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(54) **Title**: DETERMINING SENSITIVITY OF CELLS TO B-RAF INHIBITOR TREATMENT BY DETECTING KRAS MUTATION AND RTK EXPRESSION LEVELS

(57) **Abstract**: The present invention relates to prognostic methods for identifying tumors that are not susceptible to B-Raf inhibitor treatment by detecting mutations in a K-ras gene or protein or by detecting overexpression of RTKs and/or their ligands. Kits are also disclosed for carrying out the methods.

DETERMINING SENSITIVITY OF CELLS TO B-RAF INHIBITOR TREATMENT BY DETECTING KRAS MUTATION AND RTK EXPRESSION LEVELS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit under 35 U.S.C. § 119(e) of U.S. Provisional Application Serial Nos. 61/236466 filed August 24, 2009 and 61/301149 filed February 3, 2010, which are incorporated herein by reference in their entirety for all purposes.

FIELD OF THE INVENTION

[0002] The present invention relates to cancer diagnostics and therapies and in particular to the detection of mutations or RTK overexpression that are diagnostic and/or prognostic and correlating the detection with cancer treatment.

BACKGROUND OF INVENTION

[0003] Receptor tyrosine kinases (RTKs) and their ligands are important regulators of tumor cell proliferation, angiogenesis, and metastasis. For example, the ErbB family of RTKs include EGFR (HER1 and ErbB1), HER2 (neu or ErbB2), HER3 (ErbB3), and HER4 (ErbB4), and have distinct ligand-binding and signaling activities. Ligands that bind to ErbB receptors include epidermal growth factor (EGF), transforming growth factor a (TGFa), heparin-binding EGF-like ligand (HB-EGF), amphiregulin (AR), betacellulin (BTC), epiregulin (EPR), epigen (EPG), heregulin (HRG), and neuregulin (NRG). These ligands bind directly to EGFR, HER3, or HER4 and trigger multiple downstream signaling cascades, including the RAS-ERK and PI3K-Akt pathways. EGF and other growth factors and cytokines, such as platelet-derived growth factor (PDGF), signal via Ras. Ras mutations permanently lock Ras in its active, GTP-bound state (Wislez, M, et al., *Cancer Drug Discovery and Development: EGFR Signaling Networks in Cancer Therapy*, Eds: J. D. Haley and W. J. Gullick, Humana Press, pp. 89-95, 2008).

[0004] MET is another RTK whose activation by its ligand hepatocyte growth

factor (HGF) induces MET kinase catalytic activity, which triggers transphosphorylation of the tyrosines Tyr 1234 and Tyr 1235. These two tyrosines engage various signal transducers, thus initiating a whole spectrum of biological activities driven by MET. HGF induces sustained RAS activation, and thus prolonged MAPK activity.

[0005] K-ras is one of ras genes that undergo mutation in various cancers. The mutation of the K-ras gene at codons 12 and 13 takes part in tumorigenesis which leads to functional modification of p21-ras protein, a K-ras gene product, resulting in transferring excessive growth signals to a cell nuclei to stimulate cell growth and division. Therefore, identification of mutations of K-ras gene has been widely used as a useful tool in cancer diagnosis, e.g., pancreatic, colorectal and non-small cell lung cancers, and studies have suggested that it might be associated with some tumor phenotypes (Samowitz W S, et al., Cancer Epidemiol. Biomarkers Prev. 9: 1193-1197, 2000; Andreyev H J, et al., Br. J. Cancer 85: 692-696, 2001; and Brink M, et al., Carcinogenesis 24: 703-710, 2003).

[0006] Ras plays an essential role in oncogenic transformation and genesis. Oncogenic H-, K-, and N-Ras arise from point mutations limited to a small number of sites (amino acids 12, 13, 59 and 61). Unlike normal Ras, oncogenic ras proteins lack intrinsic GTPase activity and hence remain constitutively activated (Trahey, M., and McCormick, F. (1987) Science 238: 542-5; Tabin, C. J. et al. (1982) Nature. 300: 143-9; Taparowsky, E. et al. (1982) Nature. 300: 762-5). The participation of oncogenic ras in human cancers is estimated to be 30% (Almoguera, C. et al (1988) Cell. 53:549-54).

[0007] Mutations are frequently limited to only one of the ras genes, and the frequency is tissue- and tumor type-specific. K-ras is the most commonly mutated oncogene in human cancers, especially the codon-12 mutation. While oncogenic activation of H-, K-, and N-Ras arising from single nucleotide substitutions has been observed in 30% of human cancers (Bos, J. L. (1989) Cancer Res 49, 4682-9), over 90% of human pancreatic cancer manifest the codon 12 K-ras mutation (Almoguera, C. et al. (1988) Cell 53, 549-54; Smit, V. T. et al. (1988) Nucleic Acids Res 16, 7773-82; Bos, J. L. (1989) Cancer Res 49, 4682-9). Pancreatic ductal adenocarcinoma, the most common cancer of the pancreas, is notorious for its rapid onset and resistance to treatment. The high frequency of K-ras mutations in human pancreatic tumors

suggests that constitutive Ras activation plays a critical role during pancreatic oncogenesis. Adenocarcinoma of the exocrine pancreas represents the fourth-leading cause of cancer-related mortality in Western countries. Treatment has had limited success and the five-year survival remains less than 5% with a mean survival of 4 months for patients with surgically unresectable tumors (Jemal, A et al (2002) CA Cancer J Clin 52, 23-47; Burris, H. A., 3rd et al. (1997) J Clin Oncol 15, 2403-13). This point mutation can be identified early in the course of the disease when normal cuboidal pancreatic ductal epithelium progresses to a flat hyperplastic lesion, and is considered causative in the pathogenesis of pancreatic cancer (Hruban, R. H. et al (2000) Clin Cancer Res 6, 2969-72; Tada, M. et al. (1996) Gastroenterology 110, 227-31). The regulation of oncogenic K-ras signaling in human pancreatic cancer, however, remains largely unknown.

[0008] K-ras mutations are present in 50% of the cancers of colon and lung (Bos, J. L. et al. (1987) Nature. 327: 293-7; Rodenhuis, S. et al. (1988) Cancer Res. 48: 5738-41). In cancers of the urinary tract and bladder, mutations are primarily in the H-ras gene (Fujita, J. et al. (1984) Nature. 309: 464-6; Visvanathan, K. V. et al. (1988) Oncogene Res. 3: 77-86). N-ras gene mutations are present in 30% of leukemia and liver cancer. Approximately 25% of skin lesions in humans involve mutations of the Ha-Ras (25% for squamous cell carcinoma and 28% for melanomas) (Bos, J. L. (1989) Cancer Res. 49:4683-9; Migley, R. S, and Kerr, D. J. (2002) Crit Rev Oncol Hematol. 44:109-20). 50-60% of thyroid carcinomas are unique in having mutations in all three genes (Adjei, A. A. (2001) J Natl Cancer Inst. 93: 1062-74).

[0009] Constitutive activation of Ras can be achieved through oncogenic mutations or via hyperactivated growth factor receptors such as the EGFRs. Elevated expression and/or amplification of the members of the EGFR family, especially the EGFR and HER2, have been implicated in various forms of human malignancies (as reviewed in Prenzel, N. et al. (2001) Endocr Relat Cancer. 8: 11-31). In some of these cancers (including pancreas, colon, bladder, lung), EGFR/HER2 overexpression is compounded by the presence of oncogenic Ras mutations. Abnormal activation of these receptors in tumors can be attributed to overexpression, gene amplification, constitutive activation mutations or autocrine growth factor loops (Voldborg, B. R. et al. (1997) Ann Oncol. 8: 1197-206). For growth factor receptors, especially the EGFRs, amplification or/and overexpression of these receptors frequently occur in the

cancers of the breast, ovary, stomach, esophagus, pancreatic, lung, colon and neuroblastoma.

[0010] The RAS-MAPK signaling pathway controls cell growth, differentiation and survival. This signaling pathway has long been viewed as an attractive pathway for anticancer therapies, based on its central role in regulating the growth and survival of cells from a broad spectrum of human tumors, and mutations in components of this signaling pathway underlie tumor initiation in mammal cells (Sebolt-Leopold et al (2004) Nat Rev Cancer 4, pp 937-47).

[0011] The RAS-MAPK signaling pathway is activated by a variety of extracellular signals (hormones and growth factors), which activate RAS by exchanging GDP with GTP. Ras then recruits RAF to the plasma membrane where its activation takes place. As noted above, mutations in components of the signaling pathway, resulting in constitutive activation, underlie tumor initiation in mammalian cells. For example, growth factor receptors, such as epidermal growth factor receptor (EGFR), are subject to amplifications and mutations in many cancers, accounting for up to 25% of non-small cell lung cancers and 60% of glioblastomas. Braf is also frequently mutated, particularly in melanomas (approximately 70% of cases) and colon carcinomas (approximately 15% of cases). Moreover, ras is the most frequently mutated oncogene, occurring in approximately 30% of all human cancers. The frequency and type of mutated ras genes (H-ras, K-ras or N-ras) varies widely depending on the tumor type. K-ras is, however, the most frequently mutated gene, with the highest incidence detected in pancreatic cancer (approximately 90%) and colorectal cancer (approximately 45%). This makes it, as well as other components of the signaling pathway, an appropriate target for anticancer therapy. Indeed, small-molecular weight inhibitors designed to target various steps of this pathway have entered clinical trials. Moreover, sorafenib (Nexavar.RTM., Bayer HealthCare Pharmaceuticals), a RAF-kinase inhibitor resulting in RAS signaling inhibition, has recently been approved against renal cell carcinoma. Following these data, there continues to be a high level of interest in targeting the RAS-MAPK pathway for the development of improved cancer therapies.

[0012] The RAS-MAPK signaling pathway is activated by a variety of extracellular signals (hormones and growth factors), which activate RAS by exchanging GDP with GTP. Ras then recruits RAF to the plasma membrane where its

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[0013] As described in Downward, J. (2002) Nature Reviews Cancer, volume 3, pages 11-22, the RAS proteins are members of a large superfamily of low-molecular-weight GTP-binding proteins, which can be divided into several families according to the degree of sequence conservation. Different families are important for different cellular processes. For example, the RAS family controls cell growth and the RHO family controls the actin cytoskeleton. Conventionally, the RAS family is described as consisting of three members H-, N- and K-RAS, with K-RAS producing a major (4B) and a minor (4A) splice variant (Ellis, C. A and Clark, G. (2000) Cellular Signalling, 12:425-434). The members of the RAS family are found to be activated by mutation in human tumors and have potent transforming potential.

