review of synthesis and anticancer activity of some paclitaxel derivatives see: D. G. I. Kingston et al., Studies in Organic Chemistry vol. 26, entitled "New trends in Natural Products Chemistry 1986", Attaur-Rahman, P.W. Le Quesne, Eds. (Elsevier, Amsterdam, 1986) pp 219-235.

Paclitaxel has been approved for clinical use in the treatment of refractory ovarian cancer in the United States (Markman et al., Yale Journal of Biology and Medicine, 64:583, 1991; McGuire et al., Ann. Intern. Med., 111:273,1989) and for the treatment of breast cancer (Holmes et al., J. Nat. Cancer Inst., 83:1797,1991.) It is a potential candidate for treatment of neoplasms in the skin (Einzig et. al., Proc. Am. Soc. Clin. Oncol., 20:46) and head and neck carcinomas (Forastire et. al., Sem. Oncol., 20:56, 1990). The compound also shows potential for the treatment of polycystic kidney disease (Woo et. al., Nature, 368:750. 1994), lung cancer and malaria. Treatment of patients with paclitaxel results in bone marrow suppression (multiple cell lineages, Ignoff, R.J. et. al, Cancer Chemotherapy Pocket Guide, 1998) related to the duration of dosing above a threshold concentration (50nM) (Kearns, C.M. et. al., Seminars in Oncology, 3(6) p.16-23, 1995).

Docetaxel, (2R,3S)- N-carboxy-3-phenylisoserine,N-*tert*-butyl ester, 13-ester with 5 β -20-epoxy-1,2 α ,4,7 β ,10 β ,13 α -hexahydroxytax-11-en-9-one 4-acetate 2-benzoate, trihydrate; is commercially available as an injectable solution as TAXOTERE®. Docetaxel is indicated for the treatment of breast cancer. Docetaxel is a semisynthetic derivative of paclitaxel *q. v.*, prepared using a natural precursor, 10-deacetyl-baccatin III, extracted from the needle of the European Yew tree. The dose limiting toxicity of docetaxel is neutropenia.

Vinca alkaloids are phase specific anti-neoplastic agents derived from the periwinkle plant. Vinca alkaloids act at the M phase (mitosis) of the cell cycle by binding specifically to tubulin. Consequently, the bound tubulin molecule is unable to polymerize into microtubules. Mitosis is believed to be arrested in metaphase with cell death following. Examples of vinca alkaloids include, but are not limited to, vinblastine, vincristine, and vinorelbine.

Vinblastine, vincaleukoblastine sulfate, is commercially available as VELBAN® as an injectable solution. Although, it has possible indication as a second line therapy of various solid tumors, it is primarily indicated in the treatment of testicular cancer and various lymphomas including Hodgkin's Disease; and lymphocytic and histiocytic lymphomas. Myelosuppression is the dose limiting side effect of vinblastine.

Vincristine, vincaleukoblastine, 22-oxo-, sulfate, is commercially available as ONCOVIN® as an injectable solution. Vincristine is indicated for the treatment of acute leukemias and has also found use in treatment regimens for Hodgkin's and non-Hodgkin's malignant lymphomas. Alopecia and neurologic effects are the most common side effect of vincristine and to a lesser extent myelosuppression and gastrointestinal mucositis effects occur.

Vinorelbine, 3',4'-didehydro -4'-deoxy-C'-norvincaleukoblastine [R-(R*,R*)-2,3-dihydroxybutanedioate (1:2)(salt)], commercially available as an injectable solution of vinorelbine tartrate (NAVELBINE®), is a semisynthetic vinca alkaloid. Vinorelbine is indicated as a single agent or in combination with other chemotherapeutic agents, such as cisplatin, in the treatment of various solid tumors, particularly non-small cell lung, advanced breast, and hormone refractory prostate cancers. Myelosuppression is the most common dose limiting side effect of vinorelbine.

Platinum coordination complexes are non-phase specific anti-cancer agents, which are interactive with DNA. The platinum complexes enter tumor cells, undergo, aquation and form intra- and interstrand crosslinks with DNA causing adverse biological effects to the tumor. Examples of platinum coordination complexes include, but are not limited to, cisplatin and carboplatin.

Cisplatin, cis-diamminedichloroplatinum, is commercially available as PLATINOL® as an injectable solution. Cisplatin is primarily indicated in the treatment of metastatic testicular and ovarian cancer and advanced bladder cancer. The primary dose limiting side effects of cisplatin are nephrotoxicity, which may be controlled by hydration and diuresis, and ototoxicity.

Carboplatin, platinum, diammine [1,1-cyclobutane-dicarboxylate(2-)-O,O'], is commercially available as PARAPLATIN® as an injectable solution. Carboplatin is primarily indicated in the first and second line treatment of advanced ovarian carcinoma. Bone marrow suppression is the dose limiting toxicity of carboplatin.

Alkylating agents are non-phase anti-cancer specific agents and strong electrophiles. Typically, alkylating agents form covalent linkages, by alkylation, to DNA through nucleophilic moieties of the DNA molecule such as phosphate, amino, sulfhydryl, hydroxyl, carboxyl, and imidazole groups. Such alkylation disrupts nucleic acid function leading to cell

death. Examples of alkylating agents include, but are not limited to, nitrogen mustards such as cyclophosphamide, melphalan, and chlorambucil; alkyl sulfonates such as busulfan; nitrosoureas such as carmustine; and triazenes such as dacarbazine.

Cyclophosphamide, 2-[bis(2-chloroethyl)amino]tetrahydro-2H-1,3,2-oxazaphosphorine 2-oxide monohydrate, is commercially available as an injectable solution or tablets as CYTOXAN®. Cyclophosphamide is indicated as a single agent or in combination with other chemotherapeutic agents, in the treatment of malignant lymphomas, multiple myeloma, and leukemias. Alopecia, nausea, vomiting and leukopenia are the most common dose limiting side effects of cyclophosphamide.

Melphalan, 4-[bis(2-chloroethyl)amino]-L-phenylalanine, is commercially available as an injectable solution or tablets as ALKERAN®. Melphalan is indicated for the palliative treatment of multiple myeloma and non-resectable epithelial carcinoma of the ovary. Bone marrow suppression is the most common dose limiting side effect of melphalan.

Chlorambucil, 4-[bis(2-chloroethyl)amino]benzenebutanoic acid, is commercially available as LEUKERAN® tablets. Chlorambucil is indicated for the palliative treatment of chronic lymphatic leukemia, and malignant lymphomas such as lymphosarcoma, giant follicular lymphoma, and Hodgkin's disease. Bone marrow suppression is the most common dose limiting side effect of chlorambucil.

Busulfan, 1,4-butanediol dimethanesulfonate, is commercially available as MYLERAN® TABLETS. Busulfan is indicated for the palliative treatment of chronic myelogenous leukemia. Bone marrow suppression is the most common dose limiting side effects of busulfan.

Carmustine, 1,3-[bis(2-chloroethyl)-1-nitrosourea, is commercially available as single vials of lyophilized material as BiCNU®. Carmustine is indicated for the palliative treatment as a single agent or in combination with other agents for brain tumors, multiple myeloma, Hodgkin's disease, and non-Hodgkin's lymphomas. Delayed myelosuppression is the most common dose limiting side effects of carmustine.

Dacarbazine, 5-(3,3-dimethyl-1-triazeno)-imidazole-4-carboxamide, is commercially available as single vials of material as DTIC-Dome®. Dacarbazine is indicated for the treatment of metastatic malignant melanoma and in combination with other agents for the second line treatment of Hodgkin's Disease. Nausea, vomiting, and anorexia are the most common dose limiting side effects of dacarbazine.

Antibiotic anti-neoplastics are non-phase specific agents, which bind or intercalate with DNA. Typically, such action results in stable DNA complexes or strand breakage, which disrupts ordinary function of the nucleic acids leading to cell death. Examples of antibiotic

anti-neoplastic agents include, but are not limited to, actinomycins such as dactinomycin, anthracyclins such as daunorubicin and doxorubicin; and bleomycins.

Dactinomycin, also known as Actinomycin D, is commercially available in injectable form as COSMEGEN®. Dactinomycin is indicated for the treatment of Wilm's tumor and rhabdomyosarcoma. Nausea, vomiting, and anorexia are the most common dose limiting side effects of dactinomycin.

Daunorubicin, (8S-cis)-8-acetyl-10-[(3-amino-2,3,6-trideoxy- α -L-lyxo-hexopyranosyl)oxy]-7,8,9,10-tetrahydro-6,8,11-trihydroxy-1-methoxy-5,12 naphthacenedione hydrochloride, is commercially available as a liposomal injectable form as DAUNOXOME® or as an injectable as CERUBIDINE®. Daunorubicin is indicated for remission induction in the treatment of acute nonlymphocytic leukemia and advanced HIV associated Kaposi's sarcoma. Myelosuppression is the most common dose limiting side effect of daunorubicin.

Doxorubicin, (8S, 10S)-10-[(3-amino-2,3,6-trideoxy- α -L-lyxo-hexopyranosyl)oxy]-8-glycoloyl, 7,8,9,10-tetrahydro-6,8,11-trihydroxy-1-methoxy-5,12 naphthacenedione hydrochloride, is commercially available as an injectable form as RUBEX® or ADRIAMYCIN RDF®. Doxorubicin is primarily indicated for the treatment of acute lymphoblastic leukemia and acute myeloblastic leukemia, but is also a useful component in the treatment of some solid tumors and lymphomas. Myelosuppression is the most common dose limiting side effect of doxorubicin.

Bleomycin, a mixture of cytotoxic glycopeptide antibiotics isolated from a strain of *Streptomyces verticillus*, is commercially available as BLENOXANE®. Bleomycin is indicated as a palliative treatment, as a single agent or in combination with other agents, of squamous cell carcinoma, lymphomas, and testicular carcinomas. Pulmonary and cutaneous toxicities are the most common dose limiting side effects of bleomycin.

Topoisomerase II inhibitors include, but are not limited to, epipodophyllotoxins.

Epipodophyllotoxins are phase specific anti-neoplastic agents derived from the mandrake plant. Epipodophyllotoxins typically affect cells in the S and G₂ phases of the cell cycle by forming a ternary complex with topoisomerase II and DNA causing DNA strand breaks. The strand breaks accumulate and cell death follows. Examples of epipodophyllotoxins include, but are not limited to, etoposide and teniposide.

