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(54) **METHODS OF INDUCING CANCER CELL DEATH AND TUMOR REGRESSION**

VERFAHREN ZUR INDUKTION VON KREBSZELLENTOD UND TUMORREGRESSION

PROCEDES POUR L'INDUCTION DE LA MORT DE CELLULES CANCEREUSES ET LA REGRESSION DE TUMEURS

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EP 1 165 078 B1

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Description

FIELD OF THE INVENTION

[0001] This invention provides the use of an FPT inhibitor or an additional Ras signaling pathway inhibitor in the manufacture of a pharmaceutical composition for treating subjects afflicted with cancers, including tumors and metastatic disease, by administering an FPT inhibitor and an additional Ras signaling pathway inhibitor. In particular, this invention provides the use of an FPT inhibitor or an additional Ras signaling pathway inhibitor in the manufacture of a pharmaceutical composition for use in methods of treating cancer comprising the combined use of (1) a farnesyl protein transferase ("FPT") inhibitor and (2) an additional Ras signaling pathway inhibitor to induce a synergistic level of cancer cell death (apoptotic cell death in particular), thus permitting low dose treatment regimens.

BACKGROUND OF THE INVENTION

[0002] Figure 1 of the present specification shows a simplified linear depiction of a signal transduction pathway that leads to cellular proliferation. This pathway is referred to herein as the "Ras signaling pathway" because Ras is a central relay in this pathway, receiving signals from upstream elements (e.g., growth factor receptors) and transmitting them to downstream elements.

[0003] The signaling pathways initiated by growth factor receptors which lead to cellular proliferation, and in some cases malignant transformation, are being elucidated. Many growth factor receptors such as those for epidermal growth factor (EGF) and platelet-derived growth factor (PDGF), as well as EGF receptor-related molecules (e.g. Her-2/Neu/ErbB2), possess an intrinsic tyrosine kinase activity which is activated by ligand-induced receptor dimerization (Heldin, 1995). This results in autophosphorylation of the receptor on tyrosine residues and the binding of proteins containing Src-homology 2 (SH2) domains.

[0004] Two such SH2 proteins are Grb2 and SHC which indirectly thereof) administered in an amount of 350 mg/day, in two divided doses, on a continuous dosing regimen.

[0005] In another preferred example of combination therapy in the treatment of cancers (*e.g.*, pancreatic, lung or bladder cancer), the FPT inhibitor is SCH 66336, as identified previously, administered orally in an amount of 70 mg/day, in two divided doses, on a continuous dosing regimen; and the additional Ras signaling pathway inhibitor is U0126 (or an analogue thereof) administered in an amount of 350 mg/day, in two divided doses, on a continuous dosing regimen.

[0006] In the use according to this invention, an FPT inhibitor is to be administered concurrently or sequentially with an additional Ras pathway inhibitor. Thus, it is not necessary that, for example, the additional Ras pathway inhibitor and the FPT inhibitor should be administered simultaneously or essentially simultaneously. The advantage of a simultaneous or essentially simultaneous administration is well within the determination of the skilled clinician.

[0007] Also, in general, the FPT inhibitor and the additional Ras pathway inhibitor do not have to be administered in the same pharmaceutical composition, and may, because of different physical and chemical characteristics, have to be administered by different routes. For example, the FPT inhibitor may be administered orally to generate and maintain good blood levels thereof, while the additional Ras pathway inhibitor may be administered intravenously. The determination of the mode of administration and the advisability of administration, where possible, in the same pharmaceutical composition, is well within the knowledge of the skilled clinician. The initial administration can be made according to established protocols known in the art, and then, based upon the observed effects, the dosage, modes of administration and times of administration can be modified by the skilled clinician.

[0008] The particular choice of FPT inhibitor and additional Ras pathway inhibitor will depend upon the diagnosis of the attending physicians and their judgement of the condition of the patient and the appropriate treatment protocol.

activate the plasma membrane-associated, small GTP-binding protein Ras. Ras activation also occurs in response to ligand binding to seven transmembrane domain G-protein coupled receptors (e.g. Gutkind, 1998). Activation of Ras and other growth factor receptor-regulated signaling pathways ultimately leads to changes in the cytoskeleton and gene expression which are necessary for cellular proliferation, differentiation, and transformation (reviewed in Campbell et al., 1998).

[0009] The 3 human *ras* genes (Ha-Ras. N-Ras, and Ki-Ras) encode 4 proteins (due to alternative splicing of the Ki-Ras mRNA). Under normal circumstances, Ras proteins cycle between an active (GTP-bound) state and an inactive (GDP-bound) state. Ras activation occurs by exchange of bound GDP for GTP, which is facilitated by a family of guanine nucleotide exchange factors. Ras inactivation occurs by hydrolysis of bound GTP to GDP. This reaction is facilitated by GTPase activating proteins (GAPs) (Trahey and McCormick, 1987). In many human cancers. Ras proteins become oncogenically activated by mutations which destroy their GTPase activity, and thus deregulate Ras signaling (reviewed in Campbell et al., 1998).

[0010] Multiple candidate Ras effectors exist that may serve downstream of Ras in signal transduction and oncogenic transformation, including members of the Rho family of small GTPases, phosphatidylinositol-3 kinase (PI3K) and the

serine/threonine protein kinase c-Raf-1 (reviewed in Campbell et al., 1998). Raf-mediated signaling is the best characterized Ras effector pathway. Activated Ras recruits Raf to the membrane where Raf activation occurs. Activated Raf is the initial component of a kinase cascade, the Mitogen-Activated Protein Kinase (MAPK) cascade (reviewed in Lowy and Willumsen, 1993; Campbell et al., 1998). Raf phosphorylates and activates the MEK1 and MEK2 (MAPK/ERK kinase) protein kinases which, in turn, phosphorylate and activate the Extracellular signal Regulated Kinases ERK1 and ERK2 (also known as MAPK1 and MAPK2). Unlike their downstream targets, ERK1,2, the MEK1,2 proteins are highly specific enzymes whose only known substrates are the ERK1,2 proteins. Upon activation, ERK1 and ERK2 phosphorylate (and thus regulate) a variety of target proteins, including nuclear transcription factors, leading to the ultimate cellular response. This linear pathway of Ras signaling is diagrammed in Figure 1.

[0011] The importance of these signaling pathways in the abnormal growth of cancer cells is indicated by the finding that growth factor receptor and Ras pathway components are often mutated and/or overexpressed in cancer. For example, Ras is mutationally activated in about 30% of human cancers including a high percentage of major epithelial cancers such as lung, colon and pancreatic cancers. Additionally, overexpression of growth factor receptors occurs in a number of cancers (e.g. overexpression of the Her-2/Neu receptor occurs in about 30% of human breast cancer). These observations have led to the pursuit and development of agents designed to block individual components of either signal transduction pathway. While such agents hold potential as novel cancer therapeutics, many inhibitors of signal transduction are thought to act in a cytostatic rather than a cytotoxic fashion by blocking the cell's progression through the cell cycle. This distinguishes them from traditional cancer chemotherapy drugs in being less toxic but also possessing less dramatic antitumor activity.

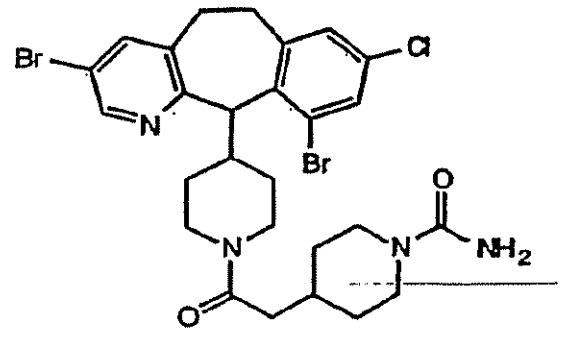
[0012] WO 97/45412 discloses the use of a MEK inhibitor and a farnesyl protein transferase (FPT) inhibitor in the treatment of cancer. The teaching does not disclose any unexpected benefits from the administration of the two drugs in combination. Potentiating agents, such as fused-ring benzocycloheptapyridine compounds, which enhance the efficacy of anti-neoplastic agents are disclosed in WO 92/11034. In document WO 97/23478, novel tricyclic amide compounds which are capable of inhibiting farnesyl protein transferase are demonstrated to be useful in the treatment of proliferation diseases. The novel compounds include SCH66336, which is further demonstrated by Liu, M *et al*, (1998) *Cancer Research*, vol. 58, no. 21, pages 4947-4956, to have anti-tumor activity in human tumor zenograft models and Wap-*ras* transgenic mice. Alessi, D.R. *et al*, (1995) *J Biol. Chem*, vol. 270, no. 46, pages 27489-27494, demonstrate the activity of the compound PD 098059 as a specific inhibitor of the activation of mitogen-activated protein kinase kinase *in vitro* and *in vivo*

[0013] There remains a challenge to provide new and improved methods of treating cancer. For instance, to treat tumorigenic cancer cells, it would be highly desirable to provide new methods that achieve a dramatic and selective induction of cancer cell death while minimizing potential toxic side effects against normal, untransformed cells. The present invention provides just such methods of treatment.

SUMMARY OF THE INVENTION

[0014] The present invention provides the use of a farnesyl protein transferase (FPT) inhibitor in the manufacture of a pharmaceutical composition for treating cancer in a patient by administering:

(1) an FPT inhibitor of the following formula

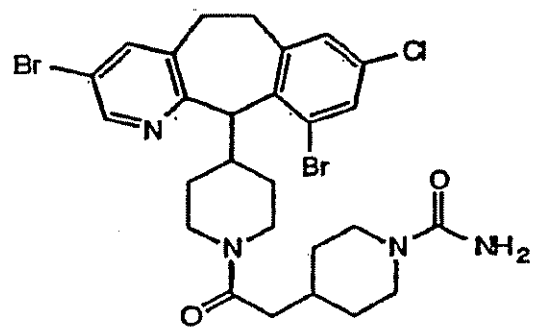


(+) - enantiomer

and (2) an additional Ras signalling pathway inhibitor in amounts effective to induce a synergistic level of cancer cell death.

[0015] The present invention further provides the use of an additional Ras signalling pathway inhibitor in the manufacture of a pharmaceutical composition for treating cancer in a patient by administering:

(1) an FPT inhibitor of the following formula



(+) - enantiomer

and (2) an additional Ras signalling pathway inhibitor in amounts effective to induce a synergistic level of cancer cell death.

[0016] The compositions of the present invention achieve an unexpectedly dramatic induction of cancer cell death (apoptotic cell death in particular). The effects are synergistic, and highly selective against transformed cells (particularly tumorigenic cancer cells), thus enabling the use of low doses to minimize potential toxic side effects against normal, untransformed cells. Moreover, the compositions of the present invention were surprisingly found to have a long-lasting, sustained effect on blocking cell signaling, again while minimizing potential toxic side effects against normal, untransformed cells. None of these effects, let alone their magnitude, could have been predicted prior to the present invention. Furthermore, taking advantage of the surprising synergy and sustained, long-lasting effects of this invention, special low-dose compositions are provided so that cancer cell death is effectively achieved while, at the same time, maintaining low risk of potential toxic side effects on normal, untransformed cells. The compositions of the present invention are particularly useful for the treatment of various tumorigenic cancers, especially epithelial cancers, (*e.g.*, pancreatic cancer, ovarian cancer, prostate cancer, lung cancer, breast cancer, colorectal cancer, and bladder cancer), and melanoma.

BRIEF DESCRIPTION OF THE DRAWINGS

[0017] The FPT Inhibitory Compound referred to in Figures 1 through 7 (sometimes referred to as "SCH 66336") is as follows:

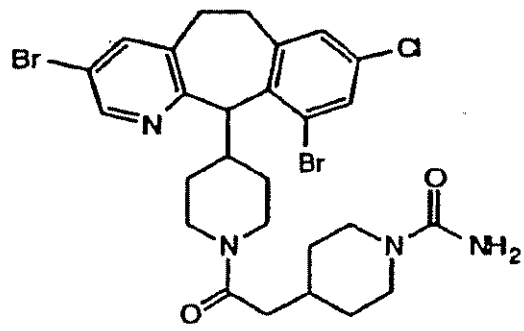


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Figure 1: Ras Signal Transduction: Schematic representation of the components of the Ras/MAPK signal transduction pathway. This linear pathway from growth factor receptor to ERK activation was the first Ras-mediated

pathway to be elucidated. Also indicated are steps targeted by various inhibitors including the FPT inhibitor SCH 66336 and the MEK inhibitors PD098059 and U0126.

Figure 2: The dose-dependent apoptotic response to **treatment with PD098059 is enhanced by addition of SCH 66336:** H-Ras-CVLS-transformed Rat2 cells were treated for 36 hours with the indicated concentrations of PD098059 (A385-023-MOOS; Alexis Corporation), either alone or in a combination with SCH 66336. The cells were harvested by trypsin/EDTA treatment, fixed in Acctone/Methanol (50%:50%) at -20°C for 30 min, extensively washed with PBS, and labeled for 30 min at room temperature with PBS containing 75 μg/ml propidium iodide (PI; Calbiochem; La Jolla, CA), and 500 μg/ml RNase (Sigma; St. Louis, MO). Apoptosis was measured by propidium iodide staining of chromosomal DNA with FACS analysis of the cell population (FACS-Calibur, Becton-Dickinson; Mountain View, CA). The concentration of PD098059 was varied from 0.25 to 20 μM in the presence (▲) or absence (■) of 100 nM SCH 66336.

Figure 3: The dose-dependent apoptotic response to treatment with SCH 66336 is enhanced by addition of PD098059: H-Ras-CVLS-transformed Rat2 cells were treated for 36 hours with the indicated concentrations of SCH 66336, either alone or in a combination with PD098059. Analysis was performed as described in the description for Figure 2 above. The concentration of SCH 66336 was varied from 0.0125 to 0.75 μM in the presence (▲) or absence (■) of 2.5 μM PD098059.

Figure 4: Effect of SCH 66336 and U0126 on Apoptosis measured by FACS: H-Ras-transformed Rat2 cells were treated for 24 hours with 0 to 10 μM U0126 (#V1121; Promega Corporation; Madison, WI) in the presence or absence of 0.5 μM SCH 66336. Analysis was performed as described in the legend to Fig. 2. 1= Untreated Cells; 2= SCH 66336; 3= 1 μM U0126; 4= 1 μM U0126 + SCH 66336; 5= 5 μM U0126; 6= 5 μM U0126 + SCH 66336; 7= 10 μM U0126; 8= 10 μM U0126 + SCH 66336.