[0014] The RAS members are very closely related, having 85% amino acid sequence identity. Although the RAS proteins function in very similar ways, some indications of subtle differences between them have recently come to light. The H-ras, K-ras and N-ras proteins are widely expressed, with K-ras being expressed in almost

all cell types. Knockout studies have shown that H-ras and N-ras, either alone or in combination, are not required for normal development in the mouse, whereas K-ras is essential (Downward, J. (2002) at page 12).

[0015] Furthermore, as described in Downward, J. (2002), aberrant signaling through RAS pathways occurs as the result of several different classes of mutational damage in tumor cells. The most obvious of these mutations is in the ras genes themselves. Some 20% of human tumors have activating point mutations in ras, most frequently in K-ras (about 85% of total), then N-ras (about 15%), then H-ras (less than 1%). These mutations all compromise the GTPase activity of RAS, preventing GAPs from promoting hydrolysis of GTP on RAS and therefore causing RAS to accumulate in the GTP-bound, active form. Almost all RAS activation in tumors is accounted for by mutations in codons 12, 13 and 61 (Downward, J. (2002) at page 15).

[0016] It would be useful if cancer treatment could be tailored to the specific cancer. In particular, the present invention provides for a means of determining whether certain approved and available treatments would nevertheless not be of benefit for the particular type of cancer.

BRIEF SUMMARY OF INVENTION

[0017] The present invention relates to prognostic methods for identifying tumors that are not susceptible to B-Raf inhibitor treatment by detecting mutations in a K-ras gene or protein. The methods involve determining the presence or absence of a mutated K-ras gene or protein in a sample thereby identifying a tumor that is non-responsive to B-Raf inhibitor treatment. Kits are also disclosed for carrying out the methods.

[0018] In another aspect, the present invention relates to prognostic methods for identifying tumors that are not susceptible to B-Raf inhibitor treatment by detecting aberrant expression levels of RTKs. The methods involve determining the expression levels of certain RTKs in a sample, whereby overexpression of RTKs correlate non-responsiveness to B-Raf inhibitor treatment. Examples of RTKs that correlate to the responsiveness of B-Raf treatment include, but are not limited to EGFR and cMet. The methods also involve determining the induction levels of certain ligands of RTKs in a sample whereby abnormally high levels of ligand induction correlates with non-responsiveness to B-Raf inhibitor treatment. Examples of ligands

that correlate to the responsiveness of B-Raf treatment include, but are not limited to EGF and HGF. The methods also involve determining the levels of Ras-GTP in a sample whereby abnormally high levels of Ras-GTP correlates with non-responsiveness to B-Raf inhibitor treatment. Kits are also disclosed for carrying out the methods.

[0019] In another aspect, the present invention relates to methods of treating a tumor that is non-responsive to B-Raf inhibitor treatment. The methods include administering a B-Raf inhibitor in combination with an EGFR inhibitor.

DESCRIPTION OF THE FIGURES

[0020] Figure 1 depicts Biochemical enzyme assay data. The data show that at physiological [ATP], only GDC-0879 maintains effective potency against both B-Raf^{V600E} and WT Raf isoforms.

[0021] Figure 2 depicts Viability assays in tumor lines of different Raf/Ras mutational status.

[0022] Figure 3 depicts sustained pMEK induction by Raf inhibitors only in non-B-Raf^{V600E} lines. pMEK levels plateau relatively to inhibitors' IC_{50} against WT Raf.

[0023] Figure 4 depicts c-Raf is the Raf isoform primarily responsible for the pMEK induction by Raf inhibitors in non B-Raf^{V600E} lines.

[0024] Figure 5 depicts c-Raf specific activity induced by both inhibitors only in non-B-Raf^{V600E} lines. There was no decrease in Sprouty levels under conditions of Raf induction.

[0025] Figure 6 depicts no induction of pERK levels. Inhibitors' relative potencies correlate with their biochemical IC_{50}s.

[0026] Figure 7 depicts bell-shaped effects on pMEK levels under basal conditions. Inhibitory effects of GDC-0879 predominate after serum stimulation.

[0027] Figure 8A depicts the duration and extent of BRAF pathway inhibition determines B-Raf inhibitor, GDC-0879 efficacy in primary human tumor xenograft models. A Kaplan-Meier plot showing time to tumor doubling for patient-derived melanoma and non-small cell lung cancer tumor models treated daily with 100 mg/kg GDC-0879 or vehicle. Genotypes for BRAF, N-ras and K-ras are indicated. A statistically significant (P <0.05) delay in tumor progression was noted for MEXF

989, MEXF 276, and MEXF 355 tumors. GDC-0879 administration significantly accelerated growth of some K-ras-mutant non-small cell lung tumors, such as LXFA 1041 and LXFA 983.

[0028] Figure 8B depicts GDC-0879 treatment down-regulated ERK1/2 phosphorylation in $BRAF^{V600E}$ primary human xenograft tumors. In time course pharmacodynamic studies, mice were treated with 100 mg/kg GDC-0879 and sacrified at 1 or 8 h following the last dose (days 21-24). Immunoblots of phosphorylated and total ERK1/2 are shown. Potent phosphor-ERK1/2 inhibition sustained through 8 h was strongly correlated with $BRAF^{V600E}$ status and GDC-0879 antitumor efficacy. Total ERK1/2 expression was examined in all samples as a loading control.

[0029] Figures 9A, B, C & D depict K-ras-mutant tumor cell lines show differential sensitivity to GDC-0879 RAF and MEK inhibitors *in vivo* and *in vitro*. A and B, inhibition of MEK, but not RAF, prevented the *in vivo* growth of K-RAS-mutant HCT116 tumors. Mice were randomized when tumors reached ~200 mm^3 and treatment was initiated with either 100 mg/kg GDC-0879 (A) or 25 mg/kg MEK inhibitor (MEK Inh; B) on a daily schedule. Points, mean; bars, SE. C, GDC-0879 EC_{50} values for 130 cell lines are shown as a function of BRAF and K-RAS mutational status. GDC-0879-mediated inhibition of cell growth was strongly correlated with BRAF mutation. D, dot plots for MEK inhibitor EC_{50} values are organized according to genotype. MEK inhibition was also potent on a significant fraction of cell lines expressing wild-type BRAF. Data represents the mean of quadruplicate measurements.

[0030] Figures 10-18 depict growth in lung tumor xenografts after dosing with GDC-0879.

[0031] Figures 19A and B depict Raf inhibitors inducing RAS-dependent translocation of wildtype RAF to the plasma membrane in non-B-RAFV600E cells. (A) MeWo (RAS/RAFWT) cells were treated with GDC-0879 (2-{4-[(1E)-1-(hydroxyimino)-2,3-dihydro-1H-inden-5-yl]-3-(pyridine-4-yl)-1H-pyrazol-1-yl}ethan-1-ol), PLX4720 (N-[3-[(5-chloro-1H-pyrrolo[2,3-b]pyridin-3-yl)carbonyl]-2,4-difluorophenyl]-1-propanesulfonamide) or AZ-628 (3-(2-cyanopropan-2-yl)-N-(4-methyl-3-(3-methyl-4-oxo-3,4-dihydroquinazolin-6-ylamino)phenyl)benzamide) (all at 0.1, 1, 10 mM) for 1hr and fractionated into membrane (P100) and cytosolic (S100)

fractions. Aliquots of the membrane and cytosolic fractions were immunoblotted with the indicated antibodies. (B) HEK293T cells were transiently transfected with Venus-C-RAF (green), CFP-K-RAS (red) and mCherry-H2B (blue). Venus-tagged C-RAF co-localizes with CFP-KRAS on the plasma membrane in cells treated with 10 mM GDC-0879 or AZ-628 for 4 hours followed by live cell imaging using confocal fluorescence microscopy. Membrane translocation is blocked when the dominant negative CFP-tagged KRASS17N is transfected instead of KRASWT (right panel).

[0032] Figures 20A, B, C and D depict the importance of the role of acitve Ras plays in C-RAF activation and phospho-MEK induction by RAF inhibitors. (A) A375 (B-RAFV600E) cells were treated with GDC-0879 or PLX4720 for 1hour and lysed in hypotonic buffer for membrane fractionation. Both membrane (P100) and cytosolic (S100) fractions were immunoblotted with the indicated antibodies. (B) MeWo cells were transiently transfected with KRASWT or KRASS17N, treated with GDC-0879 or PLX4720 (at 0.1, 1, 10 mM) for 1hour and fractionated into membrane (P100) and cytosolic (S100) fractions. Aliquots of the membrane and cytosolic fractions were immunoblotted with anti-phospho- and anti-total MEK antibodies. (C) RAS-GTP levels were measured from lysates of MeWo (RAS/RAFWT), A375 (B-RAFV600E) and H2122 (KRASMT) cells with a Ras-GTP ELISA protocol using immobilized C-RAF-RBD as bait for capturing RAS-GTP. Relative luminescent units represent RAS detection of an anti-RAS antibody bound to the RBD. RAS-GTP H2122>>Mewo>A375. (D) Transfection of mutant KRASG12D (but not KRASWT) in A375 (B-RAFV600E) cells, allows the cells to induce B-RAF:C-RAF heterodimers and C-RAF kinase activation in the presence of the RAF inhibitor GDC-0879 (dosed at 0.1, 1, 10 mM). C-RAF was immunoprecipitated from control and inhibitor-treated cells and assayed for protein activity and B-RAF heterodimerization. Total C-RAF levels shown by WB in the immunoprecipitate indicate loading for each lane.

[0033] Figures 21A, B, C and D depict measurements of basal and EGF-stimulated pERK knockdown by Raf inhibitors in B-RafV600E and WT B-Raf cell lines. (A) Table of genotype and EGFR levels among lines tested. (B) Measurement of basal and stimulated pERK levels: cells were treated with 0.0004-10mM compound in serum free media for 1 hour. For stimulation 20ng/ml EGF was added for 5 min before cells were lysed. Lysates were transferred to an MSD plate where phospho- and total ERK levels were measured. (C) pERK IC50 data are plotted for the two Raf

inhibitors (CHR-265, 1-methyl-5-[[2-[5-(trifluoromethyl)-1H-imidazol-2-yl]-4-pyridinyl]oxy]-N-[4-(trifluoromethyl)phenyl]-1H-Benzimidazol-2-amine and GDC-0879) under basal and EGF-stimulated conditions. (D) Dose response curves of pERK induction upon 1-hr treatment of indicated WT B-Raf lines with Raf inhibitors.