Etoposide, 4'-demethyl-epipodophyllotoxin 9[4,6-O-(R)-ethylidene- β -D-glucopyranoside], is commercially available as an injectable solution or capsules as VePESID® and is commonly known as VP-16. Etoposide is indicated as a single agent or in combination with other chemotherapy agents in the treatment of testicular and non-small cell lung cancers. Myelosuppression is the most common side effect of etoposide. The incidence of leucopenia tends to be more severe than thrombocytopenia.

Teniposide, 4'-demethyl-epipodophyllotoxin 9[4,6-O-(R)-thenylidene- β -D-glucopyranoside], is commercially available as an injectable solution as VUMON® and is commonly known as VM-26. Teniposide is indicated as a single agent or in combination with other chemotherapy agents in the treatment of acute leukemia in children. Myelosuppression is the most common dose limiting side effect of teniposide. Teniposide can induce both leucopenia and thrombocytopenia.

Antimetabolite neoplastic agents are phase specific anti-neoplastic agents that act at S phase (DNA synthesis) of the cell cycle by inhibiting DNA synthesis or by inhibiting purine or pyrimidine base synthesis and thereby limiting DNA synthesis. Consequently, S phase does not proceed and cell death follows. Examples of antimetabolite anti-neoplastic agents include, but are not limited to, fluorouracil, methotrexate, cytarabine, mercaptopurine, thioguanine, and gemcitabine.

5-fluorouracil, 5-fluoro-2,4- (1H,3H) pyrimidinedione, is commercially available as fluorouracil. Administration of 5-fluorouracil leads to inhibition of thymidylate synthesis and is also incorporated into both RNA and DNA. The result typically is cell death. 5-fluorouracil is indicated as a single agent or in combination with other chemotherapy agents in the treatment of carcinomas of the breast, colon, rectum, stomach and pancreas. Myelosuppression and mucositis are dose limiting side effects of 5-fluorouracil. Other fluoropyrimidine analogs include 5-fluoro deoxyuridine (floxuridine) and 5-fluorodeoxyuridine monophosphate.

Cytarabine, 4-amino-1- β -D-arabinofuranosyl-2 (1H)-pyrimidinone, is commercially available as CYTOSAR-U® and is commonly known as Ara-C. It is believed that cytarabine exhibits cell phase specificity at S-phase by inhibiting DNA chain elongation by terminal incorporation of cytarabine into the growing DNA chain. Cytarabine is indicated as a single agent or in combination with other chemotherapy agents in the treatment of acute leukemia. Other cytidine analogs include 5-azacytidine and 2',2'-difluorodeoxycytidine (gemcitabine). Cytarabine induces leucopenia, thrombocytopenia, and mucositis.

Mercaptopurine, 1,7-dihydro-6H-purine-6-thione monohydrate, is commercially available as PURINETHOL®. Mercaptopurine exhibits cell phase specificity at S-phase by inhibiting DNA synthesis by an as of yet unspecified mechanism. Mercaptopurine is indicated as a single agent or in combination with other chemotherapy agents in the treatment of acute leukemia. Myelosuppression and gastrointestinal mucositis are expected side effects of mercaptopurine at high doses. A useful mercaptopurine analog is azathioprine.

Thioguanine, 2-amino-1,7-dihydro-6H-purine-6-thione, is commercially available as TABLOID®. Thioguanine exhibits cell phase specificity at S-phase by inhibiting DNA

synthesis by an as of yet unspecified mechanism. Thioguanine is indicated as a single agent or in combination with other chemotherapy agents in the treatment of acute leukemia. Myelosuppression, including leucopenia, thrombocytopenia, and anemia, is the most common dose limiting side effect of thioguanine administration. However, gastrointestinal side effects occur and can be dose limiting. Other purine analogs include pentostatin, erythrohydroxynonyladenine, fludarabine phosphate, and cladribine.

Gemcitabine, 2'-deoxy-2', 2'-difluorocytidine monohydrochloride (β -isomer), is commercially available as GEMZAR®. Gemcitabine exhibits cell phase specificity at S-phase and by blocking progression of cells through the G1/S boundary. Gemcitabine is indicated in combination with cisplatin in the treatment of locally advanced non-small cell lung cancer and alone in the treatment of locally advanced pancreatic cancer. Myelosuppression, including leucopenia, thrombocytopenia, and anemia, is the most common dose limiting side effect of gemcitabine administration.

Methotrexate, N-[4[[[(2,4-diamino-6-pteridiny)] methyl]methylamino] benzoyl]-L-glutamic acid, is commercially available as methotrexate sodium. Methotrexate exhibits cell phase effects specifically at S-phase by inhibiting DNA synthesis, repair and/or replication through the inhibition of dihydrofolic acid reductase which is required for synthesis of purine nucleotides and thymidylate. Methotrexate is indicated as a single agent or in combination with other chemotherapy agents in the treatment of choriocarcinoma, meningeal leukemia, non-Hodgkin's lymphoma, and carcinomas of the breast, head, neck, ovary and bladder. Myelosuppression (leucopenia, thrombocytopenia, and anemia) and mucositis are expected side effect of methotrexate administration.

Camptothecins, including, camptothecin and camptothecin derivatives are available or under development as Topoisomerase I inhibitors. Camptothecins cytotoxic activity is believed to be related to its Topoisomerase I inhibitory activity. Examples of camptothecins include, but are not limited to irinotecan, topotecan, and the various optical forms of 7-(4-methylpiperazino-methylene)-10,11-ethylenedioxy-20-camptothecin described below.

Irinotecan HCl, (4S)-4,11-diethyl-4-hydroxy-9-[(4-piperidinopiperidino) carbonyloxy]-1H-pyrano[3',4',6,7]indolizino[1,2-b]quinoline-3,14(4H,12H)-dione hydrochloride, is commercially available as the injectable solution CAMPTOSAR®.

Irinotecan is a derivative of camptothecin which binds, along with its active metabolite SN-38, to the topoisomerase I – DNA complex. It is believed that cytotoxicity occurs as a result of irreparable double strand breaks caused by interaction of the topoisomerase I : DNA : irinotecan or SN-38 ternary complex with replication enzymes. Irinotecan is indicated for treatment of metastatic cancer of the colon or rectum. The dose limiting side effects of

irinotecan HCl are myelosuppression, including neutropenia, and GI effects, including diarrhea.

Topotecan HCl, (S)-10-[(dimethylamino)methyl]-4-ethyl-4,9-dihydroxy-1H-pyrano[3',4',6,7]indolizino[1,2-b]quinoline-3,14-(4H,12H)-dione monohydrochloride, is commercially available as the injectable solution HYCAMTIN®. Topotecan is a derivative of camptothecin which binds to the topoisomerase I – DNA complex and prevents religation of single strand breaks caused by Topoisomerase I in response to torsional strain of the DNA molecule. Topotecan is indicated for second line treatment of metastatic carcinoma of the ovary and small cell lung cancer. The dose limiting side effect of topotecan HCl is myelosuppression, primarily neutropenia.

Rituximab is a chimeric monoclonal antibody which is sold as RITUXAN® and MABTHERA®. Rituximab binds to CD20 on B cells and causes cell apoptosis. Rituximab is administered intravenously and is approved for treatment of rheumatoid arthritis and B-cell non-Hodgkin's lymphoma.

Ofatumumab is a fully human monoclonal antibody which is sold as ARZERRA®. Ofatumumab binds to CD20 on B cells and is used to treat chronic lymphocytic leukemia (CLL; a type of cancer of the white blood cells) in adults who are refractory to treatment with fludarabine (Fludara) and alemtuzumab (Campath).

mTOR inhibitors include but are not limited to rapamycin and rapalogs, RAD001 or everolimus (Afinitor), CCI-779 or temsirolimus, AP23573, AZD8055, WYE-354, WYE-600, WYE-687 and Pp121.

Bexarotene is sold as Targretin® and is a member of a subclass of retinoids that selectively activate retinoid X receptors (RXRs). These retinoid receptors have biologic activity distinct from that of retinoic acid receptors (RARs). The chemical name is 4-[1-(5,6,7,8-tetrahydro-3,5,5,8,8-pentamethyl-2-naphthalenyl) ethenyl] benzoic acid. Bexarotene is used to treat cutaneous T-cell lymphoma (CTCL, a type of skin cancer) in people whose disease could not be treated successfully with at least one other medication.

Sorafenib marketed as Nexavar® is in a class of medications called multikinase inhibitors. Its chemical name is 4-[4-[4-chloro-3-(trifluoromethyl)phenyl]carbamoylamino]phenoxy]-N-methyl-pyridine-2-carboxamide. Sorafenib is used to treat advanced renal cell carcinoma (a type of cancer that begins in the kidneys). Sorafenib is also used to treat unresectable hepatocellular carcinoma (a type of liver cancer that cannot be treated with surgery).

The term "wild type" as is understood in the art refers to a polypeptide or polynucleotide sequence that occurs in a native population without genetic modification. As is also understood in the art, a "mutant" includes a polypeptide or polynucleotide sequence

having at least one modification to an amino acid or nucleic acid compared to the corresponding amino acid or nucleic acid found in a wild type polypeptide or polynucleotide, respectively. Included in the term mutant is Single Nucleotide Polymorphism (SNP) where a single base pair distinction exists in the sequence of a nucleic acid strand compared to the most prevalently found (wild type) nucleic acid strand.

As used herein, "genotyping" a cell including a tumor cell from a subject (or DNA or other biological sample) for a mutation or a polymorphic allele of a gene(s) means detecting which allelic or polymorphic form(s) and/or wild type or somatically mutated form(s) of the gene(s) or gene expression products (*e.g.*, hnRNA, mRNA or protein) are present or absent in a subject (or a sample). Related RNA or protein expressed from such gene may also be used to detect polymorphic variation. For purposes of the present invention, "genotyping" includes the determination of somatic as well as genotypic mutations from a sample. As used herein, an allele may be 'detected' when other possible allelic variants have been ruled out; *e.g.*, where a specified nucleic acid position is found to be neither adenine (A), thymine (T) or cytosine (C), it can be concluded that guanine (G) is present at that position (*i.e.*, G is 'detected' or 'diagnosed' in a subject). Sequence variations may be detected directly (by, *e.g.* sequencing, for example, EST sequencing or partial or full genome sequencing) or indirectly (*e.g.*, by restriction fragment length polymorphism analysis, or detection of the hybridization of a probe of known sequence, or reference strand conformation polymorphism), or by using other known methods.