Figure 5: Effect of SCH 66336 and PD098059 on Apoptosis measured by Caspase Activation: H-Ras-transformed Rat2 and parental Rat2 cells were treated for 24 hours with 20 μM PD098059, 0.5 μM SCH 66336, or a combination of the two drugs. Cells were lysed in a detergent buffer recommended by Clontech (Apo-Alert CPP32/Caspase-3 Assay) and centrifuged at 14,000 rpm for 15 min at 4°C to pellet the cellular debris. Protein concentration of the resulting supernatant was determined by a BCA protein assay (Pierce; Rockford, IL) with 175 μg of each lysate assayed for Caspase-3 activity using a fluorogenic peptide substrate (AC-DEVD-AMC; Clontech; Palo Alto, CA) by fluorometry (CytoFluor plate reader; Perseptive Biosystems; Framingham, MA). 1= Untreated H-ras Cells; 2= H-ras cells + SCH 66336; 3= H-ras cells + PD09059; 4= H-ras cells + SCH 66336 + PD 098959; 5= Untreated Rat2 Cells; 6= Rat2 cells + SCH 66336; 7= Rat2 cells + PD09059; 8= Rat2 cells + SCH 66336 + PD 098959.

Figure 6: Effect of SCH 66336 and PD098059 on ERK1 and ERK2 phosphorylation: H-Ras-transformed Rat2 cells were treated with 20 μM PD098059 or 0.5 μM SCH 66336 for 0 to 36 hr. Cells were lysed in a detergent buffer and centrifuged at 14,000 rpm for 15 min at 4°C to pellet the cellular debris. Protein concentration of the resulting supernatant was determined by a BCA protein assay (Pierce; Rockford, IL). Cellular proteins (20 μg) were separated by 8-16% Tris-Glycine polyacrylamide gel electrophoresis (Novex; San Diego, CA). Proteins were then transferred to PVDF membranes for Western Blot analysis. Phosphorylated ERK1 and ERK2 were detected using a rabbit polyclonal antibody specific for the phosphorylated p42/44 MAPK proteins (phospho-Thr202/Tyr204 specific; #9101; New England Biolabs. Inc.; Beverly MA). Total ERK1 and ERK2 were detected using a rabbit polyclonal antibody specific for the p42/44 MAPK proteins (#9102; New England Biolabs, Inc.; Beverly, MA). Both antibodies were recognized with Goat anti-Mouse-HRP antibody (horseradish peroxidase; Chemicon; Temecula. CA) and visualized by enhanced chemiluminescence (SuperSignal West Pico Chemiluminescent Substrate; Pierce; Rockford, IL).

Figure 7: Intracellular Signal Transduction Pathways: Figure 1 diagrammed a linear pathway leading from growth factor receptors through Ras to activation of the MAPK cascade. It is clear that signaling pathways are considerably more complex with multiple branches and interconnections. Some of this complexity is illustrated here in Figure 7.

DETAILED DESCRIPTION OF THE INVENTION

[0018] The present invention provides novel methods of treating cancer by combining (1) a farnesyl protein transferase (FPT) inhibitor, and (2) an additional Ras pathway signaling inhibitor.

(1) A "farnesyl protein transferase inhibitor" or "FPT inhibitor" or "FTI" is defined herein as a compound which: (i)

potently inhibits FPT (but preferably not geranylgeranyl protein transferase I, in vitro); (ii) blocks the phenotypic change induced by a form of transforming *H-ras* which is a farnesyl acceptor (but preferably not by a form of transforming *H-ras* engineered to be a geranylgeranyl acceptor); (iii) blocks intracellular farnesylation of *ras*; and (iv) blocks abnormal cell growth.

(2) A "Ras signaling pathway inhibitor" is defined herein as an agent that blocks the activity of any protein in the signal transduction pathway shown in Fig. 1. A particularly preferred Ras signaling pathway inhibitor is a "MEK inhibitor", which is defined herein as an agent that blocks the in vitro enzyme activity of a MEK (MAPK/ERK kinase) protein (preferably inhibiting MEK1 and MEK2), and thus blocks the activation of a MAPK protein as evidenced by a block in the phosphorylation of the MAPK protein. This can be detected by western blot analysis for phosphorylated MAPK as described in, *e g.*, Dudley et al., Proc Natl Acad Sci. 92:7686-7689 (1995), and Favata et al., J Biol Chem. *273*:18623-32 (1998).

1. **FPT Inhibitors**

[0019] As single agents, or in combination with chemotherapy (see, e g., Liu *et al*, 1998), FPT inhibitors represent a leading approach for blocking the function of Ras oncoproteins. FPT catalyzes the addition of an isoprenyl lipid moiety onto a cysteine residue present near the carboxy-terminus of the Ras protein. This is the first step in a post-translational processing pathway that is essential for both Ras membrane-association and Ras-induced oncogenic transformation. A number of FPT inhibitors have been reported, including a variety of peptidomimetic inhibitors as well as other small molecule inhibitors, most notably the tricyclic FPT inhibitors exemplified by SCH 66336. FPT inhibitors interfere with the post-translational processing of Ras proteins in cells and demonstrate antitumor activity in a wide variety of in vitro and in vivo cancer models (Bishop et al., 1995; Liu et al., 1998). The antitumor activity of SCH 66336 includes inhibition of anchorage-independent growth of a variety of human tumor cell lines in vitro and their growth as xenografts in immunocompromised mice (Liu et al., 1998). Human tumor cell lines differ significantly in their sensitivity to the growth effects of FPT inhibitors. Sensitivity or resistance does not correlate with Ras mutational status.

[0020] In several transgenic mouse tumor models (e.g. MMTV-H-Ras, WAP-H-Ras, TGFα and TGFα/neu) significant tumor regressions are induced by treatment with FPT inhibitors. These regressions are associated with an increase in apoptosis (Liu et al 1998; Barrington et al., 1998; Norgaard et al., 1999). FPT inhibitors can also induce apoptosis of transformed cells in culture. The apoptotic effect in vitro has been reported to require growth in low serum or forced growth in suspension (Hung and Chaung, 1998; Lebowitz et al., 1997; Suzuki et al., 1998).

[0021] It has also been shown that FPT inhibitor treatment reduces the activity of the MAPK pathway in Ha-Ras-transformed Rat1 cells (e g. James et al., 1994). This decrease in MAPK activity correlates with a decrease in cell growth. FPT inhibitors did not reduce MAPK activity in untransformed Ratlcells.

2. **Agents Targeting MEK:**

[0022] The MAPK pathway has also been examined as a target for the development of anti-cancer therapeutics and the effects of specific inhibitors of this pathway on tumor cell lines have been described (Dudley et al., 1995; Favata et al., 1998). The best-characterized MEK inhibitor is PD098059, a small molecule that inhibits the activity of MEK1 and MEK2 via direct binding in a manner that is non-competitive with respect to either substrate (ATP or ERK protein). This results in decreased MEK1 and MEK2 phosphorylation and decreased activation of the MEK substrates, ERK1 and ERK2. PD098059 treatment blocks growth factor-mediated proliferation and anchorage-independent growth of Ras-transformed cells (Alessi et al, 1995, J Biol Chem. 270:27489-27494).

[0023] Recently, a novel MEK inhibitor, U0126, was reported which binds to MEK with a higher affinity than PD098059 (Favata et al., 1998). For more detailed information on MEK inhibitors, and methods of preparing MEK inhibitors, reference can be made, e g., to international patent publications WO 99/01421 (January 14, 1999) and WO 99/01426 (January 14, 1999).

3. **Agents Targeting Growth Factor Receptors:**

[0024] Two primary approaches have been taken to block growth factor receptor signaling pathways: (i) monoclonal antibodies directed against the receptor; (ii) inhibitors of the receptor tyrosine kinase activity; and (iii) antisense nucleic acids to block protein expression. Anti-receptor monoclonal antibodies include those targeting the erbB2 receptor (e.g. Genentech's HERCEPTIN®/trastuzumab) and those targeting the EGF receptor. The best characterized anti-EGF receptor antibody is the chimeric antibody C225 (Goldstein et al. (1995), Clin Cancer Res. *1*:1311-1318). Both HERCEPTIN® and C225 have demonstrated efficacy in preclinical tumor models in which their cognate receptors are expressed.

[0025] Small molecule inhibitors of tyrosine kinase activity have also been reported with at least two of these compounds

already in human clinical trials: Sugen's PDGF receptor inhibitor, SU101, which is in phase III clinical trials for glioma and earlier stage trials for other cancer indications, and Pfizer's EGF receptor inhibitor, CP-358,774, which is in early phase clinical trials (Moyer et al. (1997), Cancer Res. *57*: 4838-4848).

4 Other Signaling Antagonists:

[0026] In addition to the approaches described above, other elements of the Ras signaling pathway and other signal transduction pathways have been targeted for cancer drug discovery. The SH2 proteins (SHC and Grb2), which link growth factor receptors to Ras activation, have been targeted by peptidomimetic agents that block the binding of SH2 domains to phosphotyrosine-containing protein sequences.

[0027] The protein kinase Raf, which links Ras to MEK1,2 activation, has also been targeted both by small molecule kinase inhibitors and by antisense approaches. The latter approach (ISIS-5132) is in phase II clinical trials (Monia et al., 1996)

[0028] Other relevant intracellular signaling targets include the phospho-lipid kinase PI3K (phosphatidylinositol-3 kinase) and protein kinase C.

[0029] In preferred embodiments, the methods of the present invention can be used to treat tumorigenic cancer cells by having a significant effect on cell death (e.g. by apoptosis) in the case of the cancerous cells (i.e., having a significant effect on cell death beyond mere arrest of growth) while, at the same time, the active agents can be administered in relatively low doses (and/or less frequently) to minimize potential toxic side effects against normal, untransformed cells. In addition, the present invention provides new methods of treating cancer by providing a longer, more sustained effect on blocking cell signaling, while, at the same time, minimizing the risk of potential toxic side effects against normal cells.

[0030] Thus, the present invention also provides methods of inducing a synergistic level of cancer cell death (*e.g.*, apoptosis) in a cancer patient, comprising administering, concurrently or sequentially, effective amounts of (1) a FPT inhibitor and (2) an additional Ras signaling pathway inhibitor (*i.e.*, in amounts sufficient to induce a synergistic level of cancer cell death as measured, *e.g.*, by the propidium iodide fluorescence assay described in Dengler *et al*, (1995) Anticancer Drugs *6*:522-32. Similarly, methods are provided herein for killing cancer cells in a cancer patient (as measured by the assay of Dengler *et al* 1995) comprising administering effective amounts of (1) a FPT inhibitor and (2) an additional Ras signaling pathway inhibitor.

[0031] Furthermore, in preferred embodiments, the methods of the present invention include methods for treating tumors and regressing tumor volume (*e.g.*, as measured by CAT scan) in a patient in need of such treatment (*e.g.*, a mammal such as a human) by administering, concurrently or sequentially, (1) an FPT inhibitor and (2) an additional Ras signaling pathway inhibitor in amounts sufficient to achieve. Examples of tumors which may be treated include, but are not limited to, epithelial cancers, e.g., prostate cancer, lung cancer (e.g., lung adenocarcinoma), pancreatic cancers (*e.g.*, pancreatic carcinoma such as, for example, exocrine pancreatic carcinoma), breast cancers, colon cancers (*e.g.*, colorectal carcinomas, such as, for example, colon adenocarcinoma and colon adenoma), ovarian cancer, bladder carcinoma, and cancers of the liver. Other cancers that can be treated include melanoma, myeloid leukemias (for example. acute myelogenous leukemia), sarcomas, thyroid follicular cancer, and myclodysplastic syndrome.

[0032] Pharmaceutical compositions comprising an FPT inhibitor and an additional Ras signaling pathway inhibitor, for the treatment of cancer (including induction of cancer cell death and tumor regression), and preparation of such compositions, are also provided by the present invention.

[0033] As used herein the following terms have the following meanings unless indicated otherwise:

[0034] "Growth factor receptor inhibitor": an agent that blocks the signal transduction properties of a growth factor receptor. These may act as direct inhibitors of the receptor's tyrosine kinase activity or by inhibiting ligand-stimulated activation of the receptor kinase activity as described in Levitzki and Gazit, 1995 (Science. 267:1782-1788).

[0035] "Tyrosine kinase inhibitor": an agent that blocks the tyrosine phosphorylation activity by either being competitive with ATP or via an allosteric interaction with the enzyme as described in Levitzki and Gazit, 1995.

[0036] "Protein kinase inhibitor": an agent that blocks protein phosphorylation activity on serine, threonine, or tyrosine residues as described in Levitzki and Gazit, 1995.

[0037] "p185 erbB2 / HER2 / neu receptor inhibitor" or "erbB2 receptor inhibitor": an agent that blocks the signal transduction properties of the erbB2 receptor by either inhibiting the receptor's tyrosine kinase activity or blocking ligand-stimulation of the receptor's kinase activity as described in Levitzki and Gazit, 1995.

[0038] "PDGF receptor tyrosine kinase inhibitor": an agent that blocks the signal transduction properties of the platelet-derived growth factor (PDGF) receptor by either inhibiting the receptor's tyrosine kinase activity or blocking PDGF-stimulation of the receptor's kinase activity as described in Kovalenko, M., et al. (1994). Cancer Res. *54*:6106-6114.

[0039] "EGF receptor tyrosine kinase inhibitor": an agent that blocks the signal transduction properties of the epidermal growth factor (EGF) receptor by either inhibiting the receptor's tyrosine kinase activity or blocking EGF-stimulation of the receptor's kinase activity as described in Fry et al (1994), Science *9*:1093-1095.

[0040] "An antibody directed against the extracellular domain of a growth factor receptor": such antibody blocks the

biological activity of the growth factor receptor by inhibiting the binding of ligand and/or preventing ligand-stimulated activation of the receptor tyrosine kinase as described in Mendelsohn, J. (1992) J Nat'l Cancer Inst Monogr *13*:125-131.

[0041] "A monoclonal antibody which targets the p185 erbB2 / HER2 / neu receptor" or "A monoclonal antibody which targets the erbB2 receptor": such antibody blocks the biological activity of the HER2 receptor as shown by inhibiting the binding of ligand and/or preventing ligand-stimulated activation of the growth factor receptor kinase as described in Pegram et al., 1998; See also Carter et al. (1992), Proc. Nat'l Acad. Sci. *89*:4285-4289.

[0042] "A monoclonal antibody which targets the EGF receptor": shown by a monoclonal antibody which inhibits EGF binding and/or EGF-stimulated kinase activity as described in Mendelsohn, J. (1992) J Nat'l Cancer Inst Monogr *13*: 125-131.