[0034] Figures 22A and B depict EGF stimulation rendering phospho-MEK levels and cellular proliferation of B-RAF V600E mutant cell lines resistant to RAF inhibitor. (A) Cells were treated with 0.0004-10mM compound in serum free media for 1 hour. For stimulation 20ng/ml EGF was added for 5 min before cells were lysed. Lysates were transferred to an MSD plate where phospho- and total MEK levels were measured. Phospho-MEK (pMEK) IC50 data are plotted for the two Raf inhibitors indicated under basal and EGF-stimulated conditions. GDC-0879 is more effective in knocking down phospho-MEK levels because it has a lower adjusted IC50 against wildtype C-RAF and B-RAF isoforms than PLX4720. (B) EGF treatment renders B-RAFV600E cells resistant to RAF inhibitors but combination with Tarceva (or MEK inhibitor, e.g. PD-0325901) overcomes that resistance. Cells were dosed with indicated inhibitors, either alone or in combination in the presence of 20ng/ml EGF in the media.

[0035] Figure 23 depicts EGF stimulation inducing B-RAF and C-RAF activity in B-RAFV600E mutant lines (LOX, 888 are melanoma while HT29 is colon). All cell lines express surface EGFR levels. 888 is homozygous for the B-RAF V600E allele, all others are lines are heterozygous, therefore carry a wildtype B-RAF allele as well . The heterozygous cell lines induce both B-RAF and C-RAF activity, while the homozygous line induces only C-RAF activity. This wildtype RAF activity can not be inhibited by B-RAF V600E selective RAF inhibitors, therefore the phospho-MEK induced levels by EGF are resistant to RAF inhibition in these lines, while endogenous phospho-MEK levels driven by B-RAF V600E are sensitive to B-RAF V600E selective RAF inhibitors.

[0036] Figure 24 depicts a trend towards a negative correlation between high EGF mRNA levels (x axis) and RAF inhibitor IC50 (uM, in y axis). Cellular efficacy data are shown for B-RAF V600E melanoma cell lines and represent RAF inhibitors that are biochemically selective for the B-RAF V600E isoform with lower respective biochemical and cellular potencies against wildtype RAF isoforms.

[0037] Figure 25 depicts RAS-GTP levels in various tumor types. RAS-GTP

levels are low in K-RAS^{WT} tumors, and high in tumors bearing mutated K-RAS, for example, H2122 tumors. Ras-GTP levels were determined by RBD-Elisa assay.

[0038] Figure 26 depicts Ras-GTP levels in B-Raf V600E cells with (+EGF) and without (NI) induction of EGF. EGF stimulation increases Ras-GTP levels in BRAF V600E cells.

[0039] Figure 27 depicts pERK levels in B-Raf V600E cells with (stim) and without (unstim) induction of EGF. EGF stimulation increases Ras-GTP levels in BRAF V600E cells leading to an increase in pERK levels in B-RAF V600E cell lines through activation of C-Raf (see C-Raf activation shown in Figure 23). All 4 cell lines are B-Raf V600E mutant, but among those, A375 has the lowest Ras-GTP levels (lowest levels of active Ras) and does not show robust induction of pMEK and pERK levels in response to EGF. A375 cells are known to be sensitive to Raf inhibitors.

[0040] Figure 28 depicts pMEK levels in B-Raf V600E cells with (stim) and without (unstim) induction of EGF. EGF stimulation increases Ras-GTP levels in BRAF V600E cells leading to an increase in pMEK levels in B-RAF V600E cell lines through activation of C-Raf (see C-Raf activation shown in Figure 23).

[0041] Figure 29 summarizes certain RAF inhibitor (GDC-0879, PLX-4720 and "Raf inh a" which is 2,6-difluoro-N-(3-methoxy-1H-pyrazolo[3,4-b]pyridin-5-yl)-3-(propylsulfonamido)benzamide) potencies for blocking cellular pERK induction in response to EGF stimulation. BRAF V600E cells expressing EGFR were serum starved and then either left unstimulated (-EGF) or stimulated with EGF (+EGF) in the presence of the indicated RAF inhibitors at different doses. pERK inhibition curves were generated and IC_{50} values graphed. GDC-0879, as shown in Figure 1, can more efficiently block wildtype RAF signaling while the remaining two inhibitors are BRAF V600E selective.

[0042] Figure 30 depicts how HGF stimulation (+HGF) leads to pERK induction in cells overexpressing c-MET. This induction is not blocked by RAF inhibitors. However, basal pERK levels that are driven by BRAF V600E are effectively blocked by RAF inhibitors. This demonstrates that c-MET signaling is also through wildtype RAF isoforms.

[0043] Therefore, aberrant expression of receptor tyrosine kinase (RTKs), including EGFR, or aberrant induction by the corresponding ligands, can render cells resistant to RAF inhibitors.

[0044] Figure 31 shows how EGFR expression is associated with resistance to RAF inhibitors among B-RAFV600E cells. This graph represent cellular viability EC50 values (uM) of B-RAF V600E mutant melanoma and colon cell lines that were treated with a RAF inhibitor for 4 days before viability determination. EGFR levels were determined by western blot and classified as negative when no band could be detected by western blot with an anti-EGFR antibody of cell lysates. Among EGFR positive cell lines, there is a range of expression from low to moderate and high. The single EGFR negative cell line that is resistant (>20uM EC50) is PTEN null.

[0045] Figures 32A-C depict combination studies of RAF inhibitor and EGFR inhibitor (Tarceva) in colon tumor lines with different levels of EGFR expression.

[0046] In Figure 32A, western blot of lysates from two BRAF V600E colon lines shows their different levels of total EGFR: COLO201 has low EGFR levels while CX-1 has relatively high EGFR levels.

[0047] In Figure 32B, the effects of combination treatment of COLO201 cells with either RAF inhibitor alone, Tarceva alone or combination of RAF inhibitor and Tarceva are shown.

[0048] In Figure 32C, the effects of combination treatment of CX-1 cells with either RAF inhibitor alone, Tarceva alone or combination of RAF inhibitor and Tarceva are shown. Neither RAF inhibitor alone nor Tarceva alone suppress proliferation as effectively as the combination. Both inhibitors show good synergy when administered together to CX-1 cells.

[0049] Therefore, among EGFR expressing BRAFV600E cells, high levels of EGFR predict strong synergy between RAF inhibitors and EGFR inhibitors. Particularly in colon cancer, where high EGFR expression is prevalent among BRAFV600E tumors, combination of these RAF inhibitors and Tarceva show synergy in inhibiting proliferation of tumor cells.

[0050] Figure 33 shows a mechanistic basis for synergy between RAF inhibitors and Tarceva in BRAFV600E tumor cells expressing high EGFR levels. Western blot were prepared of cells treated for either 1 hour or 24 hours with either no inhibitors (lanes 1, 5, 9, 13) or with RAF inhibitor alone (lanes 2, 6, 10, 14), Tarceva alone (lanes 3, 7, 11, 15) or combination of RAF inhibitor and Tarceva (lanes 4, 8, 12, 16) at a concentration equal to their cellular EC50 value. The 24 hour timepoint shows that ERK phosphorylation in B-RAFV600E mutant cells with high EGFR

expression (CX-1) has reduced sensitive to inhibition by RAF inhibitors and requires RAF inhibitor and EGFR inhibitor combination for maximal efficacy. A portion of the activation signal to ERK comes from wildtype RAF that is activated downstream of EGFR and may not be blocked by the BRAF V600E selective RAF inhibitor.

[0051] Figures 34A – C show results from the interaction and efficacy of the RAF inhibitor a and Erlotinib (Tarceva) given in combination to NCR nude (Taconic) mice bearing subcutaneous HT-29 BRAF V600E human colorectal carcinoma xenografts. In Figure 34A, RAF inh a was given at 100 mg/kg with increasing doses of Tarceva. In Figure 34B, Tarceva was given to all animals with increasing concentrations of RAF inh a. Increased efficacy was observed when both compounds were administered in combination. In Figure 34C, lysates from tumors treated with the indicated doses of inhibitors in Figures 34A and B were analyzed for phospho-ERK (pERK) levels by western blot. The RAF inhibitor a and Tarceva synergized in decreasing phospho-ERK levels in the tumors when co-administered in mice.

DETAILED DESCRIPTION OF THE INVENTION

[0052] In one embodiment, the subject matter disclosed herein relates to a method of identifying a patient nonresponsive to treatment with a B-Raf inhibitor, comprising determining the amount of expression or induction of RTKs and/or their ligands. The methods involve determining the expression or induction levels of certain RTKs and/or their ligands in a sample, whereby overexpression of RTKs and/or their ligands correlate non-responsiveness to B-Raf inhibitor treatment. In an embodiment, the sample expresses the B-Raf V600E mutant. Examples of RTKs that correlate to the responsiveness of B-Raf treatment include, but are not limited to EGFR and cMet. The methods also involve determining the levels of expression of certain ligands of RTKs in a sample whereby abnormally high levels of ligand expression correlates with non-responsiveness to B-Raf inhibitor treatment. Examples of ligands that correlate to the responsiveness of B-Raf treatment include, but are not limited to EGF and HGF.

[0053] In one embodiment, the subject matter disclosed herein relates to a method of identifying a patient nonresponsive to treatment with a B-Raf inhibitor, comprising determining the amount of Ras-GTP in a sample, whereby elevated amounts indicate a patient will not respond to said B-Raf inhibitor treatment. In one

example, the elevated amounts are greater than amounts found in normal unstimulated samples. Methods for measuring the levels of Ras-GTP in a sample are known, for example, ELISA assays are used (e.g. Ras-GTPase ELISA assays from Upstate, Inc.). In one example, the method further comprises administering an effective amount of a MEK or ERK inhibitor to said nonresponsive patient. In another example, the method further comprises administering an effective amount of an inhibitor of EGFR signaling. In another example, the method further comprises administering an effective amount of an inhibitor of EGFR signaling in combination with a B-Raf inhibitor.

[0054] In one embodiment, the subject matter disclosed herein relates to a method of identifying a patient nonresponsive to treatment with a B-Raf inhibitor, comprising determining the level of EGF or EGFR expression in a sample, whereby overexpressed levels of either EGF or EGFR indicate a patient will not respond to said B-Raf inhibitor treatment. In one example, the amount of EGF mRNA is determined. Methods for measuring the levels of EGF and EGFR expression in a sample are known, for example, ELISA immunoassays are used (e.g. QUANTIKINE® immunoassays from R&D Systems, Inc.). In one example, the method further comprises administering an effective amount of a MEK or ERK inhibitor to said nonresponsive patient. In another example, the method further comprises administering an effective amount of an inhibitor of EGFR signaling. In another example, the method further comprises administering an effective amount of an inhibitor of EGFR signaling in combination with a B-Raf inhibitor.