The sequence of any nucleic acid including a gene or PCR product or a fragment or portion thereof may be sequenced by any method known in the art (*e.g.*, chemical sequencing or enzymatic sequencing). "Chemical sequencing" of DNA may denote methods such as that of Maxam and Gilbert (1977) (Proc. Natl. Acad. Sci. USA 74:560), in which DNA is randomly cleaved using individual base-specific reactions. "Enzymatic sequencing" of DNA may denote methods such as that of Sanger (Sanger, et al., (1977) Proc. Natl. Acad. Sci. USA 74:5463).

Conventional molecular biology, microbiology, and recombinant DNA techniques including sequencing techniques are well known among those skilled in the art. Such techniques are explained fully in the literature. See, *e.g.*, Sambrook, Fritsch & Maniatis, Molecular Cloning: A Laboratory Manual, Second Edition (1989) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (herein "Sambrook, et al., 1989"); DNA Cloning: A Practical Approach, Volumes I and II (D. N. Glover ed. 1985); Oligonucleotide Synthesis (M. J. Gait ed. 1984); Nucleic Acid Hybridization (B. D. Hames & S. J. Higgins eds. (1985)); Transcription And Translation (B. D. Hames & S. J. Higgins, eds. (1984)); Animal Cell Culture (R. I. Freshney, ed. (1986)); Immobilized Cells And Enzymes (IRL Press, (1986)); B.

Perbal, A Practical Guide To Molecular Cloning (1984); F. M. Ausubel, et al. (eds.), Current Protocols in Molecular Biology, John Wiley & Sons, Inc. (1994)

The Peptide Nucleic Acid (PNA) affinity assay is a derivative of traditional hybridization assays (Nielsen et al., *Science* 254:1497-1500 (1991); Egholm et al., *J. Am. Chem. Soc.* 114:1895-1897 (1992); James et al., *Protein Science* 3:1347-1350 (1994)). PNAs are structural DNA mimics that follow Watson-Crick base pairing rules, and are used in standard DNA hybridization assays. PNAs display greater specificity in hybridization assays because a PNA/DNA mismatch is more destabilizing than a DNA/DNA mismatch and complementary PNA/DNA strands form stronger bonds than complementary DNA/DNA strands.

DNA microarrays have been developed to detect genetic variations and polymorphisms (Taton *et al.*, *Science* 289:1757-60, 2000; Lockhart et al., *Nature* 405:827-836 (2000); Gerhold *et al.*, *Trends in Biochemical Sciences* 24:168-73 (1999); Wallace, R. W., *Molecular Medicine Today* 3:384-89 (1997); Blanchard and Hood, *Nature Biotechnology* 149:1649 (1996)). DNA microarrays are fabricated by high-speed robotics, on glass or nylon substrates, and contain DNA fragments with known identities ("the probe"). The microarrays are used for matching known and unknown DNA fragments ("the target") based on traditional base-pairing rules.

The term "at least one mutation" in a polypeptide or a gene encoding a polypeptide and grammatical variations thereof means a polypeptide or gene encoding a polypeptide having one or more allelic variants, splice variants, derivative variants, substitution variants, deletion variants, truncation variants, and/or insertion variants, fusion polypeptides, orthologs, and/or interspecies homologs. By way of example, at least one mutation of a protein would include a protein in which part of all of the sequence of a polypeptide or gene encoding the protein is absent or not expressed in the cell for at least one protein produced in the cell. For example, a TRAIL protein may be produced by a cell in a truncated form and the sequence of the truncated form may be wild type over the sequence of the truncate. A deletion may mean the absence of all or part of a gene or protein encoded by a gene. Additionally, some of a protein expressed in or encoded by a cell may be mutated while other copies of the same protein produced in the same cell may be wild type. By way of another example a mutation in a TRAIL protein would include a TRAIL protein having one or more amino acid differences in its amino acid sequence compared with wild type of the same TRAIL protein.

As used herein "genetic abnormality" is meant a deletion, substitution, addition, translocation, amplification and the like relative to the normal native nucleic acid content of a cell of a subject.

The terms "polypeptide" and "protein" are used interchangeably and are used herein as a generic term to refer to native protein, fragments, peptides, or analogs of a polypeptide sequence. Hence, native protein, fragments, and analogs are species of the polypeptide genus.

The terminology "X#Y" in the context of a mutation in a polypeptide sequence is art-recognized, where "#" indicates the location of the mutation in terms of the amino acid number of the polypeptide, "X" indicates the amino acid found at that position in the wild-type amino acid sequence, and "Y" indicates the mutant amino acid at that position. For example, the notation "G12S" with reference to the K-ras polypeptide indicates that there is a glycine at amino acid number 12 of the wild-type K-ras sequence, and that glycine is replaced with a serine in the mutant K-ras sequence.

The term "TRAIL protein" as used herein means the TNF-related apoptosis-inducing ligand, a protein involved in apoptosis or cell death. TRAIL is often referred to as a cytokine. TRAIL can also be called CD253 or tumor necrosis factor superfamily member 10 (TNFSF10), Apo-2L, or TL2, among other names known in the art. It is also known in the art that TRAIL binds death receptors DR4 (TRAIL-RI) and DR5 (TRAIL-RII), as well as DcR1 and DcR2. The gene and protein sequences are known in the art. For example, the gene sequence of TRAIL in GenBank is known in the art and is represented by GenBank number U37518; the protein sequence of TRAIL in UniProtKB is known in the art and is represented by UniProtKB number P50591. The OMIM entry is: <http://omim.org/entry/603598>.

As used herein "gene encoding a TRAIL protein" means any part of a gene or polynucleotide encoding any TRAIL protein. Included within the meaning of this term are exons encoding TRAIL.

As used herein the term "amplification" and grammatical variations thereof refers to the presence of one or more extra gene copies in a chromosome complement. Amplification of the HER2 gene has been correlated with certain types of cancer. Amplification of the HER2 gene has been found in human salivary gland and gastric tumor-derived cell lines, gastric and colon adenocarcinomas, and mammary gland adenocarcinomas. Semba et al., *Proc. Natl. Acad. Sci. USA*, 82:6497-6501 (1985); Yokota et al., *Oncogene*, 2:283-287 (1988); Zhou et al., *Cancer Res.*, 47:6123-6125 (1987); King et al., *Science*, 229:974-976 (1985); Kraus et al., *EMBO J.*, 6:605-610 (1987); van de Vijver et al., *Mol. Cell. Biol.*, 7:2019-2023 (1987); Yamamoto et al., *Nature*, 319:230-234 (1986).

As used herein, "expression" of a protein or polypeptide and grammatical variations thereof means that a given cell makes an mRNA or protein product from the corresponding gene. Expression may be detected in a number of ways known by one of skill in the art, including detecting portions of an mRNA or protein product either directly or indirectly.

As used herein "overexpressed" and "overexpression" of a protein or polypeptide and grammatical variations thereof means that a given cell produces an increased number of a certain protein relative to a normal cell. By way of example, a protein may be overexpressed by a tumor cell relative to a non-tumor cell. Additionally, a mutant protein may be overexpressed compared to wild type protein in a cell. As is understood in the art, expression levels of a polypeptide in a cell can be normalized to a housekeeping gene such as actin. In some instances, a certain polypeptide may be underexpressed in a tumor cell compared with a non-tumor cell.

As used herein "nucleic acid necessary for expression of at least one gene product" refers to a nucleic acid sequence that encodes any portion of a gene and/or is operably linked to a nucleic acid encoding a gene product but does not necessarily comprise encoding sequence. By way of example, a nucleic acid sequence necessary for the expression of at least one gene product includes, but is not limited to, enhancers, promoters, regulatory sequences, start codons, stop codons, polyadenylation sequences, and/or encoding sequences. Expression levels of a polypeptide in a particular cell can be effected by, but not limited to, mutations, deletions and/or substitutions of various regulatory elements and/or non-encoding sequence in the cell genome.

The terms "mutant B-raf protein" refers to a B-raf polypeptide comprising at least one mutation. Certain exemplary mutant B-raf polypeptides include, but are not limited to, allelic variants, splice variants, derivative variants, substitution variants, deletion variants, and/or insertion variants, fusion polypeptides, orthologs, and interspecies homologs. In certain embodiments, a mutant B-raf polypeptide includes additional residues at the C- or N-terminus, such as, but not limited to, leader sequence residues, targeting residues, amino terminal methionine residues, lysine residues, tag residues and/or fusion protein residues. Certain B-raf mutants include but are not limited to BRAF having an amino acid substitution selected from the group consisting of R462I, I463S, G464V, G464E, G466A, G466E, G466V, G469A, G469E, D594V, F595L, G596R, L597V, L597R, T599I, V600E, V600D, V600K, V600R, T119S, and K601E. See, for example, FIG. 2 of Halilovic and Solvit (2008) *Current Opinion in Pharmacology* 8:419-26. BRAF encodes a RAS-regulated kinase that mediates cell growth and malignant transformation via kinase pathway activation.

The term "polynucleotide" as referred to herein means a polymeric form of nucleotides of at least 10 bases in length, either ribonucleotides or deoxynucleotides or a modified form of either type of nucleotide. The term includes single and double stranded forms of DNA.

The term "oligonucleotide" referred to herein includes naturally occurring and modified nucleotides linked together by naturally occurring, and non-naturally occurring oligonucleotide linkages. Oligonucleotides are a polynucleotide subset generally comprising

a length of 200 bases or fewer. Preferably oligonucleotides are 10 to 60 bases in length and most preferably 12, 13, 14, 15, 16, 17, 18, 19, or 20 to 40 bases in length. Oligonucleotides are usually single stranded, e.g. for probes, although oligonucleotides may be double stranded, e.g. for use in the construction of a gene mutant. Oligonucleotides can be either sense or antisense oligonucleotides.

An oligonucleotide probe, or probe, is a nucleic acid molecule which typically ranges in size from about 8 nucleotides to several hundred nucleotides in length. Such a molecule is typically used to identify a target nucleic acid sequence in a sample by hybridizing to such target nucleic acid sequence under stringent hybridization conditions. Hybridization conditions have been described in detail above.

PCR primers are also nucleic acid sequences, although PCR primers are typically oligonucleotides of fairly short length which are used in polymerase chain reactions. PCR primers and hybridization probes can readily be developed and produced by those of skill in the art, using sequence information from the target sequence. (See, for example, Sambrook et al., *supra* or Glick et al., *supra*).