[0043] "An antisense molecule directed against a growth factor receptor or other component in the Ras signal pathway": a modified oligonucleotide which interferes with messenger RNA translation (and hence protein expression) of any protein component in the pathway as described in Wang et al., 1998 or Resnicoff, 1998. For a general discussion of antisense technology. see, *e g*, *Antisense DNA and RNA*, (Cold Spring Harbor Laboratory, D. Melton, ed., 1988).

[0044] "Concurrently": (1) simultaneously in time, or (2) at different times during the course of a common treatment schedule; and

[0045] "Sequentially": (1) administration of one component of the method ((a) FPT inhibitor, or (b) an additional Ras pathway inhibitor) followed by administration of the other component; after adminsitration of one component, the second component can be administered substantially immediately after the first component, or the second component can be administered after an effective time period after the first component; the effective time period is the amount of time given for realization of maximum benefit from the administration of the first component.

[0046] "Downstream" is defined herein as a protein activity (within the Ras signaling pathway) which is regulated by Ras either directly via protein:protein binding or indirectly by a Ras-regulated effector protein. Thus, with reference to Figure 1. an "element downstream from Ras" can be, *e g*, Mek1,2 or Erk1,2.

[0047] "Upstream" is defined herein as a protein activity (within the Ras signaling pathay) which would regulate the activity of Ras either directly via protein:protein binding or indirectly by regulating another protein which directly binds to and regulates Ras activity. Thus, an "element upstream of Ras" can be, e.g., erbB2, PDGF receptor, IGF receptor, or EGF receptor.

[0048] "Cell death" as described herein is the death of a cell induced either under physiological conditions or by acute injury resulting in the disassembly of the cell organelles and proteins and the abolition of metabolic processes as reviewed in Raff, M. (1998) Nature. *396*:119-122. Cell death can be measured, *e g*, by the propidium iodide flow cytometry assay described in Dengler *et al.*, (1995) Anticancer Drugs. 6:522-32.

[0049] "Apoptosis" as described herein as a form of cell death (programmed cell death) that exhibits stereotypic morphological changes as reviewed in Raff, M. (1998). Nature. 396:119-122. Apoptosis can be measured, *e g*, by the propidium iodide flow cytometry assay described in Dengler *et al*, (1995) Anticancer Drugs. *6*:522-32, or by the *in situ* terminal deoxynucleotidyl transferase and nick translation assay (TUNEL analysis) described in Gorczyca, (1993) Cancer Res *53*:1945-51

[0050] "Synergistic" or "synergistic level" is defined herein as an effect achieved by the combination of two components that is greater than the sum of the effects of either of the two components alone (keeping the amount of the component constant). Thus, for example, the phrase "amounts effective to induce a synergistic level of cancer cell death" refers to amounts of two components that achieve a level of cancer cell death (*e g*, cell death by apoptosis as measured by the propidium iodide flow cytometry assay described in Dengler *et al.*, (1995) Anticancer Drugs. *6*:522-32, or by the *in situ* terminal deoxynucleotidyl transferase and nick translation assay (TUNEL analysis) described in Gorczyca, (1993) Cancer Res 53:1945-51), which is greater than the sum of the effects of either of the two components alone.

[0051] "Sustained effect" is defined herein as a prolonged/enhanced apoptotic response to combination treatment with a FPT I and a MEK1,2 inhibitor in comparison to single treatment alone. The consequences of a "sustained effect" can be monitored either by measurement of MAPK activity or cell death or apoptosis, as described in previously. The effective time course for inhibition of MAPK pathway by the individual drugs is dose dependent. However, the experiments herein show that the MEK1,2 inhibitors optimally inhibit the MAPK pathway at or prior to 6 hr of treatment, while SCH 66336 demonstrates optimal MAPK pathway inhibition 12-18 hr after treatment The MAPK inhibitory effect of SCH 66336 has been shown to last as long as 72 hr after treatment. Thus, combination of the two drugs can result in a "sustained" inhibition of the MAPK pathway for a long period of time, preferably for a period starting at or just prior to 6 hours after treatment. and preferably continuing through to 36 hours, more preferably 72 hours, post treatment. (See; *e g*, Fig. 6).

[0052] The phrase "killing cancer cells" means induction of cancer cell death of transformed, tumorigenic cancer cells.

USE OF CHEMOTHERAPY AND/OR RADIATION THERAPY AS ADDITIONAL AGENTS IN THE TREATMENTS OF THE PRESENT INVENTION

[0053] Chemotherapeutic agents and/or radiation can optionally be added to treatment regimens of the present in-

vention (in addition to the combination of (1) a farnesyl protein transferase (FPT) inhibitor, and (2) an additional Ras pathway signaling inhibitor). For use of chemotherapy and/or radiation therapy in combination with only an FPT inhibitor, reference can be made to Liu, M., et al. Cancer Res. *58*:4947-4956 (1998) and U.S. Patent Application USSN 09/217,335. expressly incorporated herein by reference.

[0054] Classes of compounds that can be used as the chemotherapeutic agent include: alkylating agents, antimetabolites. natural products and their derivatives, hormones and steroids (including synthetic analogs), and synthetics. Examples of compounds within these classes are given below.

[0055] Alkylating agents (including nitrogen mustards, ethylenimine derivatives, alkyl sulfonates, nitrosoureas and triazenes): Uracil mustard, Chlormethine, Cyclophosphamide (Cytoxan®), Ifosfamide, Melphalan, Chlorambucil, Pipobroman, Triethylene-melamine, Triethylenethiophosphoramine, Busulfan, Carmustine, Lomustine, Streptozocin, Dacarbazine, and Temozolomide.

[0056] Antimetabolites (including folic acid antagonists, pyrimidine analogs, purine analogs and adenosine deaminase inhibitors): Methotrexate, 5-Fluorouracil, Floxuridine, Cytarabine, 6-Mercaptopurine, 6-Thioguanine, Fludarabine phosphate. Pentostatine, and Gemcitabine.

[0057] Natural products and their derivatives (including vinca alkaloids, antitumor antibiotics, enzymes, lymphokines and epipodophyllotoxins): Vinblastine. Vincristine. Vindesine, Bleomycin, Dactinomycin, Daunorubicin, Doxorubicin, Epirubicin. Idarubicin, paclitaxel (paclitaxel is commercially available as Taxol®), Mithramycin, Deoxyco-formycin, Mitomycin-C, L-Asparaginase, Interferons (especially IFN- α), Etoposide, and Teniposide.

[0058] Hormones and steroids (including synthetic analogs): 17α-Ethinylestradiol, Diethylstilbestrol, Testosterone, Prednisone, Fluoxymesterone, Dromostanolone propionate, Testolactone, Megestrolacetate, Tamoxifen, Methylprednisolone, Methyltestosterone, Prednisolone, Triamcinolone, Chlorotrianisene, Hydroxyprogesterone, Aminoglutethimide, Estramustine. Medroxyprogesteroneacetate, Leuprolide, Flutamide, Toremifene, Zoladex.

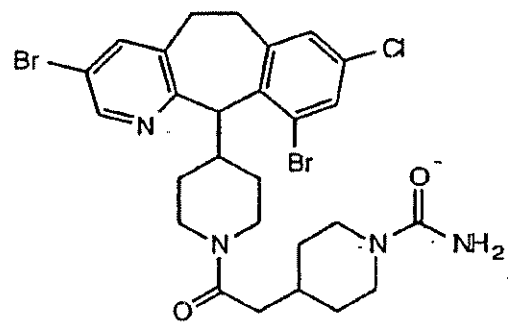
[0059] Synthetics (including inorganic complexes such as platinum coordination complexes): Cisplatin, Carboplatin, Hydroxyurea, Amsacrine, Procarbazine, Mitotane, Mitoxantrone, Levamisole, and Hexamethylmelamine.

[0060] Methods for the safe and effective administration of most of these chemotherapeutic agents are known to those skilled in the art. In addition, their administration is described in the standard literature. For example, the administration of many of the chemotherapeutic agents is described in the "Physicians' Desk Reference" (PDR), e.g., 1996 edition (Medical Economics Company. Montvale, NJ 07645-1742, USA); the disclosure of which is incorporated herein by reference thereto.

EXAMPLES

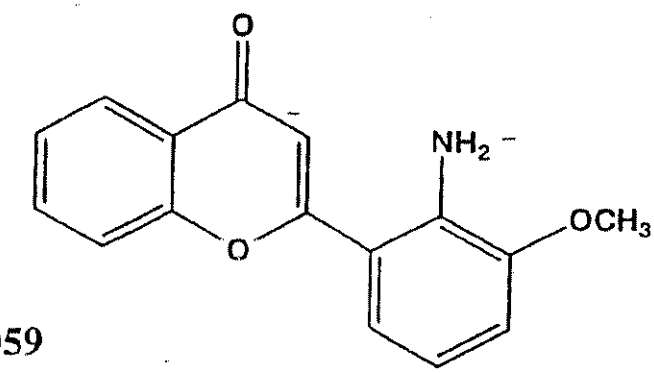
[0061] The examples provided below describe the effect of the combination of an FPT inhibitor (SCH 66336) with a MEK inhibitor (either PD098059 or U0126) on programmed cell death (apoptosis) in H-Ras-transformed Rat2 cells. Similar to tumor cell lines, these cells exhibit a fully transformed phenotype including the ability to grow anchorage-independently in soft agar and as xenografts in nude mice.

[0062] The FPT inhibitory compound used in the following examples (SCH 66336, Schering-Plough Research Institute) has the following formula:



(+) - enantiomer

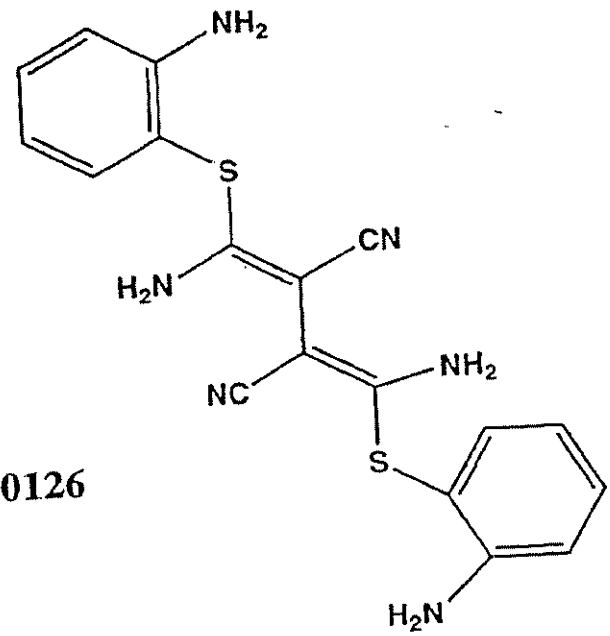
[0063] "PD 098059", a particular MEK inhibitor. has the following chemical structure:



PD 098059

PD 098059 is described in more detail in Dudley et al, 1995. The Dudley et al. reference mentions that the lyophilized solid must be reconstituted into DMSO for the reagent concentrations used in the experiments described here.

[0064] "U0126", another example of a MEK inhibitor, has the following chemical structure:



U0126

U0126 is described in more detail in Favata et al., 1998. The Favata et al. reference also mentions that the lyophilized solid must be reconstituted into DMSO for the reagent concentrations used in the experiments described here.

Materials and Methods

Cell lines and treatments.

[0065] In all cases the Ras sequences contained a Gly^{12} to Val activating mutation. H-ras (G 12V;CVLL) represents a Ser^{189} to Leu mutation which generates a geranylgeranlyated form of the H-ras protein. The cDNAs representing these H-ras proteins were subcloned into the pMV7 plasmid for the generation of stable Ras expressing-Rat2 cell lines by retroviral transduction and selection with the neomycin gene (Kirschmeier et al., 1988). The stable cell lines presented here represent individual clones of Ras-expressing, neomycin-selected cells. H-ras (G12V)/Rat2, H-ras (G12V;CVLL) /Rat2, and parental Rat2 cells were propogated in DMEM containing 10% fetal calf serum, penicillum, streptomycin,

non-essential amino acids, L-glutamine, and for the Ras-transformants, 200ug/ml Geneticin (Gibco/BRL; Gaithersburg, MD). All ras transformed cells demonstrated a fully transformed phenotype including anchorage-independent growth and tumorgenic capabilities.

[0066] PD098059 (A385-023-M005; Alexis Corporation; San Diego, CA) and U0126 (#V1121; Promega Corporation; Madison. WI) and were used according to Dudley et al. (1995) and Favata et al. (1998).

FACS analysis.

[0067] FACS analysis was performed using standard protocols. The cells were harvested by trypsin/EDTA treatment, the trypsin was neutralized with DMEM containing 10% FCS, and the cells were pelleted at 500 x g for 5 min. The cells were washed with PBS, pelleted, resuspended in 0.5 ml PBS, and fixed with 2 ml ice cold acetone:methanol (1:1) for 30 min at -20°C. To label chromosomal DNA with propidium iodide (PI), the fixed cells were washed twice with PBS prior to resuspending at 1 x 10^6 cells/ml in PBS, 75 μg/ml of PI (Calbiochem; La Jolla, CA),500 μg/ml RNase (Sigma; St. Louis, MO). for a 30 min incubation at RT. The cells were filtered through a 35 μm strainer cap (Becton Dickinson; Franklin Lakes, NJ) and stored at 4°C prior to FACS analysis on a FACS-Calibur (Becton Dickinson; Mountain View. CA). Quantification was performed using CellQuest (Becton Dickinson; Mountain View, CA).

Caspase activity assay.

[0068] H-ras transformed Rat2 and parental Rat2 cells were treated with 20 μM PD098059, 0.5 μM SCH 66336 or a combination of the two drugs for 36 h at 37BC. The cells were harvested by trypsin/EDTA, pelleted at 500 x g for 5 min. washed with PBS, and repelleted. The cells were resuspended/lysed in a lysis buffer (ApoAlert CPP32/Caspase-3 Assay Kit; Clontech laboratories; Palo Alto, CA) containing "Complete" protease inhibitors (Boehringer Mannheim; Germany), incubated for 10 min on ice and centrifuged for 3 min at 12,000 rpm at 4°C as recommended in the Clontech protocol. The protein concentration of the cell lysates was determined using the BCA protein assay (Pierce; Rockford, IL) and approximately 30 μg of each lysate assayed for Caspase-3 activity by fluorometry (CytoFluor plate reader; Perseptive Biosystems; Framingham. MA) using a fluorogenic peptide substrate (Ac-DEVD-AFC; Clontech; Palo Alto, CA).