[0055] In one embodiment, the subject matter disclosed herein relates to a method of identifying a patient nonresponsive to treatment with a B-Raf inhibitor, comprising determining the level of HGF or cMET expression in a sample, whereby overexpressed levels of either HGF or cMET indicate a patient will not respond to said B-Raf inhibitor treatment. In one example, the patient expresses B-Raf V600E. In one example, the amount of HGF mRNA is determined. Methods for measuring the levels of HGF and cMET expression in a sample are known, for example, quantitative RT-RealTime PCR assays are used. In another example, ELISA immunoassays are used (e.g. PhosphoDetect® cMET ELISA kits from EMD Chemicals, Inc, or the cMET Human ELISA kit from Invitrogen, Inc.). In one example, the method further comprises administering an effective amount of a cMET

or HGF inhibitor to said nonresponsive patient. In another example, the method further comprises administering an effective amount of a cMET or HGF inhibitor in combination with a B-Raf inhibitor.

[0056] In one embodiment, the subject matter disclosed herein relates to a method of identifying a patient nonresponsive to treatment with a B-Raf inhibitor, comprising determining the presence or absence of a K-ras mutation, whereby the presence of a K-ras mutation indicates a patient will not respond to said B-Raf inhibitor treatment. In one example, the method further comprises administering an effective amount of a MEK or ERK inhibitor to said nonresponsive patient. In another example, the method further comprises administering an effective amount of an inhibitor of EGFR signaling. In another example, the method further comprises administering an effective amount of an inhibitor of EGFR signaling in combination with a B-Raf inhibitor.

[0057] In certain embodiments, the subject matter disclosed herein relates to a method of determining whether a tumor will respond to treatment with a B-Raf inhibitor, comprising determining in a sample of said tumor the presence of a mutant K-ras protein or gene whereby the presence of a mutant K-ras protein or gene indicates that the tumor will not respond to treatment with a B-Raf inhibitor. In one example, the method further comprises administering an effective amount of a MEK or ERK inhibitor to said nonresponsive tumor. In another example, the method further comprises administering an effective amount of an inhibitor of EGFR signaling. In another example, the method further comprises administering an effective amount of an inhibitor of EGFR signaling in combination with a B-Raf inhibitor.

[0058] In certain embodiments, a method of predicting whether a patient will be nonresponsive to treatment with a B-Raf inhibitor is provided. In certain embodiments, the method comprises determining the presence or absence of a K-ras mutation in a tumor of the patient, wherein the K-ras mutation is in codon 12 or codon 13. In certain embodiments, if a K-ras mutation is present, the patient is predicted to be nonresponsive to treatment with a B-Raf inhibitor.

[0059] In certain embodiments, a method of predicting whether a tumor will be nonresponsive to treatment with a B-Raf inhibitor is provided. In certain embodiments, the method comprises determining the presence or absence of a K-ras

mutation in a sample of said tumor, wherein the K-ras mutation is in codon 12 or codon 13. In certain embodiments, the presence of the K-ras mutation indicates that the tumor will be nonresponsive to treatment with a B-Raf inhibitor.

[0060] In certain embodiments, a method of stratifying a human subject in a treatment protocol is provided. The method comprises determining the presence of a mutant K-ras gene or protein thereof in a sample from the subject whereby the presence of a mutant K-ras gene or protein indicates that the subject will not respond to B-Raf inhibitor treatment, and excluding the subject from treatment with a B-Raf inhibitor. This method can include stratifying the subject to a particular subgroup in, for example, a clinical trial. In another embodiment, the method further comprises administering an effective amount of a MEK or ERK inhibitor to said subject having said mutant K-ras gene or protein. In another example, the method further comprises administering an effective amount of an inhibitor of EGFR signaling. In another example, the method further comprises administering an effective amount of an inhibitor of EGFR signaling in combination with a B-Raf inhibitor.

[0061] In an embodiment, a method of classifying a breast, lung, colon, ovarian, thyroid, melanoma or pancreatic tumor is provided. The method comprises the steps of: obtaining or providing a tumor sample; detecting expression or activity of a (i) a gene encoding the B-Raf V600E mutant and (ii) a gene encoding a k-Ras mutant in the sample. The method can further comprise classifying the tumor as belonging to a tumor subclass based on the results of the detecting step; and selecting a treatment based on the classifying step, wherein said treatment is other than a B-Raf V600E specific inhibitor if said k-RAS mutant is overexpressed in said tumor sample. In one example, the treatment comprises administering an effective amount of a MEK or ERK inhibitor to said nonresponsive tumor. In another example, the method further comprises administering an effective amount of an inhibitor of EGFR signaling. In another example, the method further comprises administering an effective amount of an inhibitor of EGFR signaling in combination with a B-Raf inhibitor.

[0062] In another embodiment, a method of treating colorectal or lung cancer is provided. The method comprises determining whether the cancer is K-ras or B-Raf driven, whereby in treating such a cancer that is determined to be K-ras driven, the treatment does not include a B-Raf inhibitor. In one example, the treatment comprises

administering an effective amount of a MEK or ERK inhibitor to said K-ras driven cancer. Also provided is a kit comprising specific material for detecting whether the cancer is K-ras driven or B-Raf driven and instructions for identifying a patient or tumor that is non-responsive to B-Raf inhibitor treatment.

[0063] In certain embodiments, determining the presence or absence of one or more K-ras mutations in a subject comprises determining the presence or amount of expression of a mutant K-ras polypeptide in a sample from the subject. In certain embodiments, determining the presence or absence of one or more K-ras mutations in a subject comprises determining the presence or amount of transcription or translation of a mutant K-ras polynucleotide in a sample from the subject.

[0064] In certain embodiments, determining the presence or absence of one or more K-ras mutations in a subject comprises determining the presence or amount of expression of a polypeptide comprising at least one amino acid sequence selected from the group consisting of the following SEQ ID NOs. listed in US2009/0075267: SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, and SEQ ID NO: 16. In certain embodiments, determining the presence or absence of one or more K-ras mutations in a subject comprises determining the presence or amount of transcription or translation of a polynucleotide encoding at least one amino acid sequence selected from the group consisting of the following SEQ ID NOs. listed in US2009/0075267: SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, and SEQ ID NO: 16 in a sample from the subject.

[0065] In certain embodiments, determining the presence or absence of a polynucleotide encoding a mutant K-ras polypeptide is provided. In certain embodiments, a method of determining the presence or absence of a polynucleotide encoding a mutant K-ras polypeptide in a sample comprises (a) exposing a sample to a probe which hybridizes to a polynucleotide encoding a region of a mutant K-ras polypeptide, wherein the region comprises at least one K-ras mutation selected from G12S, G12V, G12D, G12A, G12C, G13A, and G13D, and (b) determining the presence or absence of a polynucleotide encoding a mutant K-ras polypeptide in the sample. In certain embodiments, a method of determining the presence or absence of a mutant K-ras polypeptide in a sample comprises (a) exposing a sample to a probe which hybridizes to a polynucleotide encoding a region of a mutant K-ras

polypeptide, wherein the region comprises at least one K-ras mutation selected from G12S, G12V, G12D, G12A, G12C, G13A, and G13D, and (b) determining the presence or absence of a mutant K-ras polypeptide in the sample.

[0066] In certain embodiments, determining the presence or absence of a polynucleotide encoding a mutant B-Raf polypeptide is provided. In certain embodiments, a method of determining the presence or absence of a polynucleotide encoding a mutant B-Raf polypeptide in a sample comprises (a) exposing a sample to a probe which hybridizes to a polynucleotide encoding a region of a mutant B-Raf polypeptide, wherein the region comprises a V600E mutation, and (b) determining the presence or absence of a polynucleotide encoding a mutant B-Raf polypeptide in the sample. In certain embodiments, a method of determining the presence or absence of a mutant B-Raf polypeptide in a sample comprises (a) exposing a sample to a probe which hybridizes to a polynucleotide encoding a region of a mutant B-raf polypeptide, wherein the region comprises a V600E mutation, and (b) determining the presence or absence of a mutant B-Raf polypeptide in the sample.

[0067] In certain embodiments, a kit for detecting a polynucleotide encoding a mutant K-ras polypeptide in a subject is provided. In certain such embodiments, the kit comprises a probe which hybridizes to a polynucleotide encoding a region of a mutant K-ras polypeptide, wherein the region comprises at least one K-ras mutation selected from G12S, G12V, G12D, G12A, G12C, G13A, and G13D. In certain embodiments, the kit further comprises two or more amplification primers. In certain embodiments, the kit further comprises a detection component. In certain embodiments, the kit further comprises a nucleic acid sampling component. The kit can optionally contain material for detecting a B-Raf mutation. These materials are known in the art. The combination of a kit capable of detecting K-ras and B-Raf mutant genes or proteins is particularly useful in treating colon and lung cancer. Included in the kit are instructions for identifying a patient or tumor that is non-responsive to B-Raf inhibition when the cancer is K-ras driven. RAS-driven cancers are known in the art. A Ras-driven cancer is any cancer or tumor in which abherent activity of a Ras protein results in production of a transformed cell or the formation of cancer or a tumor.

[0068] In certain embodiments, for those samples, tumors, cancers, subjects or patients determined to be unresponsive to a B-Raf inhibitor, the methods further

comprise administering an effective amount of a MEK inhibitor to said unresponsive samples, tumors, cancers, subjects or patients.

[0069] In certain embodiments, for those samples, tumors, cancers, subjects or patients determined to be unresponsive to a B-Raf inhibitor, the methods further comprise administering an effective amount of a ERK inhibitor to said unresponsive samples, tumors, cancers, subjects or patients. In another example, the method further comprises administering an effective amount of an inhibitor of EGFR signaling. In another example, the method further comprises administering an effective amount of an inhibitor of EGFR signaling in combination with a B-Raf inhibitor.

[0070] EGFR signaling can be inhibited by a variety of methods, including inhibiting EGFR kinase activity, binding to the extracellular domain of EGFR to inhibit activation or by inhibiting the activity and signaling of EGF ligand.

[0071] Inhibitors of EGFR signaling are known in the art and include, for example, erlotinib (TARCEVA®), gefitinib (IRESSA®), lapatinib, pelitinib, Cetuximab, panitumumab, zalutumumab, nimotuzumab and matuzumab, and those described in U.S. Pat. No. 5,747,498.

[0072] B-Raf inhibitors are known in the art and include, for example, sorafenib, PLX4720, PLX-3603, GSK2118436, GDC-0879, N-(3-(5-(4-chlorophenyl)-1H-pyrrolo[2,3-b]pyridine-3-carbonyl)-2,4-difluorophenyl)propane-1-sulfonamide, and those described in WO2007/002325, WO2007/002433, WO2009111278, WO2009111279, WO2009111277, WO2009111280 and U.S. Pat. No. 7,491,829.