As is known in the art, several primers are known for use in PCR for detecting expression of genes such as TRAIL, as well as Braf mutations. For example, primers for detecting mutations in Braf are presented in several research articles and US patents including, but not limited to, Brose, et al. Cancer Research 62:6997-7000 (2002), Xu, et al. Cancer research 63:4561-4567 (2003), as well as US Patent No. 7,745,128, and several commercially available kits (see Dxs Diagnostic Innovations, Applied Biosystems, and Quest diagnostics).

TRAIL expression, as well as cancers that are either wild type or mutant for Ras/Raf and either wild type or mutant for PI3K/Pten are identified by known methods. For example, TRAIL expression or wild type or mutant Ras/Raf or PI3K/PTEN tumor cells can be identified by DNA amplification and sequencing techniques, DNA and RNA detection techniques, including, but not limited to Northern and Southern blot, respectively, and/or various biochip and array technologies. Wild type and mutant polypeptides, including elevated levels of such polypeptides, can be detected by a variety of techniques including, but not limited to immunodiagnostic techniques such as ELISA, Western blot or immunocytochemistry.

TRAIL expression and/or elevated levels of TRAIL can be identified using what is known in the art as Cytokine and Angiogenesis Analysis (CAF Analysis) which is known in the art. Circulating cytokines and angiogenic factors (CAF) profiles have shown potential for identification of prognostic and predictive markers in subjects with cancer. Biomarkers that provide information about the outcome of cancer subjects are classified by whether they are prognostic or predictive. Prognostic markers are those which provide information about outcome regardless of treatment. Predictive markers identify sub-populations of subjects

that are most likely to benefit from a particular therapy. Certain biomarkers can provide both prognostic and predictive information. In suitable embodiments, the level of TRAIL in a patient is determined using CAF Analysis. In further embodiments, the CAF Analysis is performed by quantitative PCR or microarray analysis or any of a number of other molecular techniques known to one of skill in the art.

In any of the embodiments herein, a mammal or human can be determined to have elevated level of TRAIL by any of a number of means known in the art. For example, the elevated level of trail can be determined by performing Cytokine and Angiogenesis Factor (CAF) analysis where TRAIL is included as one of the cytokine and angiogenesis factors. The determining can involve measuring levels of TRAIL protein expression, for example in a blood or plasma sample. Determining that TRAIL is elevated can involve measuring the level of TRAIL protein expression relative to a control sample, such as a reference sample or blood sample of a mammal, e.g. human not in need of treatment for cancer.

Suitably, circulating TRAIL levels are elevated 1%, 2%, 3%, 4%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100%. In further embodiments, TRAIL is elevated, e.g., relative to a control sample or a sample from a human not in need of treatment for cancer by a fold difference or elevated 2 fold, 3 fold, 4 fold, 5 fold, or 10 fold.

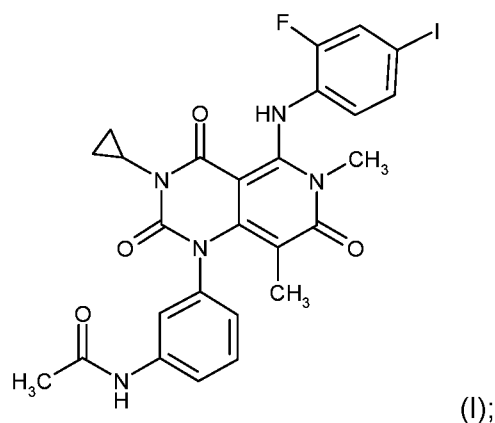
In suitable embodiments of the invention are provided method of treating cancer in a mammal, e.g. in a human, e.g., in a human patient in need of treatment for cancer, comprising determining if said mammal has an elevated level of TRAIL, and if said mammal has an elevated level of TRAIL, administering at least one tyrosine kinase inhibitor or a pharmaceutical composition comprising at least one tyrosine kinase inhibitor. In further suitable embodiments of the invention, a tyrosine kinase inhibitor is provided for use in a mammal, e.g. in a human, e.g., in a human patient in need of treatment for cancer, wherein said mammal is determined to have an elevated level of TRAIL, e.g. an elevated level of circulating blood levels of TRAIL. In other further suitable embodiments of the invention, a tyrosine kinase inhibitor is provided for use in a mammal, wherein said mammal is classified as a responder, and wherein a responder is characterized by an elevated level of TRAIL, e.g. an elevated level of circulating blood levels of TRAIL.

In suitable embodiments of the invention are provided method of treating cancer in a mammal, e.g. in a human, e.g., in a human patient in need of treatment for cancer, comprising determining if said mammal has an elevated level of TRAIL, and if said mammal has an elevated level of TRAIL, administering a MEK inhibitor or pharmaceutically acceptable salt or solvate thereof as described herein, or a pharmaceutical composition comprising a MEK inhibitor as described herein. In other suitable embodiments of the invention are provided methods of treating cancer in a mammal, e.g. in a human, e.g., in a

human patient in need of treatment for cancer, comprising identifying the mammal, e.g. human, by the presence of an elevated level of TRAIL and administering a MEK inhibitor or a pharmaceutical composition comprising a MEK inhibitor to the identified mammal, e.g. human. In other suitable embodiments of the invention are provided methods of treating cancer in a mammal, e.g. in a human, e.g., in a human patient in need of treatment for cancer, comprising identifying the mammal, e.g. human, as a responder based on an elevated level of TRAIL, and administering a MEK inhibitor or a pharmaceutical composition comprising a MEK inhibitor to the identified responder.

In other suitable embodiments of the invention, a MEK inhibitor or a pharmaceutically acceptable salt or solvate thereof as described herein is provided for use in a mammal, e.g. in a human, e.g., in a human patient in need of treatment for cancer, wherein said mammal is determined to have an elevated level of TRAIL, e.g. an elevated level of circulating blood levels of TRAIL. In other suitable embodiments of the invention, a MEK inhibitor or a pharmaceutically acceptable salt or solvate thereof as described herein, or a pharmaceutical composition comprising a MEK inhibitor as described herein, is provided for use in a mammal, wherein said mammal is classified as a responder, and wherein a responder is characterized by an elevated level of TRAIL, e.g. an elevated level of circulating blood levels of TRAIL.

In any of the embodiments herein, e.g. in any of the embodiments of the invention, the MEK inhibitor is suitably trametinib. In other further embodiments, the MEK inhibitor is Compound A or a solvate or salt thereof, or Compound A². In further suitable embodiments of the invention, the MEK inhibitor comprises a compound of Structure (I):

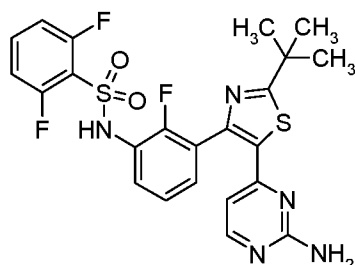


or a pharmaceutically acceptable salt or solvate thereof. In any of the embodiments, the MEK inhibitor, e.g. having Structure (I), is in the sodium salt form. In any of the embodiments described herein, the MEK inhibitor having Structure (I) is alternatively in the form of a dimethyl sulfoxide solvate. In any of the embodiments described herein, the MEK

inhibitor, or pharmaceutically acceptable salt or solvate (e.g. the dimethyl sulfoxide solvate) is administered or provided as a pharmaceutical composition as described herein.

In any of the embodiments of the invention, e.g., the methods or uses described herein, the cancer is selected from melanoma, pancreatic cancer, colorectal cancer, and non-small cell lung carcinoma. In further embodiments, the cancer is suitably selected from pancreatic cancer and non-small cell lung cancer.

In further embodiments, the methods comprising administering at least one Braf inhibitor or a pharmaceutically acceptable salt thereof to said mammal in addition to the MEK inhibitor. The Braf inhibitor is suitably a compound of Structure (II):



(II)

or a pharmaceutically acceptable salt or solvate thereof.

In other embodiments, the methods comprise, in addition to administering a MEK inhibitor or a pharmaceutically acceptable salt or solvate thereof, and/or a BRAF inhibitor or a pharmaceutically acceptable salt or solvate thereof, or pharmaceutical compositions comprising the same as describe herein, further comprises administering at least one additional anti-neoplastic agent. In a suitable embodiment, the at least one additional anti-neoplastic agent is gemcetabine. In other embodiments, the at least one additional anti-neoplastic agent in any of those known in the art to be of the same class as gemcetabine, or any anti-neoplastic agent known in the art such as those described herein. Thus also provided are a MEK inhibitor or a pharmaceutically acceptable salt or solvate thereof, and/or a BRAF inhibitor or a pharmaceutically acceptable salt or solvate thereof, or pharmaceutical compositions comprising the same as describe herein, and at least one additional anti-neoplastic agent for use for use in a mammal, e.g. in a human, e.g., a human patient in need of treatment for cancer, wherein said mammal is determined to have an elevated level of TRAIL, e.g. an elevated level of circulating blood levels of TRAIL.

In another embodiments are provided methods of treating cancer in a mammal, e.g. in a human, e.g., in a human patient in need of treatment for cancer, comprising treating with an antineoplastic agent that elevates circulating TRAIL and then administering at least one tyrosine kinase inhibitor or a pharmaceutical composition comprising at least one tyrosine kinase inhibitor. In another embodiments are provided methods of treating cancer in a mammal, e.g. in a human, e.g., a human patient in need of treatment for cancer, comprising

treating with an antineoplastic agent that elevates circulating TRAIL and then administering at least one tyrosine kinase inhibitor or a pharmaceutical composition comprising at least one MEK inhibitor, or at least one MEK inhibitor and at least one BRAF inhibitor, e.g. having Structure I or Structure II herein, or a pharmaceutically acceptable salt or solvate thereof, or pharmaceutical compositions comprising the same as described herein. Thus also provided is antineoplastic agent that elevates circulating TRAIL and at least one tyrosine kinase inhibitor or one or pharmaceutical compositions comprising the same for use in treating a mammal, e.g. a human, e.g. a human patient in need of treatment for cancer. Thus also provided is antineoplastic agent that elevates circulating TRAIL and at least one MEK inhibitor or a pharmaceutically acceptable salt or solvate thereof (as described herein) or one or pharmaceutical compositions (as described herein) comprising the same for use in treating a mammal, e.g. a human, e.g. a human patient in need of treatment for cancer.

EXAMPLES

The following examples are intended for illustration only and are not intended to limit the scope of the invention in any way.