Western Blot analysis of ERK1,2 phosphorylation status.

[0069] Cells were lysed in a detergent buffer (provided with the ApoAlert CPP32/Caspase-3 Assay Kit; Clonetech; Palo Alto, CA) and centrifuged at 14,000 rpm for 15 min at 4°C to pellet the cellular debris. Protein concentration of the resulting supernatant was determined by BCA protein assay (Pierce; Rockford, IL). Cellular proteins (20 μg) were separated on 8-16% Tris-Glycine polyacrylamide gels (Novex; San Diego, CA) and transferred to PVDF membranes for Western Blot analysis. The phosphorylated ERK 1 and ERK2 proteins were detected using a rabbit polyclonal antibody specific for the phosphorylated form of the p42/44 MAPK proteins (phospho-Thr202/Tyr204 specific; New England Biolabs, Inc.; Beverly MA). Total ERK1 and ERK2 proteins were detected using a rabbit polyclonal antibody specific for the p42/44 MAPK proteins (New England Biolabs, Inc.; Beverly, MA). A goat anti-rabbit-HRP secondary antibody (Chemicon; Temecula, CA) allowed visualization by enhanced chemiluminescence (SuperSignal West Pico Chemiluminescent Substrate; Pierce; Rockford. IL).

Results

1. Effects on apoptosis: Fluorscence-activated Cell Sorting (FACS):

[0070] Cellular apoptotic responses can be monitored in a number of ways, including analysis of chromosomal DNA fragmentation, fluorescence-activated cell sorting (FACS) of propidium iodide-stained cells, and measurement of caspase activation. To evaluate the apoptotic response to treatment with either drug, we stained the chromosomal DNA of treated cells with propidium iodide and analyzed the individual cells by FACS. Typical cell culture populations display a large peak of cells in the G1/G0 phase of the cell cycle, with a smaller peak representing G2/M phase cells. Between these 2 peaks are cells in the S phase of the cell cycle. Cells which exhibit DNA labeling which is before the G1/GO peak represent cells with fragmented DNA comprising less than the diploid amount of chromosomal DNA, and thus, undergoing cell death (Dengler, et al., 1995). This measurement gives a relative quantification of apoptosis that is comparable to other apoptosis assays including TdT-mediated dUTP nick-end labeling (TUNEL analysis); Gorczyca et al., (1993) Cancer Res. 53: 1945-51. In the experiments below, we determined the percent of the total cell population in the subG0/G1 peak as a measure of percent apoptosis.

[0071] Treatment of H-Ras-transformed Rat2 cells with PD098059 alone for 36 hours resulted in a dose-dependent increase in the percent of apoptotic cells (Fig. 2). At a concentration of 20 μM PD098059, 50% of the cells were apoptotic

When the PD098059 dose-response was repeated in the presence of 100 nM SCH 66336 a very different result was observed. In the presence of 100 nM SCH 66336 alone, 30% of the cells were apoptotic. When treated with the combination, over 60% of the cells were driven into apoptosis using as little as 2.5 μM PD098059. The concentration of PD098059 required to achieve 50% apoptosis was 20 μM when this compound was used alone, but was ≤ 1 μM when used in combination with the FPT inhibitor. This indicates that SCH 66336 significantly sensitizes cells to the pro-apoptotic effects of PD098059.

[0072] The converse experiment was also performed. Treatment with SCH 66336 alone for 36 hours induced a dose-dependent apoptotic response (Fig. 3). Using 0.75 μM SCH 66336, 70% of the cells were apoptotic. When used alone, the concentration of SCH 66336 required to induce 50% apoptosis was between 0.25 - 0.5 μM. The dose-response curve for SCH 66336 displayed a leftward shift in the presence of 2.5 μM PD098059 signifying an enhanced apoptotic response. When PD098059 was present, the concentration of SCH 66336 required to induce 50% apoptosis was 50 nM.

[0073] A similar set of experiments were performed with a structurally distinct MEK inhibitor, U0126 (Fig. 4). Similar to PD098059, U0126 is a very selective inhibitor of the MEK1,2 proteins exhibiting a potent inhibition of their kinase acitivity (Farata, et al. 1990). Treatment with U0126 alone resulted in a dose-dependent induction of apoptosis in H-Ras-transformed Rat2 cells with 17% apoptotic cells observed using a concentration of 10 μM. When this experiment was repeated in the presence of 0.5 μM SCH 66336 (a concentration which induced 14% apoptosis on its own), a greater than additive response was observed with the combination. The combination of 10 μM U0126 and 0.5 μM SCH 66336 resulted in over 50% of the cells being apoptotic.

[0074] These data demonstrate a significant increase in the proapoptotic potency in H-Ras-transformed Rat2 cells of MEK inhibitors and SCH 66336 when tested in combination. In contrast to these results, untransformed parental Rat2 cells or Rat2 cells transformed with activated Ki-Ras were insensitive to apoptosis induced by either drug alone or by the combination of SCH 66336 and PD098059 (data not shown). Lack of effect in the Ki-Ras-transformed Rat2 cells may be explained, in part, by recent observations that some Ras isoforms (Ki-Ras and N-Ras) are alternatively prenylated by geranylgeranyl transferase 1 both in vitro and in cells treated with FPT inhibitors (Zhang et al., 1997; Whyte et al., 1997).

2. **Effects on apoptosis: Caspase Activation:**

[0075] Caspases are an evolutionarily conserved family of enzymes which proteolytically degrade and dissemble the cell in response to proapoptotic signals (reviewed in Thornberry and Lazebnik, 1998). To evaluate apoptosis using this distinct biochemical endpoint, we measured caspase activity in cell lysates prepared from H-Ras-transformed Rat2 cells using a fluorometric assay for caspase 3 activity (Apo-Alert CPP32/Caspase-3 Assay; Clontech). Treatment of the H-Ras-transformed cells for 24 hr with either 0.5μ M SCH 66336 or 20 μM PD098059 alone increased caspase activity above the background level of untreated cells (Fig. 5). These results are consistent with the proapoptotic effects of either drug observed by FACS analysis (Fig. 2 at 36 hr). When H-Ras-transformed Rat2 cells were treated with the combination of both drugs a greater than additive caspase-3 response was observed, again confirming the FACS results.

[0076] Little or no caspase activation was observed in the parental Rat2 cells when treated with either single agent or a combination of both drugs (Fig. 5).

3. **Effects on MAPK phosphorylation:**

[0077] We investigated the ability of the FPT inhibitor SCH 66336 and the MEK inhibitor PD098059 to block MEK activation in H-ras-transformed Rat2 cells by measuring the phosphorylation state of its substrates ERK1 and ERK2 (44 and 42 Kd, respectively). Treatment of cells with 20 μM PD098059 decreased phosphorylation of both proteins (Fig. 6). Maximal inhibition was observed at the first time-point examined (6 hours) and phosphorylation remained suppressed throughout the 36 hour time-course. Treatment of cells with 0.5 μM SCH 66336 decreased phosphorylation of both proteins in a time- (Fig. 6) and dose-dependent manner (data not shown), with 0.5 μM SCH 66336 exhibiting maximal inhibition between 24 - 36 hours of treatment. In both cases, inhibition of phosphorylation was more profound for the 44 kDa ERK1 protein. While their phosphorylation status was decreased, the total amount of these proteins was largely unaffected by drug treatment (Fig. 6, bottom panels).

Discussion

[0078] FPT inhibitors such as SCH 66336 and MEK inhibitors such as PD098059 or UO126 target distinct steps in a common signal transduction pathway. Surprisingly, when both agents are combined, they have a greater than additive effect on apoptosis in H-Ras-transformed Rat2 cells as measured either by FACS analysis of the subG0/G1 population or by caspase activation. Without being bound to a particular theory, there are two potential explanations for this observation. First, it is possible that this combination results in a more complete or longer-lasting (sustained) inhibition of the linear pathway outlined in Fig. 1. Alternatively, the combination efficacy may be accounted for by the fact that intracellular

signaling pathways are considerably more complex and interconnected than the pathway depicted in Fig. 1. A more complex wiring diagram is shown in Fig. 7. As mentioned previously, it is clear that these pathways branch at several steps along the pathway. Growth factor receptors activate several signaling pathways via SH2-mediated interactions. Similarly, multiple Ras effectors have been identified utilizing yeast 2-hybrid and other biochemical approaches. The combined efficacy of an FPT inhibitor and a MEK inhibitor may be accounted for by their effects on distinct branches of these pathways. For example, in addition to blocking H-Ras-mediated activation of MEK, FPT inhibitors also block other Ras-effector pathways (e.g. the PI3K and Rho pathways). Similarly, there is evidence for Ras-independent activation of the MEK/MAPK pathway (Duckworth and Cantley, 1997; Morrison and Cutler, 1997). While the molecular components of this Ras-independent pathway remain to be fully delineated, this suggests that an FPT inhibitor alone may not shut-down all pathways leading to MEK/MAPK activation. Therefore, the combination of these 2 classes of inhibitors may result in a more complete blockade.

[0079] Regardless of the mechanism, the ex vivo data with the combination of SCH 66336 and PD098059 demonstrates a striking potentiation of apoptosis-inducing activity. Furthermore, this type of enhanced efficacy in combination is extendable to include other agents that target signal transduction pathways, (e.g., agents which block growth factor receptors). As stated above, such effects may result from (i) a more complete inhibition of the growth factor - Ras signaling pathway than that achieved with single agent treatment; or (ii) simultaneous inhibition of multiple signaling pathways. For example, many tumors may be driven by the action of multiple growth factors each acting in an autocrine or paracrine fashion to drive proliferation through their cognate receptors. The blockade of one of these receptor pathways using antibodies or tyrosine kinase inhibitors may exert an antitumor effect by blocking that signaling pathway, however other receptor-driven pathways will be unaffected. The addition of a FPT inhibitor may shut down signaling from these other pathways resulting in a more complete inhibition of signal transduction and, thus, exhibiting a synergistic antitumor effect. Furthermore, because growth factor receptors are known to initiate multiple signaling-cascades (e.g. Ras/MEK, phospholipase Cγ, and PI3K), inhibition of the Ras pathway with a FPT inhibitor or a MEK inhibitor may have no effect on the signaling capacity of these other pathways. Thus, the addition of a growth factor receptor antibody or tyrosine kinase inhibitor to a tumor cell treated with a FPT inhibitor may result in a more complete inhibition of signaling and have a synergistic antitumor effect by shutting down those pathways which are unaffected by the FPT inhibitor.

[0080] Similar types of synergy may also be observed by combining FPT inhibitors with agents that target other steps in these signaling pathways (e.g. Raf inhibitors, SH2 inhibitors, PI3K inhibitors, etc.).

PHARMACEUTICAL COMPOSITIONS

[0081] Inert, pharmaceutically acceptable carriers used for preparing pharmaceutical compositions of the FPT inhibitors and the Ras signaling pathway inhibitors described herein can be solid or liquid. Solid preparations include powders, tablets, dispersible granules, capsules, cachets and suppositories. The powders and tablets may comprise from about 5 to about 70% active ingredient. Suitable solid carriers are known in the art, e.g., magnesium carbonate, magnesium stearate, talc, sugar, and/or lactose. Tablets, powders, cachets and capsules can be used as solid dosage forms suitable for oral administration.

[0082] For preparing suppositories, a low melting wax such as a mixture of fatty acid glycerides or cocoa butter is first melted, and the active ingredient is dispersed homogeneously therein as by stirring. The molten homogeneous mixture is then poured into conveniently sized molds, allowed to cool and thereby solidify.

[0083] Liquid preparations include solutions, suspensions and emulsions. As an example may be mentioned water or water-propylene glycol solutions for parenteral injection. Liquid preparations may also include solutions for intranasal administration.

[0084] Aerosol preparations suitable for inhalation may include solutions and solids in powder form, which may be in combination with a pharmaceutically acceptable carrier, such as an inert compressed gas.

[0085] Also included are solid preparations which are intended for conversion, shortly before use, to liquid preparations for either oral or parenteral administration. Such liquid forms include solutions, suspensions and emulsions.

[0086] The FPT inhibitors and the additional Ras pathway inhibitors described herein may also be deliverable transdermally. The transdermal compositions can take the form of creams, lotions, aerosols and/or emulsions and can be included in a transdermal patch of the matrix or reservoir type as are conventional in the art for this purpose.

[0087] Preferably the compounds are administered orally.

[0088] Preferably, the pharmaceutical preparation is in unit dosage form. In such form, the preparation is subdivided into unit doses containing appropriate quantities of the active component, e.g., an effective amount to achieve the desired purpose.

[0089] The quantity of active compound in a unit dose of preparation may be varied or adjusted from about 0.5 mg to 1000 mg, preferably from about 1 mg to 300 mg, more preferably 5 mg to 200 mg, according to the particular application.

[0090] The actual dosage employed may be varied depending upon the requirements of the patient and the severity of the condition being treated. Determination of the proper dosage for a particular situation is within the skill of the art.

Generally, treatment is initiated with smaller dosages which are less than the optimum dose of the compound. Thereafter, the dosage is increased by small amounts until the optimum effect under the circumstances is reached. For convenience, the total daily dosage may be divided and administered in portions during the day if desired.

[0091] The amount and frequency of administration of the FPT inhibitors and the additional Ras pathway inhibitors will be regulated according to the judgment of the attending clinician (physician) considering such factors as age, condition and size of the patient as well as severity of the disease being treated. In general, dosage for an FPT inhibitor (when used as a single agent) can conceivably have an upper range of 2000 mg/day, preferably in a range of from 50 to 400 rng/day in cases where the FPT inhibitor is a fused-ring tricyclic benzocycloheptapyridine. However, in the combination therapy of the present invention, a preferred low dosage regimen of the FPT inhibitors is, *e.g.*, oral administration of an amount in the range of from 1.4 to 400 mg/day, more preferably 1.4 to 350 mg/day, even more preferably 3.5 to 70 mg/day, preferably with a B.I.D. dosing schedule. A particularly low dosage range can be 1.4 to 70 mg/day.