[0073] cMET inhibitors are known in the art, and include, but are not limited to, AMG208, ARQ197, ARQ209, PHA665752 (3Z)-5-[(2,6-dichlorobenzyl)sulfonyl]-3-[(3,5-dimethyl-4-{[(2R)-2-(pyrrolidin-1-ylmethyl)pyrrolidin-1-yl]carbonyl}-1H-pyrrol-2-yl)methylene]-1,3-dihydro-2H-indol-2-one, N-(4-(3-((3S,4R)-1-ethyl-3-fluoropiperidin-4-ylamino)-1H-pyrazolo[3,4-b]pyridin-4-yloxy)-3-fluorophenyl)-2-(4-fluorophenyl)-3-oxo-2,3-dihydropyridazine-4-carboxamide and SU11274, and those described in U.S. Pat. No. 7,723,330.

[0074] MEK inhibitors are known in the art, and include, but are not limited to, ARRY-162, AZD8330, AZD6244, U0126, GDC-0973, PD184161 and PD98059, and those described in WO2003047582, WO2003047583, WO2003047585, WO2003053960, WO2007071951, WO2003077855, WO2003077914,

WO2005023251, WO2005051300, WO2005051302, WO2007022529, WO2006061712, WO2005028426, WO2006018188, US20070197617, WO 2008101840, WO2009021887, WO2009153554, US20090275606, WO2009129938, WO2009093008, WO2009018233, WO2009013462, WO2008125820, WO2008124085, WO2007044515, WO2008021389, WO2008076415 and WO2008124085.

[0075] ERK inhibitors are known in the art, and include, but are not limited to, FR180204 and 3-(2-Aminoethyl)-5-((4-ethoxyphenyl)methylene)-2,4-thiazolidinedione, and those described in WO2006071644, WO2007070398, WO2007097937, WO2008153858, WO2008153858, WO2009105500 and WO2010000978.

[0076] Any known method for detecting a mutant K-ras gene or protein is suitable for the method disclosed herein. Particular mutations detected in exon 1 are: G12C; G12A; G12D; G12R; G12S; G12V; G13C; G13D. Methods for determining the presence of K-ras mutations are also analogous to those used to identify K-ras and EGFR mutations, for example the K-ras oligos for PCR listed as SEQ ID Nos. 55, 56, 57 and 58 as described in published U.S. Patent App. No. US2009/0202989A1, herein incorporated by reference in its entirety. By way of example, other methods for detecting a mutant K-ras gene or protein, and the primers, oligos and SEQ ID Nos. are disclosed in published U.S. Patent Application Nos. US2009/0202989A1, US2009/0075267A1, US20090143320, US20040063120 and US2007/0003936. The techniques and procedures are generally performed according to conventional methods well known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification. See e.g., Sambrook et al. Molecular Cloning: A Laboratory Manual (2d ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989)), which is incorporated herein by reference.

[0077] Certain methods of detecting a mutation in a polynucleotide are known in the art. Certain exemplary methods include, but are not limited to, sequencing, primer extension reactions, electrophoresis, picogreen assays, oligonucleotide ligation assays, hybridization assays, TaqMan assays, SNPlex assays, and assays described, e.g., in U.S. Pat. Nos. 5,470,705, 5,514,543, 5,580,732, 5,624,800, 5,807,682, 6,759,202, 6,756,204, 6,734,296, 6,395,486, and U.S. Patent Publication No. US

2003-0190646 A1.

[0078] In certain embodiments, detecting a mutation in a polynucleotide comprises first amplifying a polynucleotide that may comprise the mutation. Certain methods for amplifying a polynucleotide are known in the art. Such amplification products may be used in any of the methods described herein, or known in the art, for detecting a mutation in a polynucleotide.

[0079] Certain methods of detecting a mutation in a polypeptide are known in the art. Certain exemplary such methods include, but are not limited to, detecting using a specific binding agent specific for the mutant polypeptide. Other methods of detecting a mutant polypeptide include, but are not limited to, electrophoresis and peptide sequencing.

[0080] Certain exemplary methods of detecting a mutation in a polynucleotide and/or a polypeptide are described, e.g., in Schimanski et al. (1999) Cancer Res., 59: 5169-5175; Nagasaka et al. (2004) J. Clin. Oncol., 22: 4584-4596; PCT Publication No. WO 2007/001868 A1; U.S. Patent Publication No. 2005/0272083 A1; and Lievre et al. (2006) Cancer Res. 66: 3992-3994.

[0081] In certain embodiments, microarrays comprising one or more polynucleotides encoding one or more mutant K-ras polypeptides are provided. In certain embodiments, microarrays comprising one or more polynucleotides complementary to one or more polynucleotides encoding one or more mutant K-ras polypeptides are provided. In certain embodiments, microarrays comprising one or more polynucleotides encoding one or more mutant B-Raf polypeptides are provided. In certain embodiments, microarrays comprising one or more polynucleotides complementary to one or more polynucleotides encoding one or more mutant B-Raf polypeptides are provided.

[0082] In certain embodiments, the presence or absence of one or more mutant K-ras polynucleotides in two or more cell or tissue samples is assessed using microarray technology. In certain embodiments, the quantity of one or more mutant K-ras polynucleotides in two or more cell or tissue samples is assessed using microarray technology.

[0083] In certain embodiments, the presence or absence of one or more mutant B-Raf polynucleotides in two or more cell or tissue samples is assessed using microarray technology. In certain embodiments, the quantity of one or more mutant

B-Raf polynucleotides in two or more cell or tissue samples is assessed using microarray technology.

[0084] In certain embodiments, the presence or absence of one or more mutant K-ras polypeptides in two or more cell or tissue samples is assessed using microarray technology. In certain such embodiments, mRNA is first extracted from a cell or tissue sample and is subsequently converted to cDNA, which is hybridized to the microarray. In certain such embodiments, the presence or absence of cDNA that is specifically bound to the microarray is indicative of the presence or absence of the mutant K-ras polypeptide. In certain such embodiments, the expression level of the one or more mutant K-ras polypeptides is assessed by quantitating the amount of cDNA that is specifically bound to the microarray.

[0085] In certain embodiments, the presence or absence of one or more mutant B-raf polypeptides in two or more cell or tissue samples is assessed using microarray technology. In certain such embodiments, mRNA is first extracted from a cell or tissue sample and is subsequently converted to cDNA, which is hybridized to the microarray. In certain such embodiments, the presence or absence of cDNA that is specifically bound to the microarray is indicative of the presence or absence of the mutant B-Raf polypeptide. In certain such embodiments, the expression level of the one or more mutant B-Raf polypeptides is assessed by quantitating the amount of cDNA that is specifically bound to the microarray.

[0086] In certain embodiments, microarrays comprising one or more specific binding agents to one or more mutant K-ras polypeptides are provided. In certain such embodiments, the presence or absence of one or more mutant K-ras polypeptides in a cell or tissue is assessed. In certain such embodiments, the quantity of one or more mutant K-ras polypeptides in a cell or tissue is assessed.

[0087] In certain embodiments, microarrays comprising one or more specific binding agents to one or more mutant B-Raf polypeptides are provided. In certain such embodiments, the presence or absence of one or more mutant B-Raf polypeptides in a cell or tissue is assessed. In certain such embodiments, the quantity of one or more mutant B-raf polypeptides in a cell or tissue is assessed.

[0088] All references cited herein, including patents, patent applications, papers, textbooks, and the like, and the references cited therein, to the extent that they are not already, are hereby incorporated herein by reference in their entirety. In the

event that one or more of the documents incorporated by reference defines a term that contradicts that term's definition in this application, this application controls. The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described.

Definitions

[0089] Unless otherwise defined, scientific and technical terms used in connection with the present invention shall have the meanings that are commonly understood by those of ordinary skill in the art. Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular.

[0090] The term "B-Raf inhibitor" refers to any compound or agent that inhibits decreases the activity of a B-Raf kinase. Such an inhibitor may also inhibit other kinases, including other raf kinases. A "specific B-Raf kinase inhibitor" refers to an inhibitor that has selectivity for a mutant B-Raf, such as a mutation at the valine residue at amino acid position 600, e.g., a V600E mutation, compared to the wild-type B-Raf. Such an inhibitor is at least two times, more often at least three times or more, as potent compared to the wild-type B-Raf. The potency can also be compared in terms of IC_{50} values for cellular assays that measure growth inhibition.

[0091] The term "treatment protocol" refers to a therapeutic regimen or course of administering one or more agents to treat a disorder or disease. This includes clinical trials.

[0092] 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.

[0093] The terms "mutant K-ras polypeptide" and "mutant K-ras protein" are used interchangeably, and refer to a K-ras polypeptide comprising at least one K-ras mutation selected from G12S, G12V, G12D, G12A, G12C, G13A, and G13D. Certain exemplary mutant K-ras 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 K-ras 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.

[0094] The terms "mutant B-Raf polypeptide" and "mutant B-Raf protein" are used interchangeably, and refer to a B-Raf polypeptide comprising V600E 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.

[0095] The terms "mutant K-ras polynucleotide", "mutant K-ras oligonucleotide," and "mutant K-ras nucleic acid" are used interchangeably, and refer to a polynucleotide encoding a K-ras polypeptide comprising at least one K-ras mutation selected from G12S, G12V, G12D, G12A, G12C, G13A, and G13D.

[0096] The terms "mutant B-Raf polynucleotide", "mutant B-Raf oligonucleotide," and "mutant B-Raf nucleic acid" are used interchangeably, and refer to a polynucleotide encoding a B-Raf polypeptide comprising a V600E mutation.

[0097] The term "agent" is used herein to denote a chemical compound, a mixture of chemical compounds, a biological macromolecule, or an extract made from biological materials.

[0098] The term "pharmaceutical agent or drug" as used herein refers to a chemical compound or composition capable of inducing a desired therapeutic effect when properly administered to a patient. Other chemistry terms herein are used according to conventional usage in the art, as exemplified by The McGraw-Hill Dictionary of Chemical Terms (Parker, S., Ed., McGraw-Hill, San Francisco (1985)), incorporated herein by reference).

[0099] The term patient includes human and animal subjects.

[00100] The terms "mammal" and "animal" for purposes of treatment refers to

any animal classified as a mammal, including humans, domestic and farm animals, and zoo, sports, or pet animals, such as dogs, horses, cats, cows, etc. Preferably, the mammal is human.

[00101] The term "disease state" refers to a physiological state of a cell or of a whole mammal in which an interruption, cessation, or disorder of cellular or body functions, systems, or organs has occurred.

[00102] The terms "treat" or "treatment" refer to both therapeutic treatment and prophylactic or preventative measures, wherein the object is to prevent or slow down (lessen) an undesired physiological change or disorder, such as the development or spread of cancer. For purposes of this invention, beneficial or desired clinical results include, but are not limited to, alleviation of symptoms, diminishment of extent of disease, stabilized (i.e., not worsening) state of disease, delay or slowing of disease progression, amelioration or palliation of the disease state, and remission (whether partial or total), whether detectable or undetectable. "Treatment" can also mean prolonging survival as compared to expected survival if not receiving treatment. Those in need of treatment include those already with the condition or disorder as well as those prone to have the condition or disorder or those in which the condition or disorder is to be prevented.