Example 1: TRAIL induces apoptosis in a wide variety of transformed cell lines

Both cell-bound TRAIL and an engineered soluble form rapidly induced apoptosis in a wide variety of transformed cell lines of diverse origin (data not shown here).

Example 2: MEK114653 NSCLC Study Design, Objectives, and Outcome

The Study design was an open-label, multicenter phase 2 study in patients with KRAS- and non-KRAS mutant NSCLC, randomized 2:1 to treatment with trametinib or docetaxel. A schematic is shown in Figure 1. The primary objective was progression-free survival (PFS) and secondary objectives included the following:

- Safety and tolerability
- Overall response rate (ORR)
- Duration of response
- Overall survival (OS)
- Steady state exposure and pharmacokinetics
- Exploratory translational research.

In the study, treatment cycles discontinued if disease progression, death, or unacceptable adverse events occur. The outcome of the study was that in the randomized 2:1 to treatment with trametinib or docetaxel, trametinib did not improve PFS as second-line treatment in patients with KRAS-mutant NSCLC compared with docetaxel. The Overall

Response Rate (ORR) of 12% in trametinib-treated patients with KRAS-mutant non-small cell lung carcinoma (NSCLC) suggested that an effort to better identify responsive mutations was warranted. The safety profile of trametinib was, in general, consistent with the overall known profile of trametinib monotherapy; however, more respiratory-related adverse events (AEs) and fatal SAEs were noted in this study, possibly due to the disease under study.

Example 3: CAF Analysis in NSCLC

Analysis was done of a panel of cytokines and angiogenic factors (CAF's) measured in plasma prior to initiation of treatment with trametinib+gemcitabine or placebo+gemcitabine, and at day 15 of treatment.

TRAIL was identified as a potential high value biomarker for NSCLC patients response to trametinib. Patients with higher circulating levels of TRAIL had longer OS in NSCLC studies (and pancreatic cancer studies, as shown below). Thus, though not wishing to be bound by theory, it is believed that apoptosis machinery may be key for the efficacy of trametinib treatment.

Analysis was conducted at Aushon Biosystem (The same analysis was also done for the pancreatic MEK113487 study). Baseline analysis conducted for selected CAF's and CAF's with < 5% LOQ were identified.

The results for TRAIL in the NSCLC are summarized in the K-M plots in figures 1-6 and figure 8 which demonstrate the prognostic and predictive effects of elevated levels of circulating TRAIL on OS and PFS (exemplary data for TRAIL in the pancreatic study is shown in figure 7). Further data accompanying the figures is shown in the following tables:

Table A (to accompany Figure 4):

		Total	Deaths	Censored	% Censored	Median OS (95% CI)
Docetaxel	< median	23	10	13	56.52	4.0 (3.3, NR)
GSK1120212	< median	35	17	18	51.43	3.6 (3.0, NR)
Docetaxel	> median	16	5	11	68.75	NR
GSK1120212	> median	42	9	33	78.57	NR

Table B (to accompany Figure 7)

		Total	Deaths	Censored	% Censored	Median PFS (95% CI)
GSK1120212+Gemcitabine	> median	32	20	12	37.5	9.9 (7.9, 12.1)
Placebo+Gemcitabine	> median	39	27	12	30.77	8.3 (5.6, 10.9)
GSK1120212+Gemcitabine	< median	41	32	9	21.95	5.0 (4.0, 8.0)
Placebo+Gemcitabine	< median	32	22	10	31.25	5.7 (4.0, 9.9)

Table C (to accompany Figure 8)

	Total	Deaths	Censored	% Censored	Median PFS (95% CI)
Docetaxel < median	23	18	5	21.74	1.7 (1.4, 3.0)
GSK1120212 < median	35	25	10	28.57	1.6 (1.4, 2.9)
Docetaxel > median	16	11	5	31.25	4.2 (1.4, 6.7)
GSK1120212 > median	42	32	10	23.81	2.8 (1.5, 3.6)

Furthermore, Figure 7 demonstrates that gemcitabine dampens the apoptotic dependency of trametinib and suggests that treating first or co-treating with gemcitabine may prime patients for successful treatment with trametinib.

Additional data for TRAIL in the NSCLC are as follows:

CAF	ATRTGRP	pvalue	HazardRatio	HRLowerCL	HRUpperCL	Interaction pvalue
TRAIL	Docetaxel	0.5994	0.867	0.509	1.477	
TRAIL	GSK1120212	0.0028	0.533	0.354	0.803	0.2756

Correlation between TRAIL and Sum of Longest Diameters (SLD) at Baseline –NSCLC trial

Variable	SUMLD	PSUMLD
TRAIL	-0.09	0.3607

Change from Baseline – NSCLC trial

BICATCD	ATRTGRP	Median Delta	pValue	Between Rx pValue
TRAIL	Docetaxel	-27.4	0.004	0.0001
TRAIL	GSK1120212	4.9	0.0105	

Change from Baseline and OS – NSCLC trial

BICATCD	ATRTGRP	pValue	Hazard Ratio
TRAIL	Docetaxel	0.8583	↓
TRAIL	GSK1120212	0.1353	↓

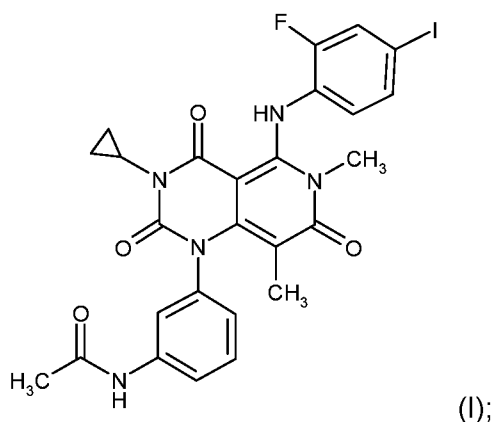
Correlation between change in CAF and max % change SLD

BICATCD	Spearman's	
	R	pValue
TRAIL	-0.12916	0.2761

While the preferred embodiments of the invention are illustrated by the Examples herein, it is to be understood that the invention is not limited to the precise instructions herein disclosed and that the right to all modifications coming within the scope of the following claims is reserved.

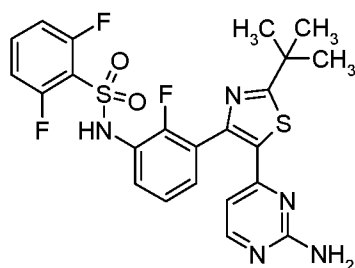
We claim:

1. A method of treating cancer in a mammal comprising determining if said mammal has an elevated level of TRAIL, and if said mammal has an elevated level of TRAIL, administering a pharmaceutical composition comprising at least one tyrosine kinase inhibitor.
2. A method of treating cancer in a mammal comprising determining if said mammal has an elevated level of TRAIL, and if said mammal has an elevated level of TRAIL, administering a pharmaceutical composition comprising at least one MEK inhibitor.
3. The method of any of the preceding claims, wherein the at least one MEK inhibitor comprises a compound of Structure (I):



or a pharmaceutically acceptable salt or solvate thereof.

4. The method of any of the preceding claims, wherein said cancer is selected from melanoma, pancreatic cancer, colorectal cancer, and non-small cell lung carcinoma.
5. The method of any of the preceding claims, wherein said cancer is selected from pancreatic cancer and non-small cell lung cancer.
6. The method of any of the preceding claims, wherein Structure (I) is in the sodium salt form.
7. The method of any of the preceding claims, wherein Structure (I) is in the form of a dimethyl sulfoxide solvate.
8. The method of any of the preceding claims, further comprising administering at least one Braf inhibitor to said mammal.
9. The method of any of the preceding claims further comprising administering at least one Braf inhibitor to said mammal, wherein said Braf inhibitor comprises a compound of Structure (II):



(II)

or a pharmaceutically acceptable salt or solvate thereof.

10. The method of any of the preceding claims, further comprising administering at least one additional anti-neoplastic agent.

11. The method of any of the preceding claims, wherein said determining comprises performing Cytokine and Angiogenesis (CAF) analysis on a sample from said mammal.

12. The method of any of the preceding claims, wherein said determining comprises measuring levels of TRAIL protein expression.

13. The method of any of the preceding claims, wherein said determining comprises measuring levels of TRAIL protein expression in a blood or plasma sample.

14. The method of any of the preceding claims, further comprising obtaining a blood sample from said mammal and measuring the level of TRAIL protein expression relative to a control.

15. The method of any of the preceding claims, further comprising obtaining a blood sample from said mammal and measuring the level of TRAIL protein expression relative to a control, wherein said control comprises a reference sample or a blood sample of a mammal not in need of treatment for cancer.

16. The method of any of the preceding claims, wherein the amount of the Structure I of a pharmaceutically acceptable salt or solvate thereof is an amount selected from 0.125mg to 10mg.

17. The method of any of the preceding claims, wherein the amount of Structure I of a pharmaceutically acceptable salt or solvate thereof is administered once daily at a dose selected from: 1.0 mg/day, 1.5 mg/day and 2.0 mg/day.

18. The method of any of the preceding claims, wherein the amount BRAF inhibitor is an amount selected from 75mg to 1,000mg.

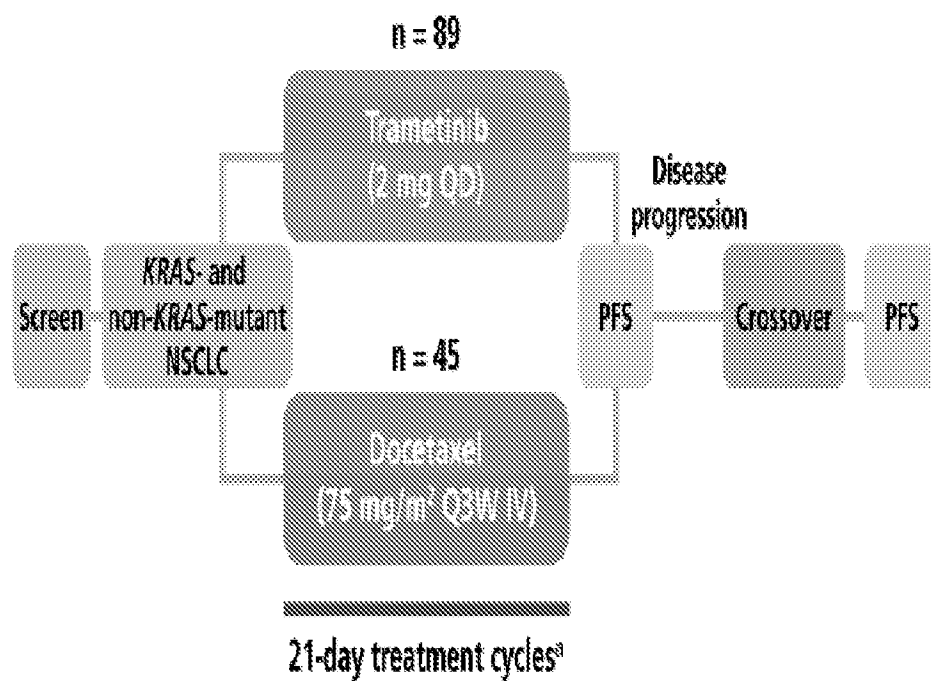
19. The method of any of the preceding claims, wherein the pharmaceutical composition comprising Structure I or a pharmaceutically acceptable salt or solvate thereof and the pharmaceutical composition comprising at least one Braf inhibitor are administered separately.