[0092] The additional Ras pathway inhibitors can be administered according to therapeutic protocols well known in the art. See, e.g., Pegram, M.D., *et al.* (1998). J Clin Oncol. *16*:2659-2671. It will be apparent to those skilled in the art that the administration of the additional Ras pathway inhibitor can be varied depending on the disease being treated and the known effects of the additional Ras pathway inhibitor on that disease. Also, in accordance with the knowledge of the skilled clinician, the therapeutic protocols (e.g., dosage amounts and times of administration) can be varied in view of the observed effects of the administered therapeutic agents (*i.e.*, additional Ras pathway inhibitor) on the patient. and in view of the observed responses of the disease to the administered therapeutic agents. In general. dosage for an additional Ras signaling pathway inhibitor (when used as a single agent) can be, *e.g.*, in the range of 5 to 2000 mg/day. However, in the combination therapy of the present invention, a preferred low dosage regimen of an additional Ras signaling pathway inhibitor *(e.g.*, a MEK inhibitor) is administration of an amount in the range of from 1 to 350 mg/day, more preferably 3.5 to 70 mg/day, preferably with a B.I.D. dosing schedule. A particularly low dosage range can be 1 to 70 mg/day.

[0093] Thus, in a preferred example of combination therapy in the treatment of cancers (*e.g.*, pancreatic, lung or bladder cancer), the FPT inhibitor can be SCH 66336, as identified previously, administered orally in an amount of 70 mg/ day, in two divided doses, on a continuous dosing regimen; and the additional Ras signaling pathway inhibitor can be PD098059 (or an analogue

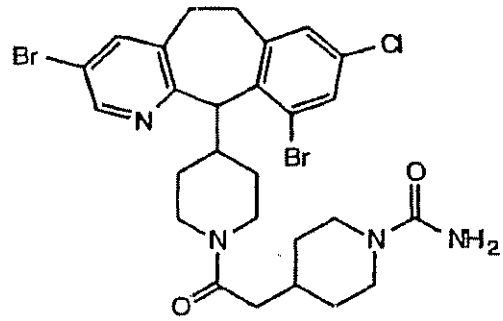
[0094] The FPT inhibitor and additional Ras pathway inhibitor may be administered concurrently (e.g., simultaneously, essentially simultaneously or within the same treatment protocol) or sequentially, depending upon the nature of the proliferative disease, the condition of the patient, and the actual choice of the additional Ras pathway inhibitor to be administered in conjunction (i.e., within a single treatment protocol) with the FPT inhibitor.

[0095] If the FPT inhibitor and additional Ras pathway inhibitor are not administered simultaneously or essentially simultaneously, then the initial order of administration of the FPT inhibitor and additional Ras pathway inhibitor may not be important. Thus, the FPT inhibitor may be administered first followed by the administration of the additional Ras pathway inhibitor; or the additional Ras pathway inhibitor may be administered first followed by the administration of the FPT inhibitor. This alternate administration may be repeated during a single treatment protocol. The determination of the order of administration, and the number of repititions of administration of each therapeutic agent during a treatment protocol, is well within the knowledge of the skilled physician after evaluation of the disease being treated and the condition of the patient. For example, the additional Ras pathway inhibitor may be administered first, and then the treatment continued with the administration of the FPT inhibitor followed, where determined advantageous, by the administration of the additional Ras pathway inhibitor, and so on until the treatment protocol is complete.

[0096] Thus, in accordance with experience and knowledge, the practising physician can modify each protocol for the administration of a component (therapeutic agent-- *i.e.*, FPT inhibitor, additional Ras pathway inhibitor) of the treatment according to the individual patient's needs, as the treatment proceeds.

[0097] The attending clinician, in judging whether treatment is effective at the dosage administered. will consider the general well-being of the patient as well as more definite signs such as relief of disease-related symptoms, inhibition of tumor growth. actual shrinkage of the tumor, or inhibition of metastasis. Size of the tumor can be measured by standard methods such as radiological studies, e.g., CAT or MRI scan, and successive measurements can be used to judge whether or not growth of the tumor has been retarded or even reversed. Relief of disease-related symptoms such as pain. and improvement in overall condition can also be used to help judge effectiveness of treatment. (Of course, as indicated previously, effective treatment using the methods of the present invention preferably results in a synergistic level of cancer cell death and/or tumor regression).

[0098] The following are examples (Examples 1-4) of capsule formulations for the FPT Inhibitory Compound:



(+) - enantiomer

EXAMPLES 1 and 2

[0099]

Capsule Formulation

Composition	Example 1 mg/capsule	Example 2 mg/capsule	% Composition
Solid Solution	100	400.0	84.2
Silicon Dioxide NF(1)	0.625	2.5	0.5
Magnesium Stearate NF(2)	0.125	0.5	0.1
Croscarmellose Sodium NF	11.000	44.0	9.3
Pluronic F68 NF	6.250	25.0	5.3
Silicon Dioxide NF(3)	0.625	2.5	0.5
Magnesium Stearate NF(4)	0.125	0.5	0.1
TOTAL	118.750	475.00	
Capsule size	No. 4	No. 0	

METHOD (Examples 1 and 2)

[0100]

Preparation of Solid Solution

Composition	g/batch	% Composition
FPT Inhibitory Compound	80	33.3
Povidone NF K29/32	160	66.6
Methylene Chloride	5000 mL	evaporates

[0101] Crystalline FPT Inhibitory Compound and the povidone were dissolved in methylene chloride. The solution was dried using a suitable solvent spray dryer. The residue was then reduced to fine particles by grinding. The powder was then passed through a 30 mesh screen. The powder was found to be amorphous by x-ray analysis.

[0102] The solid solid solution, silicon dioxide(1) and magnesium stearate(2) were mixed in a suitable mixer for 10 minutes. The mixture is compacted using a suitable roller compactor and milled using a suitable mill fitted with 30 mesh screen Croscarmellose sodium, Pluronic F68 and silicon dioxide(3) are added to the milled mixture and mixed further for 10 minutes. A premix was made with magnesium stearate(4) and equal portions of the mixture. The premix was added to the remainder of the mixture and mixed for 5 minutes. the mixture was encapsulated in hard shell gelatin capsule shells

EXAMPLES 3 and 4

[0103]

Capsule Formulation

Composition	Example 3 mg/capsule	Example 4 mg/capsule	% Composition
Solid Solution	400	200.0	80.0
Silicon Dioxide NF(1)	3.75	1.875	0.75
Magnesium Stearate NF(2)	0.125	0.625	0.25
Croscarmellose Sodium NF	40.00	20.00	8.0
Pluronic F68 NF	50.00	25.00	10
Silicon Dioxide NF(3)	3.75	1.875	0.75
Magnesium Stearate NF(4)	1.25	0.625	0.25
TOTAL	500.00	250.00	
Capsule size	No. 0	No. 2	

METHOD (Examples 3 and 4)

[0104]

Preparation of Solid Solution

Composition	g/batch	% Composition
FPT Inhibitory Compound	15	50
Povidone NF K29/32	15	50
Methylene Chloride	140 mL	evaporates
Methanol	60 mL	evaporates

[0105] Crystalline FPT Inhibitory Compound and the povidone were dissolved in a mixture of methylene chloride and methanol. The solution was dried using a suitable solvent spray dryer. The residue was then reduced to fine particles by grinding. The powder was then passed through a 30 mesh screen. The powder was found to be amorphous by x-ray analysis.

[0106] The solid solid solution, silicon dioxide(1) and magnesium stearate(2) were mixed in a suitable mixer for 10 minutes. The mixture is compacted using a suitable roller compactor and milled using a suitable mill fitted with 30 mesh screen. Croscarmellose sodium, Pluronic F68 and silicon dioxide(3) are added to the milled mixture and mixed further for 10 minutes. A premix was made with magnesium stearate(4) and equal portions of the mixture. The premix was added to the remainder of the mixture and mixed for 5 minutes. The mixture was encapsulated in hard shell gelatin capsule shells.

[0107] For information on formulations, reference can also be made to U.S. Patent Application Serial Nos. 08/997168 and 60/068387 (filed December 22, 1997).

[0108] The scope of the invention in its pharmaceutical composition aspect is not to be limited by the examples provided.

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[0109]

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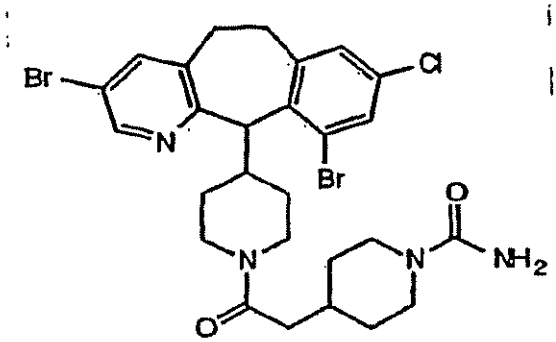
Claims

1. The use of an FPT inhibitor in the manufacture of a pharmaceutical composition for treating cancer in a patient by administering:

(1) an FPT inhibitor of the following formula

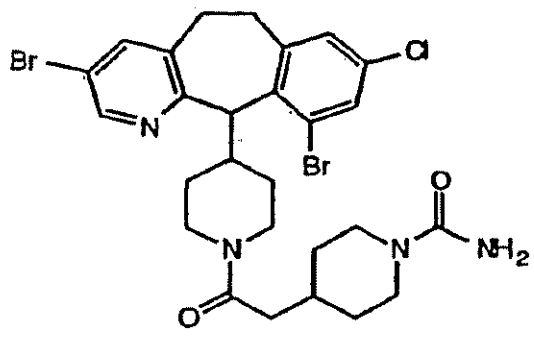


(+) - enantiomer

and (2) an additional Ras signaling pathway inhibitor, in amounts effective to induce a synergistic level of cancer cell death.

2. The use of an additional Ras signaling pathway inhibitor in the manufacture of a pharmaceutical composition for treating cancer in a patient by administering:

(1) an FPT inhibitor of the following formula

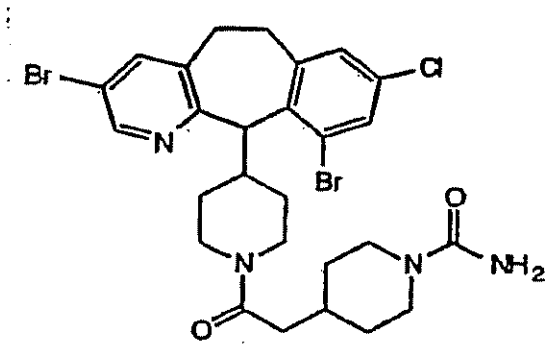


(+) - enantiomer

and (2) an additional Ras signaling pathway inhibitor, in amounts effective to induce a synergistic level of cancer cell death.

3. The use of an FPT inhibitor in the manufacture of a pharmaceutical composition for regressing tumor volume in a cancer patient by administering:

(1) an FPT inhibitor of the following formula

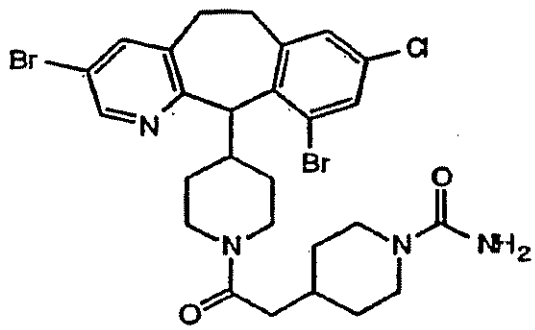


(+) - enantiomer

and (2) an additional Ras signaling pathway inhibitor, in amounts effective to induce a synergistic level of cancer cell death.

4. The use of an additional Ras signaling pathway inhibitor in the manufacture of a pharmaceutical composition for regressing tumor volume in a cancer patient by administering:

(1) an FPT inhibitor of the following formula



(+) - enantiomer

and (2) an additional Ras signaling pathway inhibitor, in amounts effective to induce a synergistic level of cancer cell death.

5. The use of any preceding claim wherein the additional Ras signaling pathway inhibitor is a kinase inhibitor.

6. The use of any of claims 1 to 4 wherein the additional Ras signaling pathway inhibitor inhibits an element downstream of Ras in the Ras signaling pathway

7. The use of any of Claims 1 to 4 wherein the additional Ras signaling pathway inhibitor is a MEK inhibitor.

8. The use of any of Claims 1 to 4 wherein the additional Ras signaling pathway inhibitor is a growth factor receptor inhibitor.

9. The use of Claim 8 wherein the growth factor receptor inhibitor is a tyrosine kinase inhibitor.

10. The use of Claim 9 wherein the tyrosine kinase inhibitor is a small molecule selected from the group consisting of (1) an erbB2 receptor inhibitor, (2) a PDGF receptor inhibitor. (3) an IGF receptor inhibitor, and (4) a EGF receptor tyrosine kinase inhibitor

11. The use of Claim 8 wherein the growth factor receptor inhibitor is an antibody directed against the extracellular

domain of a growth factor receptor.

12. The use of Claim 11 wherein the antibody is a monoclonal antibody which targets the erbB2 receptor or a monoclonal antibody which targets the EGF receptor.

13. The use of Claim 11 wherein the antibody is a monoclonal antibody which targets the erbB2 receptor.

14. The use of any preceding claim, wherein the FPT inhibitor is administered in an amount of from 1.4 to 400 mg/day.

15. The use of Claim 14, wherein the FPT inhibitor is administered in an amount of from 3.5 to 70 mg/day.

16. The use of any preceding claim, wherein the additional Ras pathway inhibitor is administered in an amount of from 1 to 350 mg/day.

17. The use of Claim 16, wherein the additional Ras pathway inhibitor is administered in an amount of from 3.5 to 70 mg/day.

18. The use of any preceding claim, wherein said FPT inhibitor, and said additional Ras pathway inhibitor are administered simultaneously.

19. The use of any of Claims 1 to 17 wherein said FPT inhibitor, and said additional Ras pathway inhibitor are administered sequentially.

20. The use of Claim 19 wherein said additional Ras pathway inhibitor is administered first.

21. The use of Claim 19 wherein said FPT inhibitor is administered first.

22. The use of any preceding claim wherein the cancer is: lung cancer, pancreatic cancer, colon cancer, ovarian cancer, cancers of the liver, myeloid leukemia, melanoma, thyroid follicular cancer, bladder carcinoma, glioma, myelodysplastic syndrome, breast cancer or prostate cancer.