[00103] The term "responsive" as used herein means that a patient or tumor shows a complete response or a partial response after administering an agent, according to RECIST (Response Evaluation Criteria in Solid Tumors). The term "nonresponsive" as used herein means that a patient or tumor shows stable disease or progressive disease after administering an agent, according to RECIST. RECIST is described, e.g., in Therasse et al., February 2000, "New Guidelines to Evaluate the Response to Treatment in Solid Tumors," J. Natl. Cancer Inst. 92(3): 205-216, which is incorporated by reference herein in its entirety.

[00104] A "disorder" is any condition that would benefit from one or more treatments. This includes chronic and acute disorders or disease including those pathological conditions which predispose the mammal to the disorder in question. Non-limiting examples of disorders to be treated herein include benign and malignant tumors, leukemias, and lymphoid malignancies. A "tumor" comprises one or more cancerous cells. Examples of cancer include, but are not limited to, carcinoma, lymphoma, blastoma, sarcoma, and leukemia or lymphoid malignancies. More

particular examples of such cancers include squamous cell cancer (*e.g.,* epithelial squamous cell cancer), lung cancer including small- cell lung cancer, non-small cell lung cancer ("NSCLC"), adenocarcinoma of the lung and squamous carcinoma of the lung, cancer of the peritoneum, hepatocellular cancer, gastric or stomach cancer including gastrointestinal cancer, pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, breast cancer, colon cancer, rectal cancer, colorectal cancer, endometrial or uterine carcinoma, salivary gland carcinoma, kidney or renal cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma, anal carcinoma, penile carcinoma, as well as head and neck cancer. In particular, the present method is suitable for breast, colorectal, ovarian, pancreatic or lung cancer. More particularly, the cancer is colon, lung or ovarian. The cancer may be a Ras-driven cancer.

[00105] A disease or condition related to a mutant K-ras includes one or more of the following: a disease or condition caused by a mutant K-ras gene or protein; a disease or condition contributed to by a mutant K-ras gene or protein; and a disease or condition that is associated with the presence of a mutant K-ras gene or protein. In certain embodiments, a disease or condition related to a mutant K-ras is a cancer.

[00106] A "disease or condition related to a mutant K-ras polypeptide" includes one or more of the following: a disease or condition caused by a mutant K-ras polypeptide; a disease or condition contributed to by a mutant K-ras polypeptide; a disease or condition that causes a mutant K-ras polypeptide; and a disease or condition that is associated with the presence of a mutant K-ras polypeptide. In certain embodiments, the disease or condition related to a mutant K-ras polypeptide may exist in the absence of the mutant K-ras polypeptide. In certain embodiments, the disease or condition related to a mutant K-ras polypeptide may be exacerbated by the presence of a mutant K-ras polypeptide. In certain embodiments, a disease or condition related to a mutant K-ras polypeptide is a cancer.

[00107] The following examples, including the experiments conducted and results achieved are provided for illustrative purpose only and are not to be construed as limiting upon the claims.

EXAMPLES

Example 1

B-RAF Deletion and Pharmacological Inhibition Enhances K-ras Driven Tumorigenesis

[00108] The Ras GTPase family controls numerous downstream signaling cascades in response to signals that regulate cellular processes including proliferation and survival. While *Ras* is one of the most prevalent targets for gain-of-function mutations in human tumors, questions remain regarding how the Ras effector pathway functions in mutant K-ras-driven tumorigenesis. Since an important function of K-ras involves B-Raf activation within the canonical MAPK signaling pathway, we initiated a study to determine B-Raf's role in the context of mutant K-ras-driven tumor promotion and maintenance. In some K-ras mutant tumors, B-Raf inhibition not only failed to show any tumor benefit, it even accelerated tumor growth. See, Fig. 8A, showing time to tumor doubling.

[00109] Adenovirus expressing the *Cre* recombinase was delivered to the lungs of genetically engineered mice possessing a conditional $K\text{-}ras^{G12D}$ allele ($K\text{-}ras^{LSL\text{-}G12D}$) and either 0, 1 or both copies of the *B-raf* gene flanked by *LoxP* sites ($B\text{-}raf^{CKO}$). This procedure results in expression of mutant $K\text{-}ras^{G12D}$ in the presence or absence of one or both *B-raf* alleles deleted within the mouse lung. Surprisingly, B-Raf deletion significantly enhances lung tumor number and burden and decreases overall survival. When a highly specific small-molecule inhibitor that targets B-Raf in a murine non-small cell lung carcinoma line harboring the $K\text{-}ras^{G12D}$ mutation was used, we observed an increase in cell proliferation and soft agar colony formation. Further investigation revealed that treating $K\text{-}ras^{G12D}$ expressing cells with the B-Raf inhibitor enhanced MEK and Erk phosphorylation. Therefore, these data suggests that while B-Raf deletion does not inhibit K-ras-driven tumor initiation and disease progression, its presence may play a pivotal role in establishing negative feedback regulation of constitutive mutant K-ras activity.

Example 2

Understanding RAF Signaling in $B\text{-}RAF^{V600E}$ Mutant Versus Wild-Type Tumors

[00110] To understand the role of the Raf pathway with different mutations in the Ras and Raf genes, we characterized two selective small molecule Raf inhibitors with distinct potency profiles against wild-type (WT) B-Raf and c-Raf versus mutant

(MT) B-Raf^{V600E}. Despite their biochemical differences, they had identical cellular profiles, being potent against B-Raf^{V600E} but not WT or Ras MT tumors. Both inhibitors induced activation of the Raf/MEK/ERK pathway in non-BRAF^{V600E} lines, via primarily the c-Raf isoform. In contrast, they inhibited phorbol ester and growth factor-stimulated Raf/MEK/ERK activity according to their predicted biochemical potencies. Thus, the cellular specificity of selective Raf inhibitors for B-Raf^{V600E} lines is not simply a reflection of their selectivity for the B-Raf^{V600E} isoform, but rather reflects the complex regulation of Raf activity in different cellular contexts. Biochemical selectivity for the B-Raf^{V600E} is not the only driver for cellular efficacy profiles of Raf inhibitors. Inhibitors induce pMEK levels selectively in non-V600E mutant lines through c-Raf. Inhibitors induce c-Raf specific activity and pMEK levels rapidly and in a dose-dependent manner according to their potency. Under basal conditions, bell-shaped curve for GDC-0879 suggests dual stimulatory vs. inhibitory effects on c-Raf. B- and c-Raf pathway status in different contexts determines Raf inhibitory pharmacodynamics. The results of the characterization are shown in Figs. 1-7.

Example 3

Growth in Lung Tumor Xenografts after Dosing with B-RAF Inhibitor

[00111] The data are shown below, and in Figs. 10-14 for Experiment H331 and Figs. 15-18 for Experiment H327.

Oncotest Exp-Nr.	H331-1	Implant / Rando / Induction time	18 days
Tumortyp Nr / Pass	LXFA 983/9N4	End result / last study day	21
Tumor Model	Lung, adeno	Therapy	Vehicle control; 10 ml/kg; Days 0-21

	Absolute tumor volume: (a*b*b)/2 [mm3] Study day (after randomization)							Survival time	Absolute body weight [g] Study day (after randomization)						
Animal-No	0	3	7	10	14	17	21	[days]	0	3	7	10	14	17	21
532 r	45.6	80.1	115.2	173.8	183.3	251.3	281.6	>22	27.5	27.7	27.0	27.6	29.0	29.7	30.2
532 l	111.6	275.7	415.2	540.7	957.6	1264.7	1454.6								
1328 r	86.2	139.3	292.8	392.9	540.0	589.8	708.7	>21	26.4	26.5	27.2	27.5	28.7	29.4	28.7
1341 r	137.3	178.9	302.6	354.8	491.9	804.8	1019.6	>21	23.6	23.6	24.0	23.8	24.0	24.0	24.6
1342 r	97.5	176.4	210.5	419.8	393.4	437.5	540.6	>21	27.5	27.4	28.1	27.5	28.0	27.7	28.8
1344 r	226.5	405.0	657.3	713.9	659.8	879.5	1140.8	>21	27.8	28.3	30.5	30.7	29.1	29.4	29.8
1348 r	148.1	196.0	343.2	445.5	563.1	770.2	919.6	>21	26.9	26.0	25.9	26.0	26.6	25.9	26.8
1348 l	134.5	246.4	421.2	514.4	715.6	749.9	881.0								
1352 r	143.7	239.1	288.0	361.3	384.6	465.5	689.1	>21	28.9	28.8	29.0	29.4	29.3	30.6	31.8
1352 l	45.6	77.2	94.2	124.9	153.6	267.2	319.4								
1361 r	126.0	290.5	510.3	562.2	778.8	832.3	1166.4	>21	28.3	28.6	28.9	29.0	30.0	29.3	29.6
1361 l	55.3	111.6	147.9	147.9	194.2	310.4	320.0								
1362 r	65.0	126.0	156.3	221.8	426.5	472.0	553.0	>22	26.8	26.0	26.4	26.3	26.5	26.3	26.1
1365 l	50.6	77.1	141.5	178.9	389.3	514.6	525.0	>22	24.3	24.4	24.0	24.9	25.3	25.3	26.1
n	14	14	14	14	14	14	14		10	10	10	10	10	10	10
Median	104.5	177.6	290.4	377.1	459.2	552.2	698.9		27.2	27.0	27.1	27.5	28.4	28.5	28.8
MIN	45.6	77.1	94.2	124.9	153.6	251.3	281.6		23.6	23.6	24.0	23.8	24.0	24.0	24.6
MAX	226.5	405.0	657.3	713.9	957.6	1264.7	1454.6		28.9	28.8	30.5	30.7	30.0	30.6	31.8
95% MIN	86.2	139.3	210.5	354.8	384.6	465.5	553.0		26.4	26.0	25.9	26.0	26.5	26.3	26.8
95% MAX	134.5	239.1	343.2	445.5	563.1	770.2	919.6		27.8	27.7	28.1	27.6	29.0	29.3	29.8