20. The method of any of the preceding claims, wherein the pharmaceutical composition comprising Structure I or a pharmaceutically acceptable salt or solvate thereof is

administered at the same time as the pharmaceutical composition comprising Structure II or a pharmaceutically acceptable salt or solvate thereof.

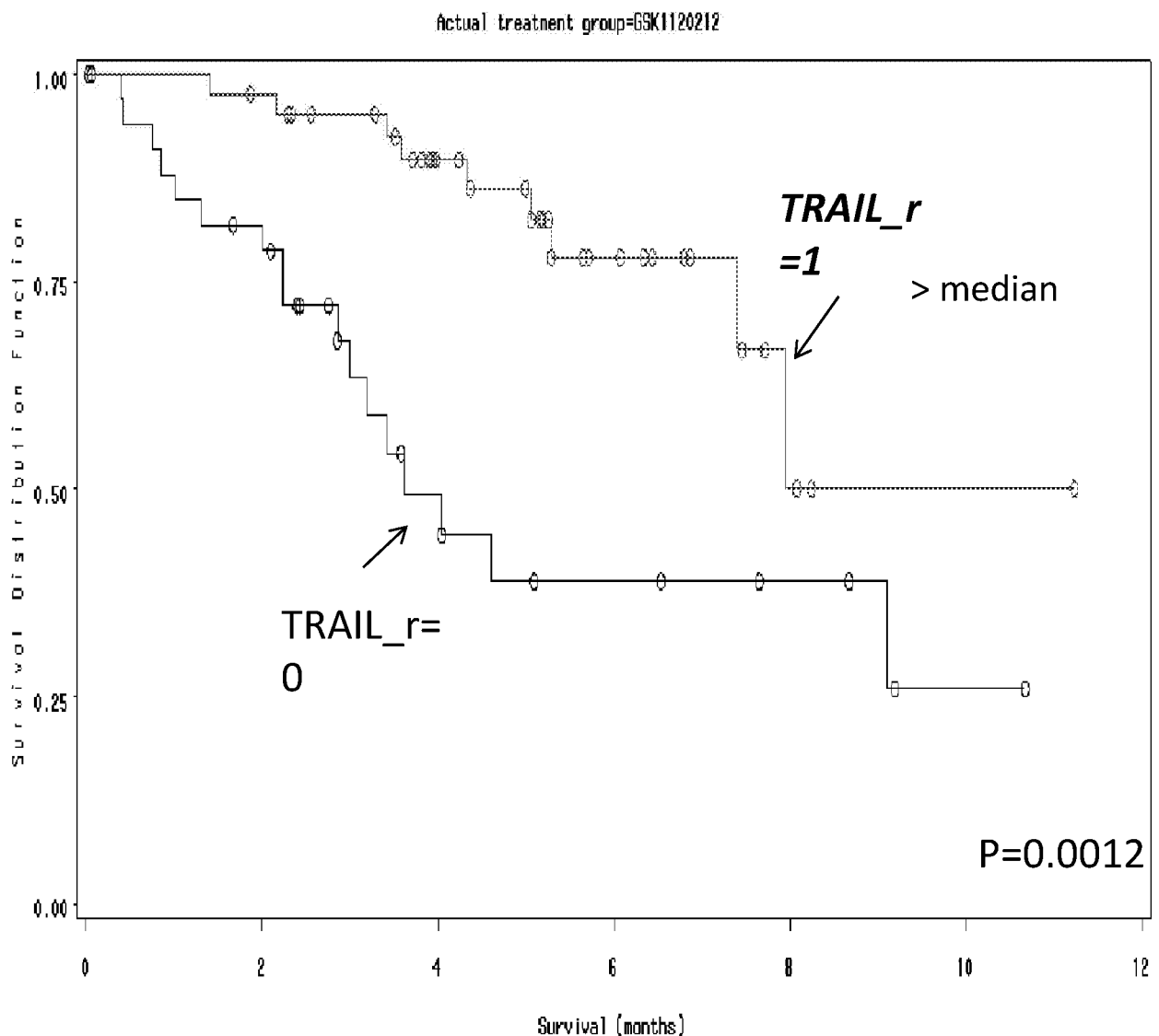
21. The method of any of the preceding claims, wherein said mammal is a human.

Figure 1: MEK114653 NSCLC Study Design



^a Treatment cycles discontinued if disease progression, death, or unacceptable adverse events occur.

Figure 2: TRAIL OS (MEK114653) NSCLC study
Trametinib vs Docetaxel



Bottom line
(black line)

Top
line

Figure 3: TRAIL OS (MEK114653) NSCLC study Trametinib vs Docetaxel

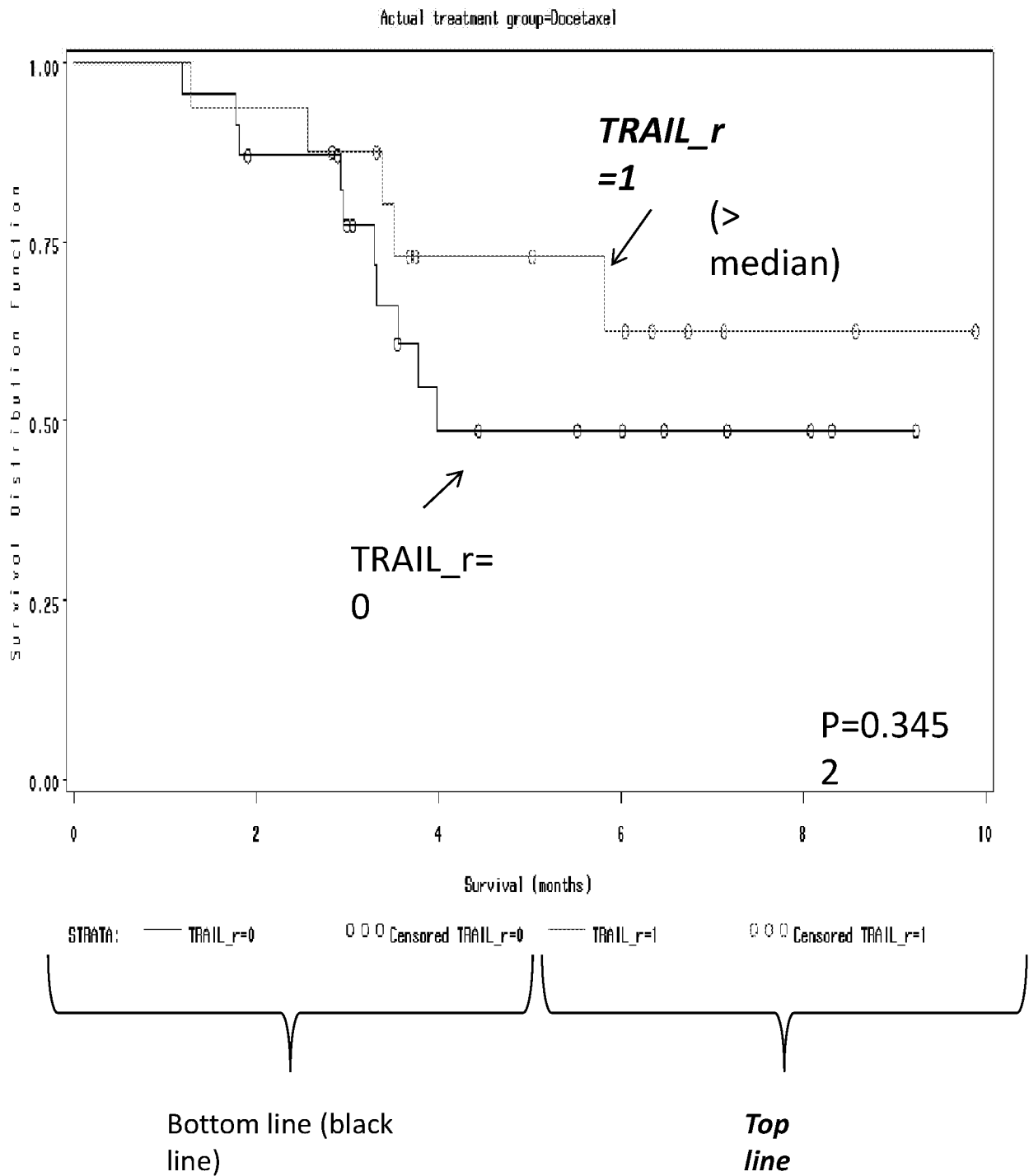
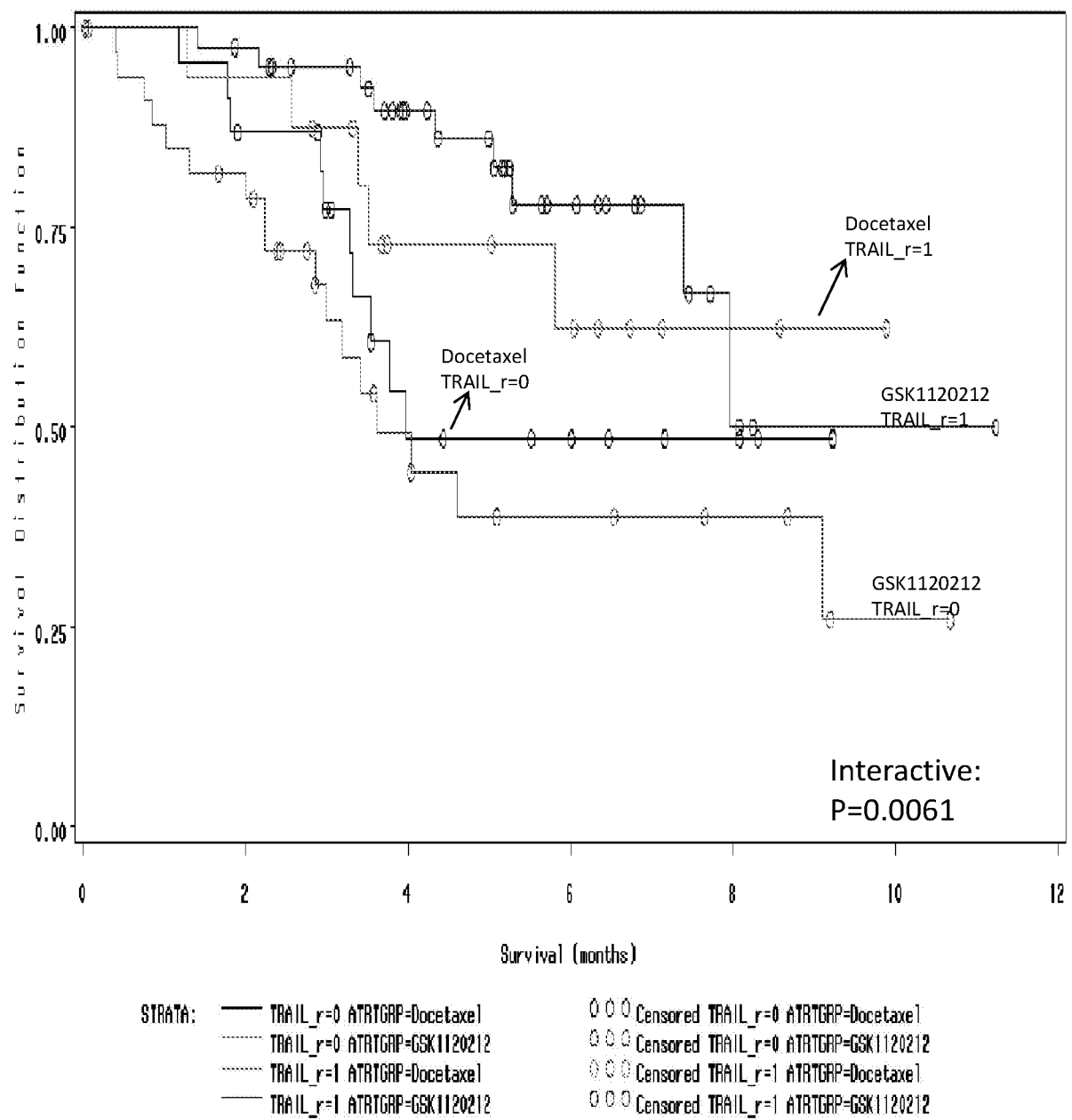