23. The use of any preceding claim wherein said treatment further comprises administering a chemotherapeutic agent.

24. The use of Claim 23 wherein said chemotherapeutic agent is selected from: Uracil mustard, Chlormethine, Cyclophosphamide, Ifosfamide, Melphalan, Chlorambucil, Pipobroman, Triethylenemelamine, Triethylenethiophosphoramine, Busulfan, Carmustine, Lomustine, Streptozocin, Dacarbazine, Temozolomide, Methotrexate, 5-Fluorouracil, Floxuridine, Cytarabine, 6-Mercaptopurine, 6-Thioguanine, Fludarabine phosphate, Pentostatine, Gemcitabine, Vinblastine, Vincristine, Vindesine, Bleomycin, Dactinomycin, Daunorubicin, Doxorubicin, Epirubicin, Idarubicin, Paclitaxel, Mithramycin, Deoxycoformycin, Mitomycin-C, L-Asparaginase, Interferons, Etoposide, Teniposide 17α-Ethinylestradiol, Diethylstilbestrol, Testosterone, Prednisone, Fluoxymesterone, Dromostanolone propionate, Testolactone, Megestrolacetate, Tamoxifen, Methylprednisolone, Methyltestosterone, Prednisolone, Triamcinolone, Chlorotrianisene, Hydroxyprogesterone, Aminoglutethimide, Estramustine, Medroxyprogesteroneacetate, Leuprolide, Flutamide, Toremifene, goserelin, Cisplatin, Carboplatin, Hydroxyurea, Amsacrine, Procarbazine, Mitotane, Mitoxantrone, Levamisole, Navelbene, CPT-11, Anastrazole, Letrazole, Capecitabine, Reloxafine, Droloxafine, gemcitabine, paclitaxel, or Hexamethylmelamine

25. The use of Claim 23 wherein said antineoplastic agent is temozolomide.

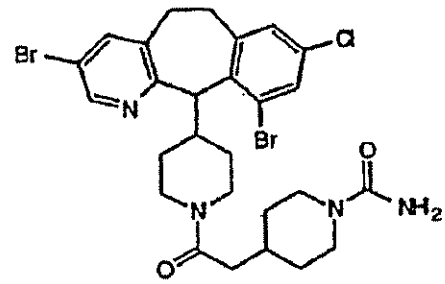
26. The use of any preceding claim wherein said treatment further comprises administering radiation.

27. The use of any preceding claim wherein cancer cell death occurs through apoptosis

Patentansprüche

1. Verwendung eines FPT-Inhibitors zur Herstellung einer pharmazeutischen Zusammensetzung zur Behandlung von Krebs bei einem Patienten durch Verabreichung

(1) eines FPT-Inhibitors mit der folgenden Formel:

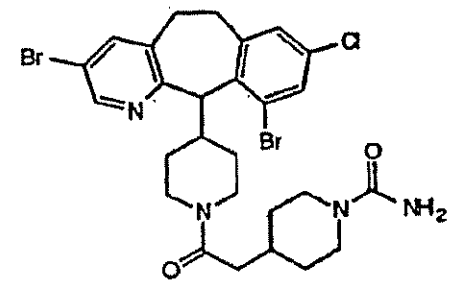


(+)-Enantiomer

und (2) eines zusätzlichen Ras-Signalweg-Inhibitors in Mengen, die wirksam sind, um einen synergistischen Grad an Krebszelltod zu induzieren.

2. Verwendung eines zusätzlichen Ras-Signalweg-Inhibitors zur Herstellung einer pharmazeutischen Zusammensetzung zur Behandlung von Krebs bei einem Patienten durch Verabreichung:

(1) eines FPT-Inhibitors mit der folgenden Formel:

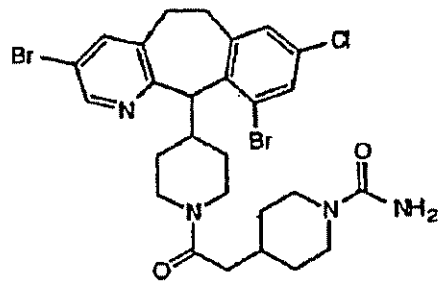


(+)-Enantiomer

und (2) eines zusätzlichen Ras-Signalweg-Inhibitors in Mengen, die wirksam sind, um einen synergistischen Grad an Krebszelltod zu induzieren

3. Verwendung eines FPT-Inhibitors zur Herstellung einer pharmazeutischen Zusammensetzung zum Herbeiführen einer Regression des Tumorvolumens bei einem Krebspatienten durch Verabreichung:

(1) eines FPT-Inhibitors mit der folgenden Formel:

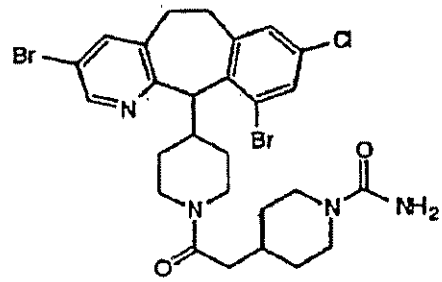


(+)-Enantiomer

und (2) eines zusätzlichen Ras-Signalweg-Inhibitors in Mengen, die wirksam sind, um einen synergistischen Grad an Krebszelltod zu induzieren.

4. Verwendung eines zusätzlichen Ras-Signalweg-Inhibitors zur Herstellung einer pharmazeutischen Zusammensetzung zum Herbeiführen einer Regression des Tumorvolumens bei einem Krebspatienten durch Verabreichung:

(1) eines FPT-Inhibitors mit der folgenden Formel:



(+)-Enantiomer

und (2) eines zusätzlichen Ras-Signalweg-Inhibitors in Mengen, die wirksam sind, um einen synergistischen Grad an Krebszelltod zu induzieren.

5. Verwendung nach einem der vorhergehenden Ansprüche, bei dem der zusätzliche Ras-Signalweg-Inhibitor ein Kinase-Inhibitor ist.

6. Verwendung nach einem der Ansprüche 1 bis 4, bei der der zusätzliche Ras-Signalweg-Inhibitor ein Ras-nachgeordnetes Element in dem Ras-Signalweg inhibiert.

7. Verwendung nach einem der Ansprüche 1 bis 4, bei der der zusätzliche Ras-Signalweg-Inhibitor ein MEK-Inhibitor ist.

8 Verwendung nach einem der Ansprüche 1 bis 4, bei der der zusätzliche Ras-Signalweg-Inhibitor ein Wachstumsfaktorrezeptor-Inhibitor ist

9. Verwendung nach Anspruch 8, bei der der Wachstumsfaktorrezeptor-Inhibitor ein Tyrosinkinase-Inhibitor ist.

10. Verwendung nach Anspruch 9, bei der der Tyrosinkinase-Inhibitor ein kleines Molekül ausgewählt aus der Gruppe

bestehend aus (1) einem erbB2-Rezeptor-Inhibitor, (2) einem PDGF-Rezeptor-Inhibitor, (3) einem IGF-Rezeptor-Inhibitor und (4) einem EGF-Rezeptor-Tyrosinkinase-Inhibitor ist

11. Verwendung nach Anspruch 8, bei der der Wachstumsfaktorrezeptor-Inhibitor ein Antikörper ist, der gegen die extrazelluläre Domäne eines Wachstumsfaktor-Rezeptors gerichtet ist.

12. Verwendung nach Anspruch 11, bei der der Antikörper ein monoklonaler Antikörper ist, der zielführend für den erbB2-Rezeptor ist, oder ein monoklonaler Antikörper ist, der zielführend für den EGF-Rezeptor ist.

13. Verwendung nach Anspruch 11, bei der der Antikörper ein monoklonaler Antikörper ist, der zielführend für den erbB2-Rezeptor ist.

14. Verwendung nach einem der vorhergehenden Ansprüche, bei der der FPT-Inhibitor in einer Menge von 1,4 bis 400 mg/Tag verabreicht wird.

15. Verwendung nach Anspruch 14, bei der der FPT-Inhibitor in einer Menge von 3,5 bis 70 mg/Tag verabreicht wird.

16. Verwendung nach einem der vorhergehenden Ansprüche, bei dem der zusätzliche Ras-Weg-Inhibitor in einer Menge von 1 bis 350 mg/Tag verabreicht wird.

17. Verwendung nach Anspruch 16, bei der der zusätzliche Ras-Weg-Inhibitor in einer Menge von 3,5 bis 70 mg/Tag verabreicht wird.

18. Verwendung nach einem der vorhergehenden Ansprüche, bei der der FPT-Inhibitor und der zusätzliche Ras-Weg-Inhibitor gleichzeitig verabreicht werden.

19. Verwendung nach einem der Ansprüche 1 bis 17, bei der der FPT-Inhibitor und der zusätzliche Ras-Weg-Inhibitor sequentiell verabreicht werden.

20. Verwendung nach Anspruch 19, bei der der zusätzliche Ras-Weg-Inhibitor zuerst verabreicht wird.

21. Verwendung nach Anspruch 19, bei der der FPT-Inhibitor zuerst verabreicht wird.

22. Verwendung nach einem der vorhergehenden Ansprüche, bei der der Krebs Lungenkrebs, Pankreaskrebs, Colonkrebs, Eierstockkrebs, Krebse der Leber, myeloide Leukämie, Melanom, Schilddrüsenfollikelkrebs, Blasenkrebs, Gliom, myelodysplastisches Syndrom, Brustkrebs oder Prostatakrebs ist.

23. Verwendung nach einem der vorhergehenden Ansprüche, bei der die Behandlung ferner die Verabreichung eines Chemotherapeutikums beinhaltet.

24. Verwendung nach Anspruch 23, bei der das Chemotherapeutikum ausgewählt ist aus Uracil-Senfgas, Chlormethin, Cyclophosphamid, Ifosfamid, Melphalan, Chlorambucil, Pipobroman, Triethylenmelamin, Triethylenthiophosphoramin, Busulfan. Carmustin, Lomustin, Streptozocin, Dacarbazin. Temozolomid, Methotrexat, 5-Fluoruracil, Floxuridin, Cytarabin, 6-Mercaptopurin, 6-Thioguanin, Fludarabinphosphat, Pentostatin, Gemcitabin, Vinblastin, Vincristin, Vindesin, Bleomycin, Dactinomycin, Daunorubicin. Doxorubicin. Epirubicin, Idarubicin, Paclitaxel, Mithramycin, Deoxycoformycin. Mitomycin-C, L-Asparaginase, Interferonen, Etoposid. Teniposid, 17-alpha-Ethinylestradiol, Diethylstilbestrol, Testosteron, Prednison, Fluoxymesteron, Dromostanolonpropionat, Testolacton, Megestrolacetat, Tamoxifen, Methylprednisolon, Methyltestosteron, Prednisolon, Triamcinolon, Chlortrianisen, Hydroxyprogesteron. Aminoglutethimid, Estramustin, Medroxyprogesteronacetat, Leuprolid, Flutamid, Toremifen, Goserelin, Cisplatin, Carboplatin. Hydroxyharnstoff, Amsacrin, Procarbazin, Mitotan, Mitoxantron, Levamisol, Navelben, CPT-11, Anastrazol, Letrazol, Capecitabin, Reloxafin, Droloxafin. Gemcitabin, Paclitaxel oder Hexamethylmelamin.

25. Verwendung nach Anspruch 23, bei der das antineoplastische Mittel Temozolomid ist.

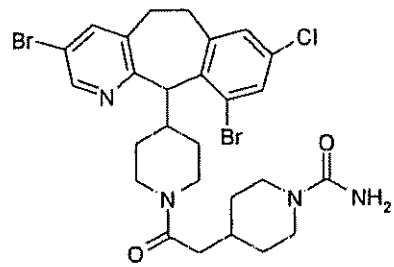
26. Verwendung nach einem der vorhergehenden Ansprüche, bei der die Behandlung ferner die Verabreichung von Strahlung beinhaltet.

27. Verwendung nach einem der vorhergehenden Ansprüche, bei der der Krebszelltod durch Apoptose erfolgt.

Revendications

1. Emploi d'un inhibiteur de FPT dans la fabrication d'une composition pharmaceutique conçue pour le traitement d'un cancer chez un patient par administration :

1) d'un inhibiteur de FTP de formule suivante :

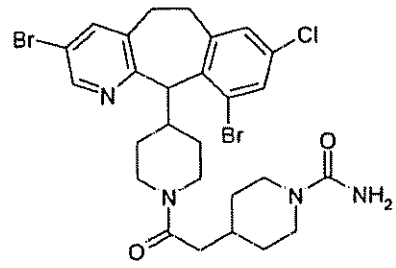


énantiomère (+)

2) et d'un inhibiteur supplémentaire de voie de signalisation Ras, en des quantités qui ont pour effet d'induire un niveau synergique de mort des cellules cancéreuses

2. Emploi d'un inhibiteur supplémentaire de voie de signalisation Ras dans la fabrication d'une composition pharmaceutique conçue pour le traitement d'un cancer chez un patient par administration :

1) d'un inhibiteur de FTP de formule suivante :

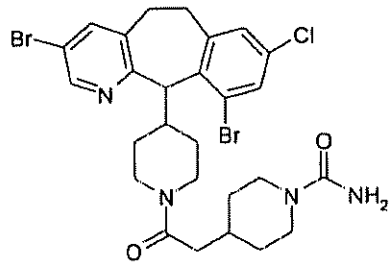


énantiomère (+)

2) et d'un inhibiteur supplémentaire de voie de signalisation Ras, en des quantités qui ont pour effet d'induire un niveau synergique de mort des cellules cancéreuses.

3. Emploi d'un inhibiteur de FPT dans la fabrication d'une composition pharmaceutique conçue pour faire diminuer le volume d'une tumeur chez un patient cancéreux par administration :

1) d'un inhibiteur de FTP de formule suivante :

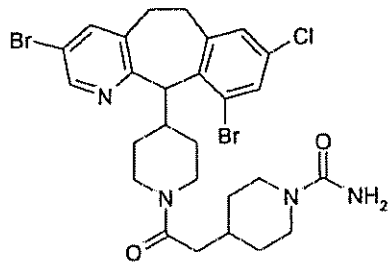


énantiomère (+)

2) et d'un inhibiteur supplémentaire de voie de signalisation Ras, en des quantités qui ont pour effet d'induire un niveau synergique de mort des cellules cancéreuses.

4. Emploi d'un inhibiteur supplémentaire de voie de signalisation Ras dans la fabrication d'une composition pharmaceutique conçue pour faire diminuer le volume d'une tumeur chez un patient cancéreux par administration :

1) d'un inhibiteur de FTP de formule suivante :



énantiomère (+)

2) et d'un inhibiteur supplémentaire de voie de signalisation Ras, en des quantités qui ont pour effet d'induire un niveau synergique de mort des cellules cancéreuses

5. Emploi conforme à l'une des revendications précédentes, dans lequel l'inhibiteur supplémentaire de voie de signalisation Ras est un inhibiteur de kinase.

6. Emploi conforme à l'une des revendications 1 à 4, dans lequel l'inhibiteur supplémentaire de voie de signalisation Ras inhibe un élément situé en aval de Ras dans la voie de signalisation Ras.