	Relative tumor volume [%]								Relative body weight [%]						
Animal-No	0	3	7	10	14	17	21		0	3	7	10	14	17	21
532 r	100.0	175.7	252.8	381.4	402.3	551.5	618.1	>22	100.0	100.7	98.2	100.4	105.5	108.0	109.8
532 l	100.0	247.0	372.0	484.5	858.1	1133.3	1303.4								
1328 r	100.0	161.5	339.6	455.7	626.4	684.2	822.1	>21	100.0	100.4	103.0	104.2	108.7	111.4	108.7
1341 r	100.0	130.3	220.4	258.4	358.2	586.1	742.5	>21	100.0	100.0	101.7	100.8	101.7	101.7	104.2
1342 r	100.0	181.0	216.0	430.7	403.6	448.8	554.6	>21	100.0	99.6	102.2	100.0	101.8	100.7	104.7
1344 r	100.0	178.8	290.2	315.2	291.3	388.3	503.7	>21	100.0	101.8	109.7	110.4	104.7	105.8	107.2
1348 r	100.0	132.3	231.7	300.8	380.2	520.0	620.9	>21	100.0	96.7	96.3	96.7	98.9	96.3	99.6
1348 l	100.0	183.2	313.1	382.4	531.9	557.4	654.8								
1352 r	100.0	166.4	200.5	251.5	267.7	324.0	479.7	>21	100.0	99.7	100.3	101.7	101.4	105.9	110.0
1352 l	100.0	169.5	206.8	274.2	337.1	586.4	701.0								
1361 r	100.0	230.6	405.0	446.2	618.1	660.6	925.7	>21	100.0	101.1	102.1	102.5	106.0	103.5	104.6
1361 l	100.0	201.8	267.4	267.4	351.2	561.3	578.7								
1362 r	100.0	193.8	240.5	341.2	656.1	726.2	850.7	>22	100.0	97.0	98.5	98.1	98.9	98.1	97.4
1365 l	100.0	152.2	279.6	353.3	769.1	1016.4	1037.0	>22	100.0	100.4	98.8	102.5	104.1	104.1	107.4
n	14	14	14	14	14	14	14		10	10	10	10	10	10	10
Median	100.0	177.3	260.1	347.3	402.9	573.7	677.9		100.0	100.2	101.0	101.3	103.0	103.8	106.0
MIN	100.0	130.3	200.5	251.5	267.7	324.0	479.7		100.0	96.7	96.3	96.7	98.9	96.3	97.4
MAX	100.0	247.0	405.0	484.5	858.1	1133.3	1303.4		100.0	101.8	109.7	110.4	108.7	111.4	110.0
95% MIN	100.0	161.5	240.5	315.2	402.3	520.0	618.1		100.0	99.6	98.5	100.0	101.4	100.7	104.2
95% MAX	100.0	193.8	290.2	382.4	531.9	726.2	850.7		100.0	100.7	103.0	104.2	104.7	105.9	107.4

Oncotest Exp-Nr.	H331-2	Implant / Rando / Induction time	18 days
Tumortyp Nr / Pass	LXFA 983/9N4	End result / last study day	21
Tumor Model	Lung, adeno	Therapy	G-026887; 100 mg/kg/day po; Days 0-21

	Absolute tumor volume: (a*b*b)/2 [mm3]								Absolute body weight [g]						
	Study day (after randomization)						Survival time		Study day (after randomization)						
Animal-No	0	3	7	10	14	17	21	[days]	0	3	7	10	14	17	21
1330 r	117.2	265.6	392.9	431.7	578.8	705.7	893.3	>21	27.2	26.8	26.3	26.7	26.8	27.2	28.6
1330 l	121.1	166.6	325.1	379.3	655.5	742.6	1083.3								
1332 l	94.1	188.5	261.6	329.5	545.9	701.1	715.5	>21	26.9	27.2	27.4	26.9	27.2	27.6	27.5
1333 r	137.3	239.1	445.5	539.0	574.3	841.5	1030.4	>21	27.3	26.7	26.3	26.7	28.2	28.5	29.3
1336 r	237.3	546.2	689.1	964.8	1411.2	1708.0	1961.1	>21	26.1	25.6	25.7	25.6	25.5	25.8	26.9
1336 l	62.5	176.4	289.1	404.0	581.0	642.0	772.8								
1339 r	80.2	210.9	348.2	612.5	1115.4	1229.4	1472.3	>21	29.4	27.9	27.1	27.6	29.1	29.5	31.1
1339 l	123.0	268.8	344.4	525.4	951.3	1327.1	1618.7								
1346 r	111.6	236.3	455.1	528.2	891.1	1247.1	1461.9	>21	24.8	24.7	24.2	25.3	25.8	25.8	26.6
1346 l	74.4	156.8	253.1	302.6	318.2	426.5	550.0								
1349 r	219.4	392.9	560.0	570.0	597.5	778.8	958.8	>21	31.2	29.4	28.9	29.1	29.4	29.5	30.6
1350 r	55.3	119.1	216.6	348.5	390.2	520.5	609.2	>22	30.2	29.2	28.1	29.1	30.2	30.6	31.0
1350 l	150.4	310.7	352.5	372.6	510.0	606.4	712.9								
1358 r	130.7	295.9	383.3	406.6	499.4	758.4	874.7	>22	27.6	27.1	27.6	28.6	28.9	29.0	28.7
1366 r	74.4	83.2	113.4	200.9	461.7	640.5	1125.0	>22	31.2	29.6	29.3	28.6	30.3	30.7	31.6
n	15	15	15	15	15	15	15		10	10	10	10	10	10	10
Median	117.2	236.3	348.2	406.6	578.8	742.6	958.8		27.5	27.2	27.3	27.3	28.6	28.8	29.0
MIN	55.3	83.2	113.4	200.9	318.2	426.5	550.0		24.8	24.7	24.2	25.3	25.5	25.8	26.6
MAX	237.3	546.2	689.1	964.8	1411.2	1708.0	1961.1		31.2	29.6	29.3	29.1	30.3	30.7	31.6
95% MIN	94.1	188.5	289.1	372.6	510.0	701.1	874.7		26.9	26.7	26.3	26.7	27.2	27.2	28.6
95% MAX	137.3	295.9	392.9	539.0	655.5	841.5	1125.0		29.4	27.9	28.1	27.6	29.1	29.5	29.3

	Relative tumor volume [%]								Relative body weight [%]						
Animal-No	0	3	7	10	14	17	21		0	3	7	10	14	17	21
1330 r	100.0	226.6	335.2	368.3	493.8	602.1	762.1	>21	100.0	98.5	96.7	98.2	98.5	100.0	105.1
1330 l	100.0	137.6	268.5	313.3	541.3	613.3	894.7								
1332 l	100.0	200.4	278.0	350.2	580.3	745.2	760.5	>21	100.0	101.1	101.9	100.0	101.1	102.6	102.2
1333 r	100.0	174.1	324.4	392.5	418.3	612.8	750.4	>21	100.0	97.8	96.3	97.8	103.3	104.4	107.3
1336 r	100.0	230.2	290.4	406.6	594.7	719.8	826.5	>21	100.0	98.1	98.5	98.1	97.7	98.9	103.1
1336 l	100.0	282.2	462.6	646.4	929.7	1027.1	1236.5								
1339 r	100.0	263.0	434.2	763.8	1390.9	1533.1	1836.0	>21	100.0	94.9	92.2	93.9	99.0	100.3	105.8
1339 l	100.0	218.5	280.0	427.1	773.4	1078.9	1316.0								
1346 r	100.0	211.7	407.8	473.3	798.5	1117.5	1309.9	>21	100.0	99.6	97.6	102.0	104.0	104.0	107.3
1346 l	100.0	210.9	340.4	406.9	427.9	573.5	739.6								
1349 r	100.0	179.1	255.3	259.8	272.4	355.0	437.1	>21	100.0	94.2	92.6	93.3	94.2	94.6	98.1
1350 r	100.0	215.3	391.6	630.2	705.6	941.4	1101.7	>22	100.0	96.7	93.0	96.4	100.0	101.3	102.6
1350 l	100.0	206.6	234.4	247.8	339.1	403.2	474.0								
1358 r	100.0	226.4	293.3	311.1	382.1	580.3	669.2	>22	100.0	98.2	100.0	103.6	104.7	105.1	104.0
1366 r	100.0	111.8	152.3	269.9	620.2	860.5	1511.3	>22	100.0	94.9	93.9	91.7	97.1	98.4	101.3
n	15	15	15	15	15	15	15		10	10	10	10	10	10	10
Median	100.0	211.7	293.3	392.5	580.3	719.8	826.5		100.0	97.9	96.5	97.9	99.5	100.8	103.5
MIN	100.0	111.8	152.3	247.8	272.4	355.0	437.1		100.0	94.2	92.2	91.7	94.2	94.6	98.1
MAX	100.0	282.2	462.6	763.8	1390.9	1533.1	1836.0		100.0	101.1	101.9	103.6	104.7	105.1	107.3
95% MIN	100.0	200.4	278.0	350.2	493.8	612.8	760.5		100.0	96.7	96.3	96.4	97.7	98.9	102.2
95% MAX	100.0	230.2	340.4	473.3	773.4	941.4	1101.7		100.0	98.5	98.5	100.0	101.1	102.6	105.1

Oncotest Exp-Nr.	H331-3
Tumortyp Nr./Pass.	LXFA 983/9N4
Tumor Model	Lung, adeno

Implant / Rando / Induction time	18 days
End result / last study day	21
Therapy	None

	Absolute tumor volume: (a*b*b)/2 [mm3] Study day (after randomization)	Survival time	Absolute body weight [g] Study day (after randomization)
Animal-No	0	[days]	0
1335 r	113.1	>0	23.0
1359 l	71.7	>0	24.1
1364 r	77.1	>0	22.5
n	3		3
Median	77.1		23.0
MIN	71.7		22.5
MAX	113.1		24.1
95% MIN	71.7		22.5
95% MAX	113.1		24.1

	Relative tumor volume [%]		Relative body weight [%]
Animal-No	0		0
1335 r	100.0	>0	100.0
1359 l	100.0	>0	100.0
1364 r	100.0	>0	100.0
n	3		3
Median	100.0		100.0
MIN	100.0		100.0
MAX	100.0		100.0
95% MIN	100.0		100.0
95% MAX	100.0		100.0

Oncotest Exp-Nr.	H331-4
Tumortyp Nr./Pass.	LXFA 983/9N4
Tumor Model	Lung, adeno
Implant / Rando / Induction time	18 days
End result / last study day	21
Therapy	Vehicle control; 10 ml/kg; Day 0

	Absolute tumor volume: (a*b*b)/2 [mm3]		Absolute body weight [g]
	Study day (after randomization)	Survival time	Study day (after randomization)
Animal-No	0	[days]	0
1326 l	384.8	>0	30.5
1329 r	156.3	>0	28.3
1338 l	157.7	>0	24.2
1345 r	252.0	>0	28.0
1347 r	171.1	>0	29.3
1353 l	179.6	>0	29.0
n	6		6
Median	175.3		28.7
MIN	156.3		24.2
MAX	384.8		30.5
95% MIN	156.3		28.0
95% MAX	252.0		29.3