Figure 4: TRAIL OS (MEK114653) NSCLC study
Trametinib vs Docetaxel



r=1, > median

Figure 5: TRAIL OS MEK113487 study
Trametinib+Gemcitabine vs Gemcitabine

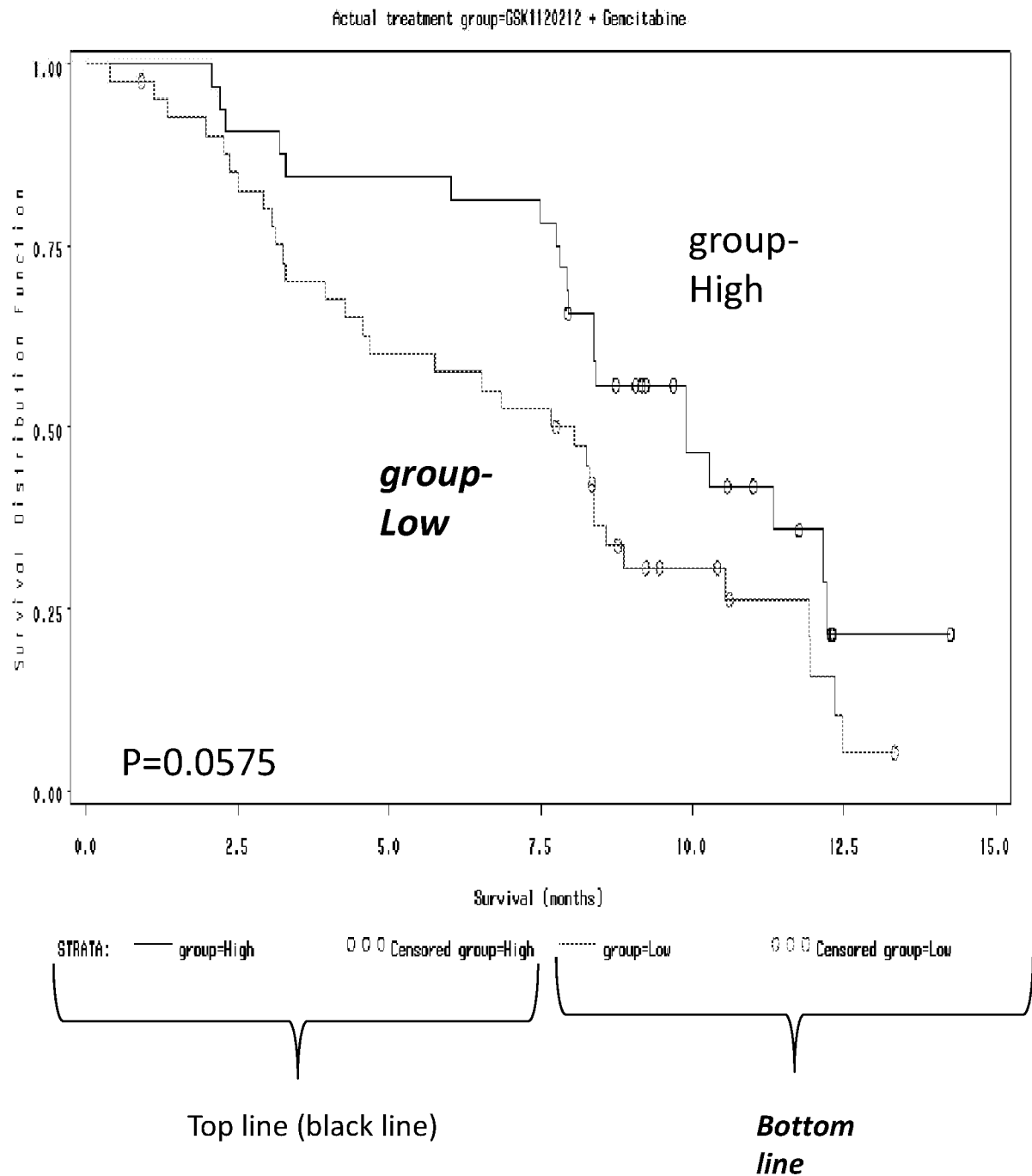


Figure 6: TRAIL OS MEK113487 study
Trametinib+Gemcitabine vs Gemcitabine GemcitabineGe
Gemcitabine