7. Emploi conforme à l'une des revendications 1 à 4, dans lequel l'inhibiteur supplémentaire de voie de signalisation Ras est un inhibiteur de MEK.

8. Emploi conforme à l'une des revendications 1 à 4, dans lequel l'inhibiteur supplémentaire de voie de signalisation Ras est un inhibiteur de récepteur de facteur de croissance.

9. Emploi conforme à la revendication 8, dans lequel l'inhibiteur de récepteur de facteur de croissance est un inhibiteur de tyrosine kinase.

10. Emploi conforme à la revendication 9, dans lequel l'inhibiteur de tyrosine kinase est une petite molécule choisie parmi :

1) un inhibiteur du récepteur erbB2,
2) un inhibiteur du récepteur de PDGF;
3) un inhibiteur de récepteur d'IGF,

4) et un inhibiteur de l'activité de tyrosine kinase du récepteur d'EGF.

11. Emploi conforme à la revendication 8, dans lequel l'inhibiteur de récepteur de facteur de croissance est un anticorps dirigé contre le domaine extracellulaire d'un récepteur de facteur de croissance.

12. Emploi conforme à la revendication 11, dans lequel l'anticorps est un anticorps monoclonal qui a pour cible le récepteur erbB2 ou un anticorps monoclonal qui a pour cible le récepteur d'EGF.

13. Emploi conforme à la revendication 11, dans lequel l'anticorps est un anticorps monoclonal qui a pour cible le récepteur erbB2.

14. Emploi conforme à l'une des revendications précédentes, dans lequel l'inhibiteur de FPT est administré en une quantité de 1,4 à 400 mg par jour.

15. Emploi conforme à la revendication 14, dans lequel l'inhibiteur de FPT est administré en une quantité de 3,5 à 70 mg par jour.

16. Emploi conforme à l'une des revendications précédentes, dans lequel l'inhibiteur supplémentaire de voie de signalisation Ras est administré en une quantité de 1 à 350 mg par jour.

17. Emploi conforme à la revendication 16, dans lequel l'inhibiteur supplémentaire de voie de signalisation Ras est administré en une quantité de 3,5 à 70 mg par jour.

18. Emploi conforme à l'une des revendications précédentes, dans lequel ledit inhibiteur de FPT et ledit inhibiteur supplémentaire de voie de signalisation Ras sont administrés simultanément.

19. Emploi conforme à l'une des revendications 1 à 17, dans lequel ledit inhibiteur de FPT et ledit inhibiteur supplémentaire de voie de signalisation Ras sont administrés successivement.

20. Emploi conforme à la revendication 19, dans lequel ledit inhibiteur supplémentaire de voie de signalisation Ras est administré en premier

21. Emploi conforme à la revendication 19, dans lequel ledit inhibiteur de FPT est administré en premier.

22. Emploi conforme à l'une des revendications précédentes, dans lequel le cancer est un cancer du poumon, un cancer du pancréas, un cancer du côlon, un cancer des ovaires, un cancer du foie, une leucémie myéloïde, un mélanome, un cancer vésiculaire de la thyroïde, un cancer de la vessie, un gliome, un syndrome myélodysplasique, un cancer du sein ou un cancer de la prostate.

23. Emploi conforme à l'une des revendications précédentes, dans lequel ledit traitement comporte en outre le fait d'administrer un agent de chimiothérapie.

24. Emploi conforme à la revendication 23, dans lequel ledit agent de chimiothérapie est choisi parmi les suivants : uracile moutarde, chlorméthine, cyclophosphamide, ifosfamide, melphalan, chlorambucil, pipobroman, triéthylène-mélamine, triéthylènethiophosphoramine, busulfan, carmustine, lomustine, streptozocine, dacarbazine, témozolomide, méthotrexate, 5-fluorouracile, floxuridine, cytarabine, 6-mercaptopurine, 6-thioguanine, fludarabine phosphate, pentostatine, gemcitabine, vinblastine, vincristine, vindésine, bléomycine, dactinomycine, daunorubicine, doxorubicine, épirubicine, idarubicine, paclitaxel, mithramycine, désoxycoformycine, mitomycine C, 1-asparaginase, interférons, étoposide, téniposide, 17α-éthynyloestradiol, diéthyl-stilbestrol, testostérone, prednisone, fluoxymestérone, dromostanolone propionate, testolactone, mégestrol acétate, tamoxifène, méthyl-prednisolone, méthyl-testostérone, prednisolone, triamcinolone, chlorotrianisène, hydroxy-progestérone, aminoglutéthimide, oestramustine, médroxyprogestérone acétate, leuprolide, flutamide, torémifène, goséréline, cisplatine, carboplatine, hydroxyurée, amsacrine, procarbazine, mitotane, mitoxantrone, lévamisole, navelbène, CPT-11, anastrazole, létrazole, capécitabine, réloxafine, droloxafine, gemcitabine, paclitaxel et hexaméthylmélamine.

25. Emploi conforme à la revendication 23, dans lequel ledit agent antinéoplasique est du témozolomide

26. Emploi conforme à l'une des revendications précédentes, dans lequel ledit traitement comporte en outre le fait

d'administrer des rayons.

27. Emploi conforme à l'une des revendications précédentes, dans lequel la mort des celules cancéreuses intervient par apoptose

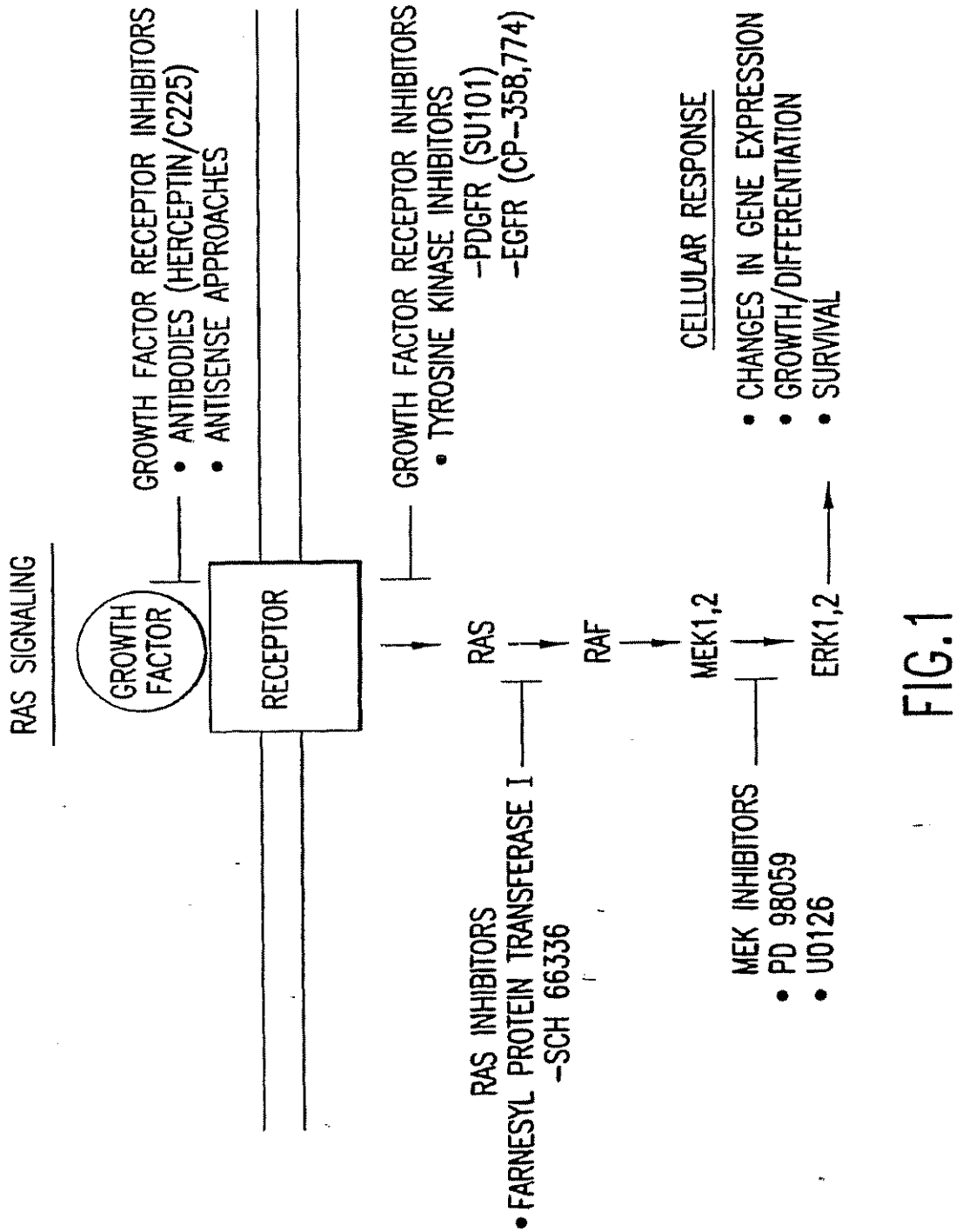

RAS SIGNALING
GROWTH FACTOR
RECEPTOR
GROWTH FACTOR RECEPTOR INHIBITORS
• ANTIBODIES (HERCEPTIN/C225)
• ANTISENSE APPROACHES
GROWTH FACTOR RECEPTOR INHIBITORS
• TYROSINE KINASE INHIBITORS
–PDGFR (SU101)
–EGFR (CP–358,774)
RAS
RAS INHIBITORS
• FARNESYL PROTEIN TRANSFERASE I
–SCH 66336
RAF
MEK1,2
MEK INHIBITORS
• PD 98059
• U0126
ERK1,2
CELLULAR RESPONSE
• CHANGES IN GENE EXPRESSION
• GROWTH/DIFFERENTIATION
• SURVIVAL

FIG. 1

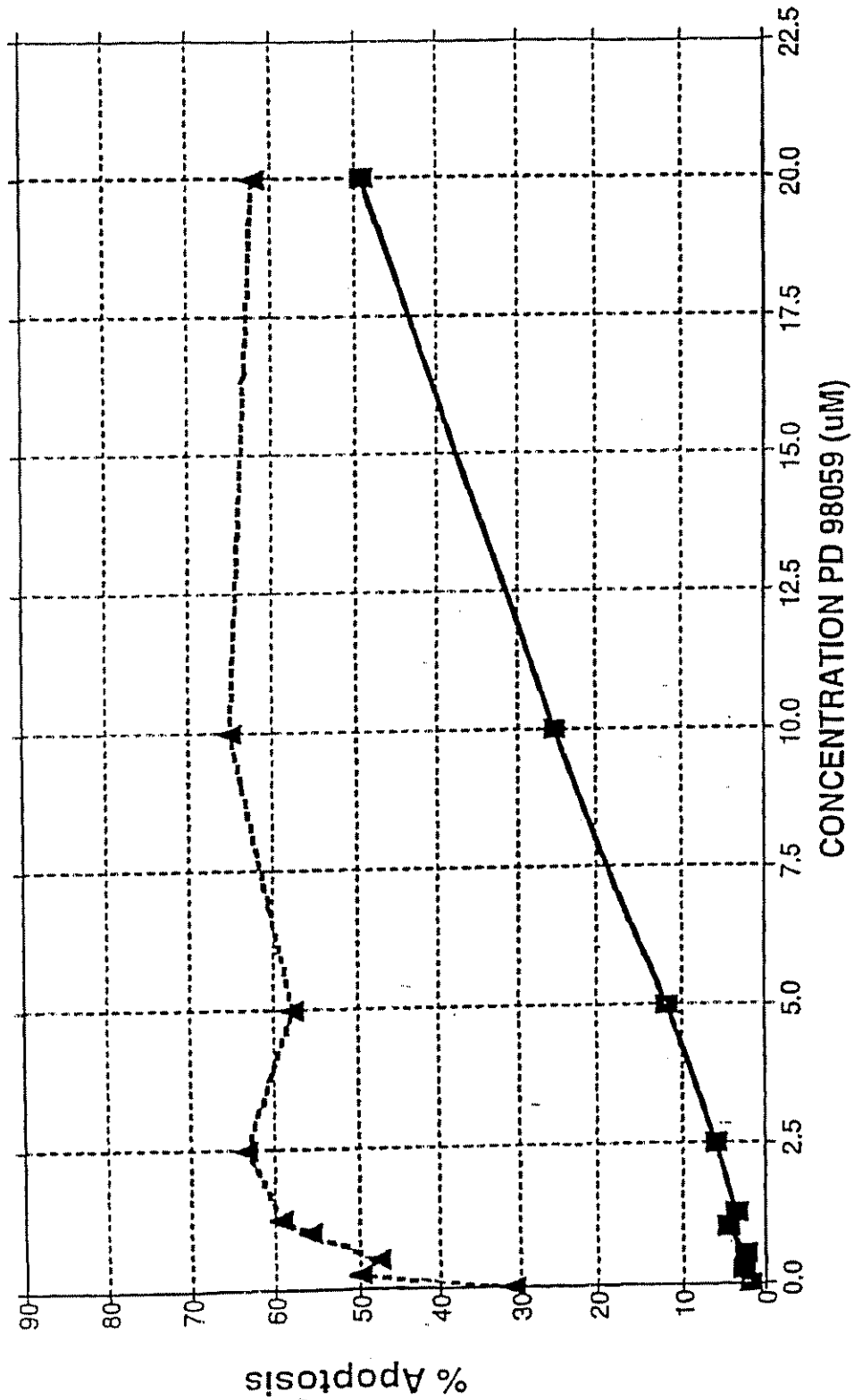

% Apoptosis
0
10
20
30
40
50
60
70
80
90
0.0
2.5
5.0
7.5
10.0
12.5
15.0
17.5
20.0
22.5
CONCENTRATION PD 98059 (uM)
PD 98059
PD 98059 +SCH 66336

FIG. 2

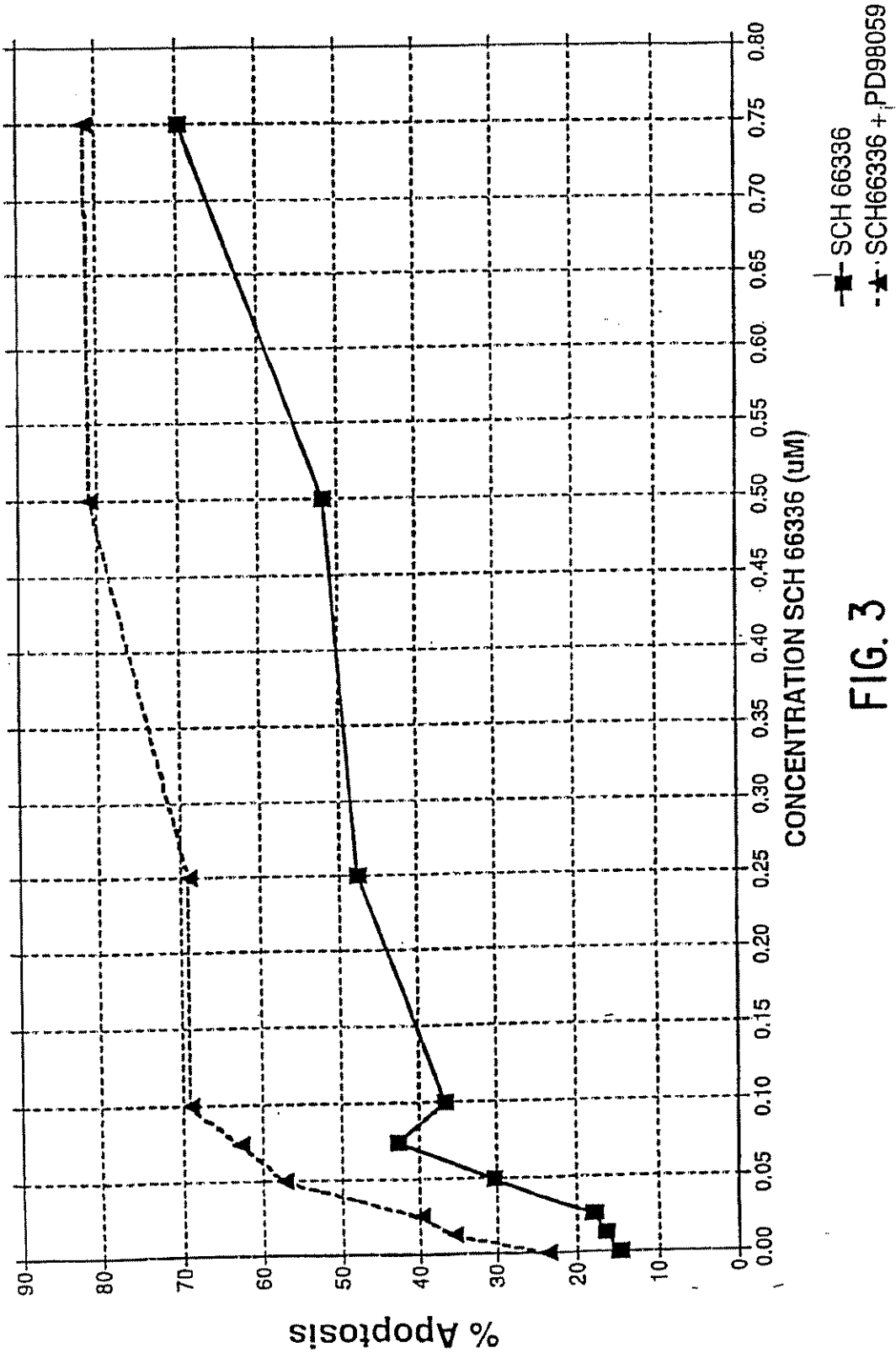

% Apoptosis
0
10
20
30
40
50
60
70
80
90
0.00
0.05
0.10
0.15
0.20
0.25
0.30
0.35
0.40
0.45
0.50
0.55
0.60
0.65
0.70
0.75
0.80
CONCENTRATION SCH 66336 (uM)
SCH 66336
SCH66336 + PD98059

FIG. 3

FIG.4

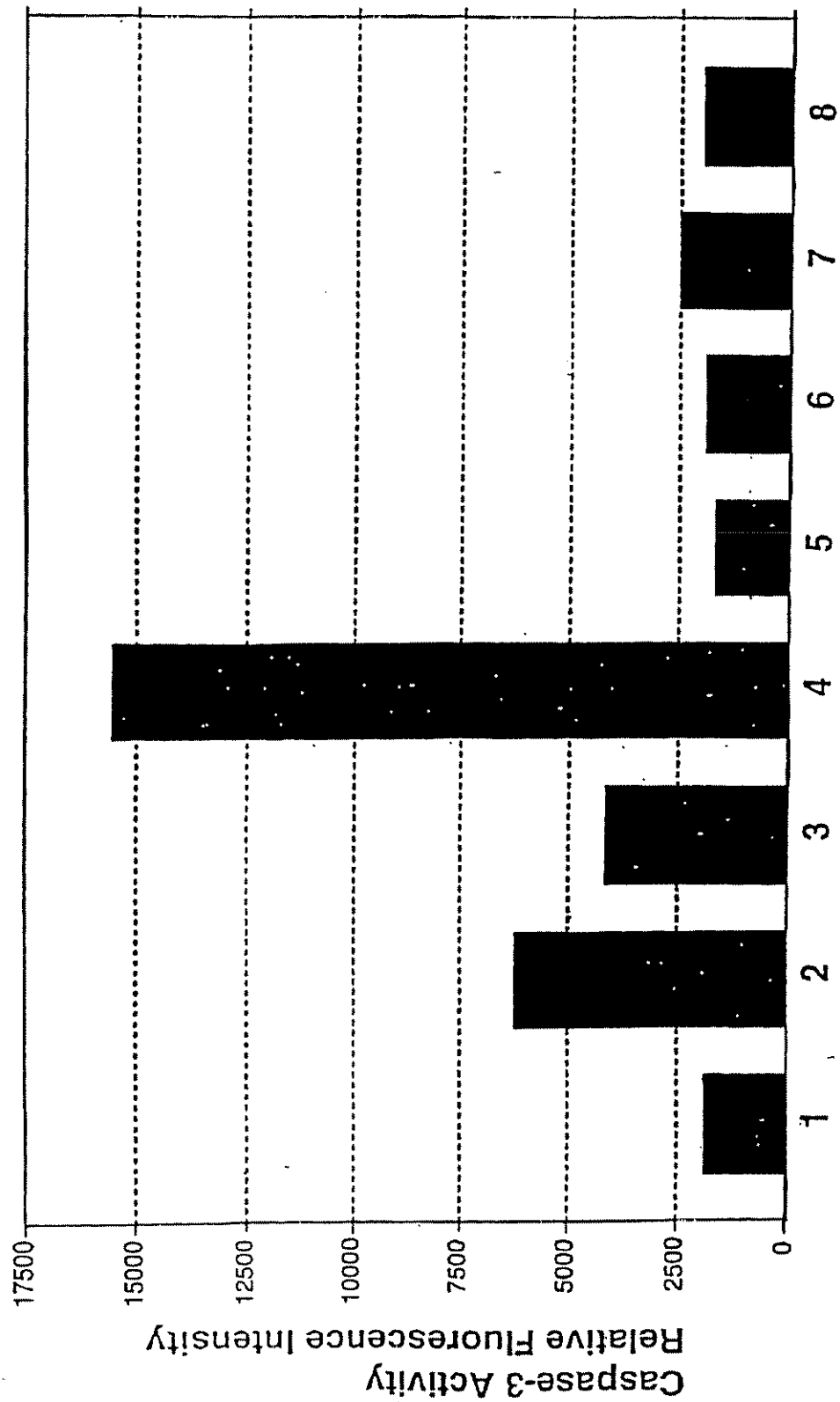

Caspase-3 Activity
Relative Fluorescence Intensity
17500
15000
12500
10000
7500
5000
2500
0
1
2
3
4
5
6
7
8

FIG.5

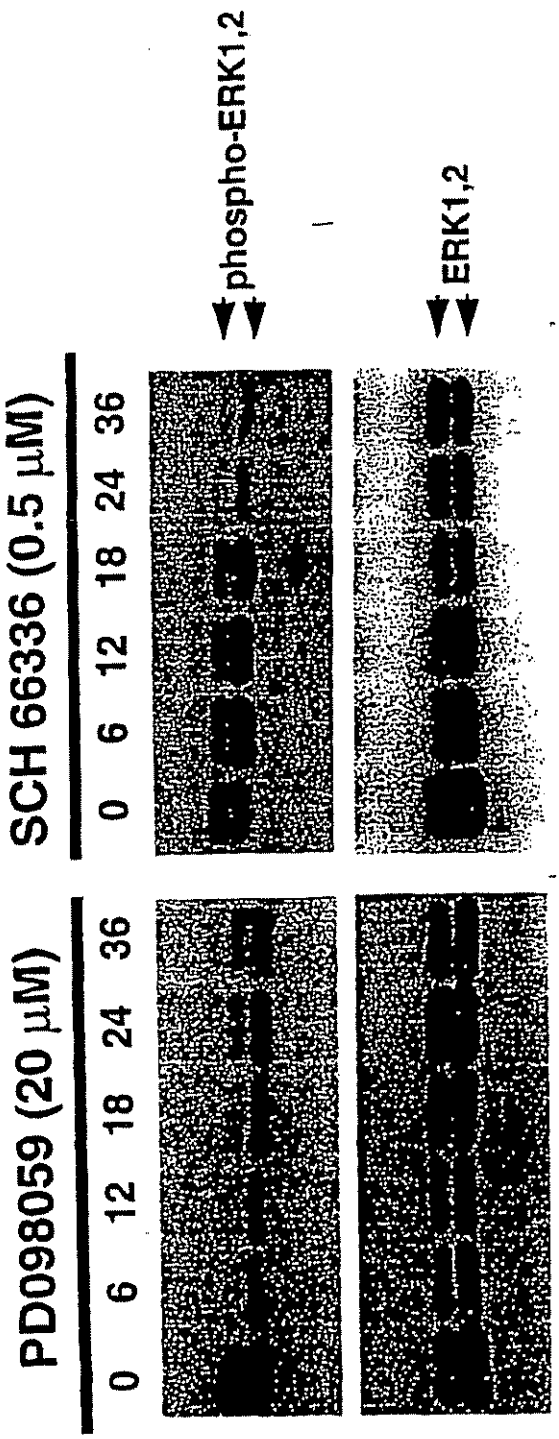

phospho-ERK1,2
ERK1,2
SCH 66336 (0.5 μM)
0 6 12 18 24 36
PD098059 (20 μM)
0 6 12 18 24 36

FIG.6

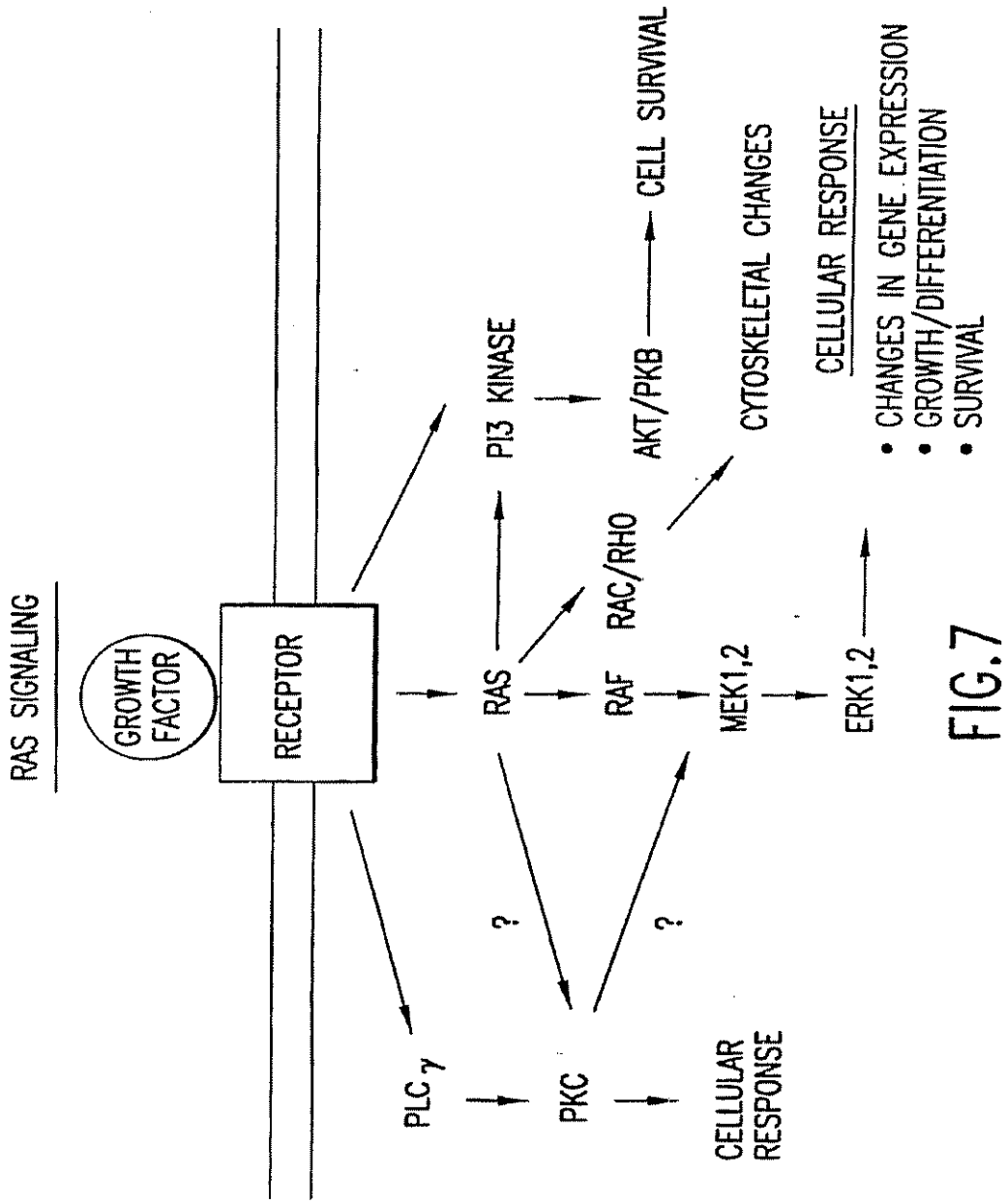

RAS SIGNALING
GROWTH FACTOR
RECEPTOR
RAS
PI3 KINASE
AKT/PKB
CELL SURVIVAL
RAF
RAC/RHO
CYTOSKELETAL CHANGES
MEK1,2
ERK1,2
CELLULAR RESPONSE
• CHANGES IN GENE EXPRESSION
• GROWTH/DIFFERENTIATION
• SURVIVAL
PLC γ
PKC
?
?
CELLULAR RESPONSE

FIG.7