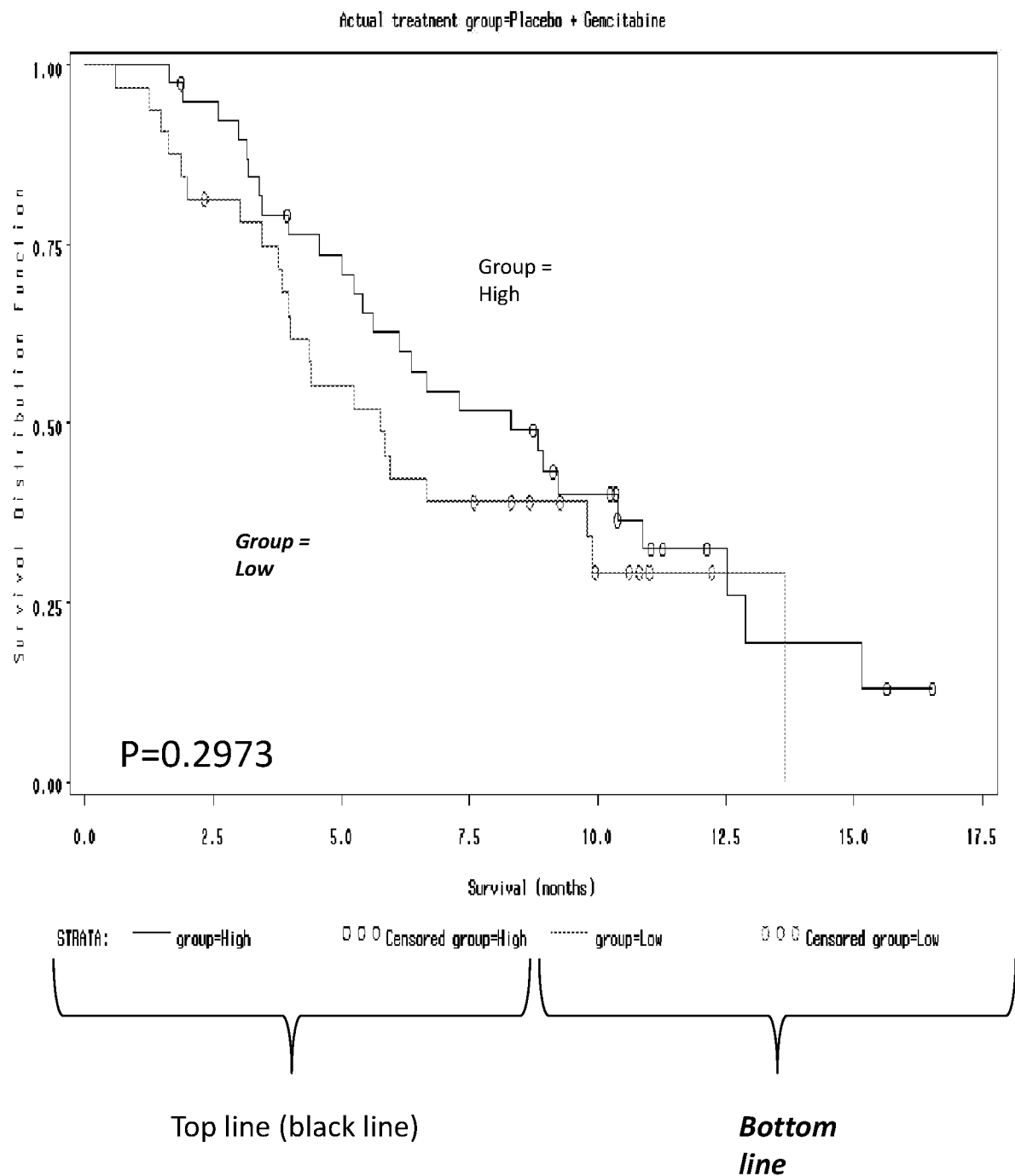
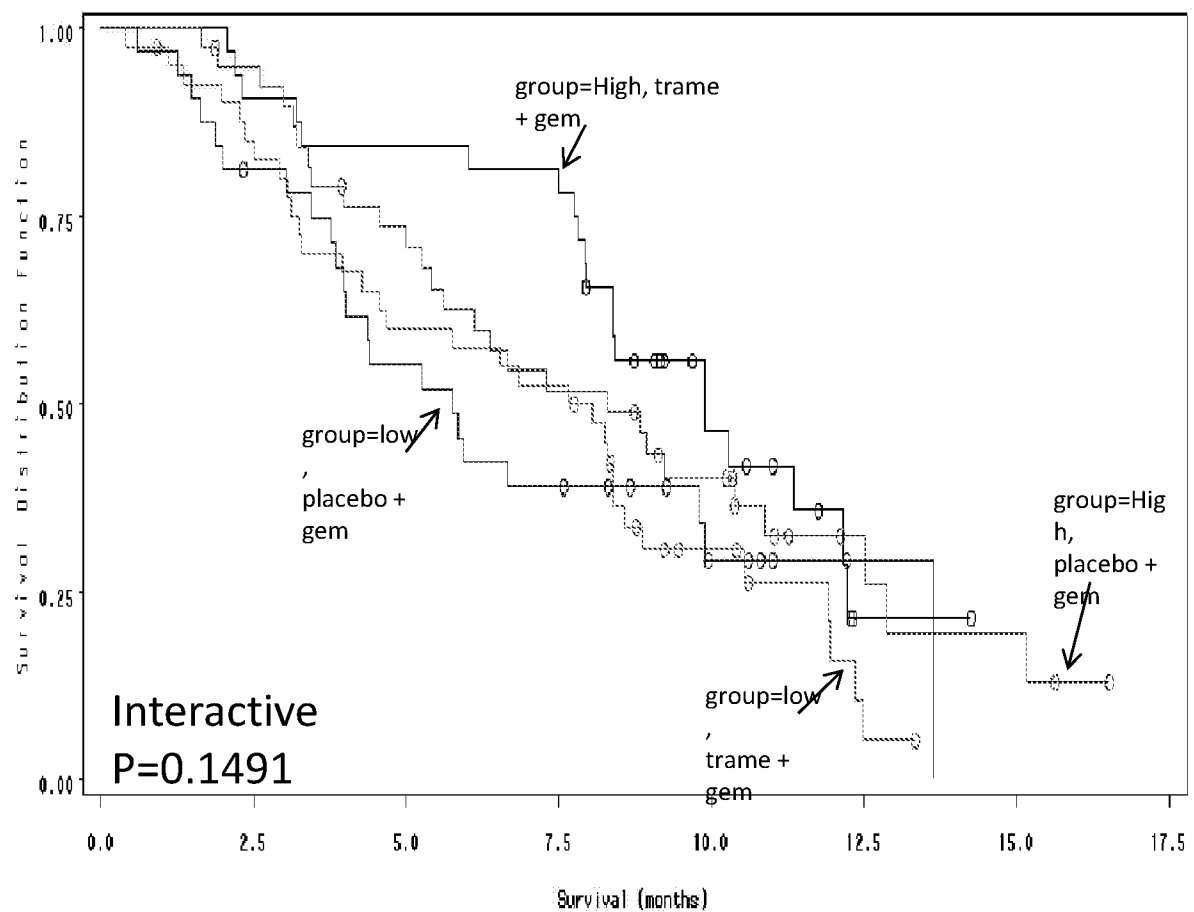


Figure 7: TRAIL OS MEK113487 study Trametinib+Gemcitabine vs Gemcitabine

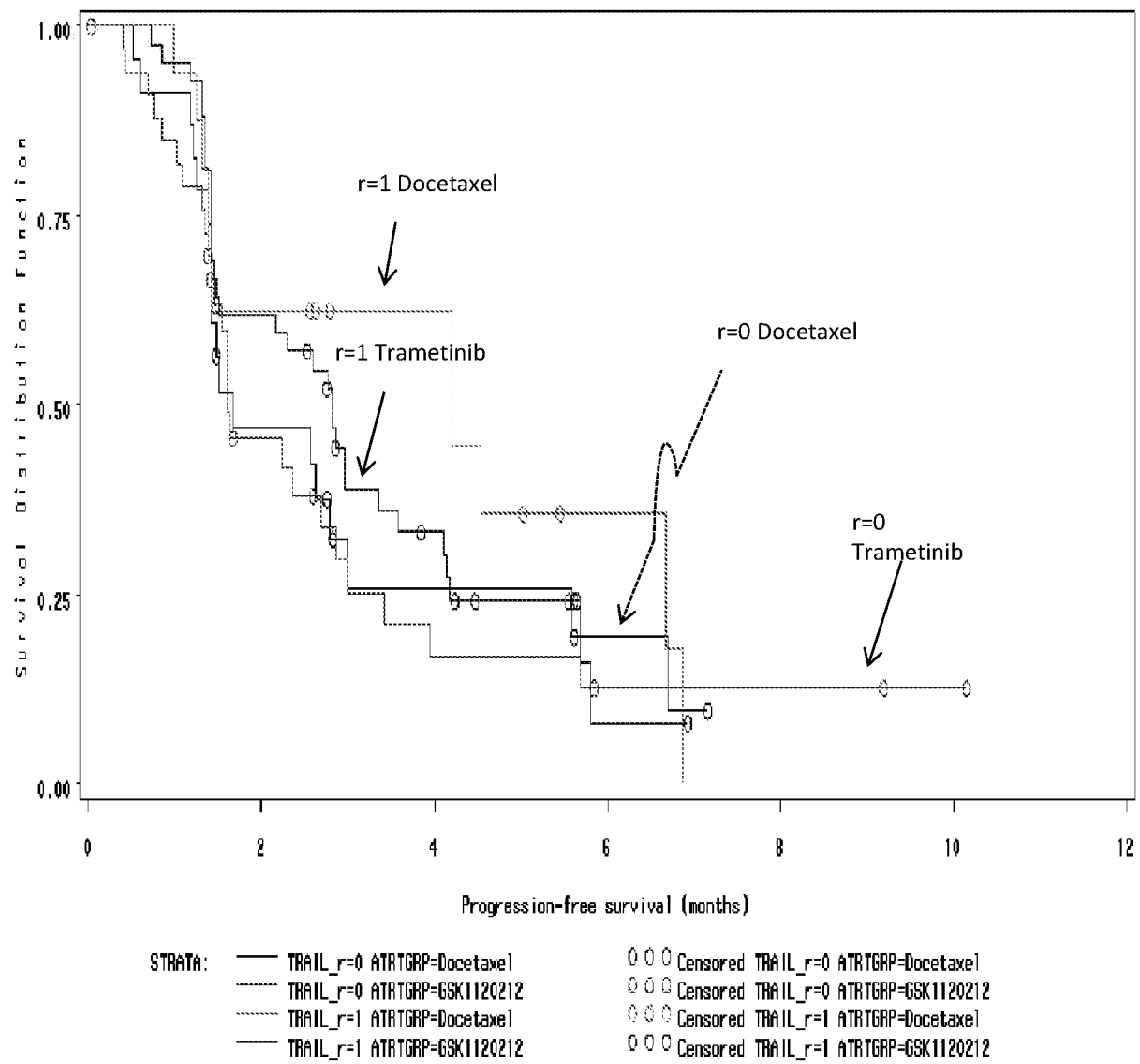


STRATA:

- group=High ATRTGRP=GSK1120212 + Gemcitabine
- group=High ATRTGRP=Placebo + Gemcitabine
- group=Low ATRTGRP=GSK1120212 + Gemcitabine
- group=Low ATRTGRP=Placebo + Gemcitabine

○ ○ ○ Censored group=High ATRTGRP=GSK1120212 + Gemcitabine
 ○ ○ ○ Censored group=High ATRTGRP=Placebo + Gemcitabine
 ○ ○ ○ Censored group=Low ATRTGRP=GSK1120212 + Gemcitabine
 ○ ○ ○ Censored group=Low ATRTGRP=Placebo + Gemcitabine

Figure 8: TRAIL PFS



r=1, > median

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2014/065599

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K31/506 A61K31/519 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, CHEM ABS Data, 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 2012/061683 A2 (GLAXOSMITHKLINE LLC [US]; ALVES-AIVADO MANUEL CARLOS [US]; BORTHAKUR G) 10 May 2012 (2012-05-10) abstract claims 1-31	1-10, 16-21
X	WO 2011/047238 A1 (GLAXOSMITHKLINE LLC [US]; DUMBLE MELISSA [US]; KUMAR RAKESH [US]; LAQU) 21 April 2011 (2011-04-21) abstract page 50 - page 51 page 55 - page 58 claims 1-11	1-5, 7-10,21



Further documents are listed in the continuation of Box C.



See patent family annex.

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

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"&" document member of the same patent family

Date of the actual completion of the international search

16 February 2015

Date of mailing of the international search report

24/02/2015

Name and mailing address of the ISA/

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Authorized officer

Damiani, Federica

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2014/065599

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2012/068468 A1 (GLAXOSMITHKLINE LLC [US]; BLEAM MAUREEN [US]; GILMER TONA M [US]; GREG) 24 May 2012 (2012-05-24) abstract claims 1-5	1-4, 8-10,21
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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/IB2014/065599

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WO 2011047238 A1	21-04-2011	AU 2010306653 A1 CA 2775803 A1 CN 102655753 A CO 6531498 A2 CR 20120155 A DO P2012000091 A EA 201290149 A1 EP 2488033 A1 JP 2013508294 A KR 20120104547 A MA 33746 B1 NZ 598913 A PE 10932012 A1 US 2012196879 A1 US 2014187566 A1 WO 2011047238 A1	10-05-2012 21-04-2011 05-09-2012 28-09-2012 05-07-2012 15-09-2013 30-11-2012 22-08-2012 07-03-2013 21-09-2012 01-11-2012 29-11-2013 04-09-2012 02-08-2012 03-07-2014 21-04-2011
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