	Relative tumor volume [%]		Relative body weight [%]
Animal-No	0		0
1326 l	100.0	>0	100.0
1329 r	100.0	>0	100.0
1338 l	100.0	>0	100.0
1345 r	100.0	>0	100.0
1347 r	100.0	>0	100.0
1353 l	100.0	>0	100.0
n	6		6
Median	100.0		100.0
MIN	100.0		100.0
MAX	100.0		100.0
95% MIN	100.0		100.0
95% MAX	100.0		100.0

Oncotest Exp-Nr.	H331-5
Tumortyp Nr./Pass	LXFA 983/9N4
Tumor Model	Lung, adeno

Implant / Rando / Induction time	18 days
End result / last study day	21
Therapy	G-026887; 100 mg/kg/day po; Day 0

	Absolute tumor volume: (a*b*b)/2 [mm3]		Absolute body weight [g]
	Study day (after randomization)	Survival time	Study day (after randomization)
Animal-No	0	[days]	0
1354 r	449.6	>0	26.5
1355 l	188.4	>0	27.2
1356 r	226.8	>0	27.3
1357 r	231.2	>0	29.3
1359 r	196.6	>0	28.1
1367 r	287.6	>0	25.5
n	6		6
Median	229.0		27.3
MIN	188.4		25.5
MAX	449.6		29.3
95% MIN	188.4		26.5
95% MAX	287.6		28.1

	Relative tumor volume [%]		Relative body weight [%]
Animal-No	0		0
1354 r	100.0	>0	100.0
1355 l	100.0	>0	100.0
1356 r	100.0	>0	100.0
1357 r	100.0	>0	100.0
1359 r	100.0	>0	100.0
1367 r	100.0	>0	100.0
n	6		6
Median	100.0		100.0
MIN	100.0		100.0
MAX	100.0		100.0
95% MIN	100.0		100.0
95% MAX	100.0		100.0

Oncotest Exp-Nr.	H331-6
Tumortyp Nr./Pass	LXFA 983/9N4
Tumor Model	Lung, adeno

Implant / Rando / Induction time	18 days
End result / last study day	21
Therapy	G-026887; 100 mg/kg/day po; Day 0

	Absolute tumor volume: (a*b*b)/2 [mm3] Study day (after randomization)	Survival time	Absolute body weight [g] Study day (after randomization)
Animal-No	0	[days]	0
1334 r	505.4	>0	27.2
1337 r	249.4	>0	27.9
1343 r	326.4	>0	25.6
n	3		3
Median	326.4		27.2
MIN	249.4		25.6
MAX	505.4		27.9
95% MIN	249.4		25.6
95% MAX	505.4		27.9

	Relative tumor volume [%]		Relative body weight [%]
Animal-No	0		0
1334 r	100.0	>0	100.0
1337 r	100.0	>0	100.0
1343 r	100.0	>0	100.0
n	3		3
Median	100.0		100.0
MIN	100.0		100.0
MAX	100.0		100.0
95% MIN	100.0		100.0
95% MAX	100.0		100.0

Oncotest Exp-Nr.	H327-1	Implant / Rando / Induction time	18 days
Tumortyp Nr./Pass	LXFA 1041/9N4	End result / last study day	20
Tumor Model	Lung, adeno	Therapy	Vehicle control; 10 ml/kg; Days 0-20

	Absolute tumor volume: (a*b*b)/2 [mm3]								Absolute body weight [g]						
	Study day (after randomization)							Survival time	Study day (after randomization)						
Animal-No	0	3	7	10	14	17	20	[days]	0	3	7	10	14	17	20
1237 r	90.9	100.9	207.8	327.2	690.0	754.7	870.8	>20	26.9	27.6	28.9	28.6	29.0	28.9	29.1
1237 l	47.6	90.8	144.9	233.4	369.8	593.0	634.6								
1238 r	84.7	109.3	156.8	263.8	363.1	480.3	546.0	>20	27.1	27.6	29.0	29.5	29.7	29.1	29.4
1238 l	133.2	170.0	290.2	352.0	887.3	1009.8	1173.6								
1242 r	87.7	159.0	334.1	425.3	705.7	876.1	1032.2	>20	27.0	26.9	27.6	27.3	27.5	27.4	27.5
1242 l	47.6	74.4	137.3	213.2	458.8	637.6	700.1								
1243 r	65.0	86.2	141.5	150.0	329.5	481.6	523.5	>20	27.7	28.6	29.1	30.4	31.5	31.9	31.5
1243 l	60.0	87.7	166.1	210.5	367.8	535.0	540.0								
1245 r	115.2	210.9	288.0	356.4	464.8	596.8	733.1	>20	30.2	30.8	31.8	32.5	33.3	31.9	27.2
1245 l	90.8	109.3	172.8	212.5	331.6	504.2	525.0								
1247 r	113.4	147.9	225.0	341.0	517.6	717.9	777.3	>20	24.8	26.1	26.5	26.4	26.9	26.6	27.0
1247 l	62.5	80.1	139.3	173.8	316.9	347.9	422.3								
1248 r	111.6	117.0	202.2	268.8	396.9	455.5	572.2	>20	26.5	27.7	28.7	28.5	29.5	29.3	29.9
1248 l	90.8	90.8	186.2	193.6	293.5	351.0	408.7								
1250 r	62.5	68.8	95.3	152.1	394.3	482.2	549.8	>21	25.9	25.4	25.6	25.2	25.4	24.9	25.0
1250 l	113.4	141.5	231.0	295.9	615.3	1137.8	1195.3								
1253 r	80.2	109.8	207.8	213.6	336.0	387.2	405.0	>21	27.0	27.4	27.7	27.6	28.0	27.8	27.2
1253 l	94.2	147.9	258.6	281.6	397.8	505.0	560.0								
1279 r	191.1	261.6	378.9	653.1	950.6	1190.9	1576.9	>21	28.1	28.0	28.6	28.3	28.9	28.8	28.7
1279 l	68.8	83.1	111.6	152.1	361.3	395.7	416.3								
n	20	20	20	20	20	20	20		10	10	10	10	10	10	10
Median	89.2	109.3	194.2	248.6	395.6	520.0	566.1		27.0	27.6	28.7	28.4	29.0	28.9	28.1
MIN	47.6	68.8	95.3	150.0	293.5	347.9	405.0		24.8	25.4	25.6	25.2	25.4	24.9	25.0
MAX	191.1	261.6	378.9	653.1	950.6	1190.9	1576.9		30.2	30.8	31.8	32.5	33.3	31.9	31.5
95% MIN	80.2	100.9	172.8	233.4	394.3	504.2	572.2		26.5	26.9	27.6	27.3	27.5	27.4	27.0
95% MAX	94.2	141.5	231.0	327.2	517.6	717.9	777.3		28.1	28.6	29.1	29.5	29.7	29.3	29.4

	Relative tumor volume [%]								Relative body weight [%]						
Animal-No	0	3	7	10	14	17	20		0	3	7	10	14	17	20
1237 r	100.0	111.0	228.5	359.8	758.7	829.8	957.5	>20	100.0	102.6	107.4	106.3	107.8	107.4	108.2
1237 l	100.0	190.7	304.4	490.5	777.1	1246.0	1333.6								
1238 r	100.0	129.1	185.1	311.5	428.7	567.1	644.6	>20	100.0	101.8	107.0	108.9	109.6	107.4	108.5
1238 l	100.0	127.6	217.9	264.3	666.1	758.1	881.1								
1242 r	100.0	181.2	380.9	484.8	804.4	998.7	1176.6	>20	100.0	99.6	102.2	101.1	101.9	101.5	101.9
1242 l	100.0	156.3	288.5	447.9	964.1	1339.8	1471.2								
1243 r	100.0	132.6	217.8	230.8	506.9	740.9	805.3	>20	100.0	103.2	105.1	109.7	113.7	115.2	113.7
1243 l	100.0	146.1	276.7	350.7	612.8	891.3	899.6								
1245 r	100.0	183.1	250.0	309.4	403.5	518.0	636.4	>20	100.0	102.0	105.3	107.6	110.3	105.6	90.1
1245 l	100.0	120.5	190.4	234.2	365.4	555.6	578.5								
1247 r	100.0	130.4	198.4	300.7	456.4	633.0	685.4	>20	100.0	105.2	106.9	106.5	108.5	107.3	108.9
1247 l	100.0	128.1	222.8	278.0	507.0	556.6	675.7								
1248 r	100.0	104.8	181.2	240.9	355.6	408.1	512.7	>20	100.0	104.5	108.3	107.5	111.3	110.6	112.8
1248 l	100.0	100.0	205.2	213.3	323.4	386.8	450.4								
1250 r	100.0	110.0	152.5	243.4	630.8	771.6	879.7	>21	100.0	98.1	98.8	97.3	98.1	96.1	96.5
1250 l	100.0	124.8	203.7	260.9	542.6	1003.4	1054.1								
1253 r	100.0	136.9	259.2	266.3	419.0	482.9	505.1	>21	100.0	101.5	102.6	102.2	103.7	103.0	100.7
1253 l	100.0	156.9	274.4	298.9	422.2	536.0	594.3								
1279 r	100.0	136.9	198.3	341.7	497.4	623.2	825.2	>21	100.0	99.6	101.8	100.7	102.8	102.5	102.1
1279 l	100.0	120.9	162.3	221.2	525.5	575.5	605.5								
n	20	20	20	20	20	20	20		10	10	10	10	10	10	10
Median	100.0	129.7	217.8	288.5	507.0	628.1	745.4		100.0	101.9	105.2	106.4	108.1	106.4	105.2
MIN	100.0	100.0	152.5	213.3	323.4	386.8	450.4		100.0	98.1	98.8	97.3	98.1	96.1	90.1
MAX	100.0	190.7	380.9	490.5	964.1	1339.8	1471.2		100.0	105.2	108.3	109.7	113.7	115.2	113.7
95% MIN	100.0	124.8	205.2	278.0	497.4	623.2	685.4		100.0	101.5	102.6	102.2	103.7	102.5	100.7
95% MAX	100.0	146.1	250.0	341.7	612.8	829.8	899.6		100.0	103.2	105.3	107.6	110.3	107.4	108.9

Oncotest Exp-Nr.	H327-2	Implant / Rando / Induction time	18 days
Tumortyp Nr./Pass	LXFA 1041/9N4	End result / last study day	20
Tumor Model	Lung, adeno	Therapy	G-026887; 100 mg/kg/day po; Days 0-20

	Absolute tumor volume: (a*b*b)/2 [mm3] Study day (after randomization)							Survival time	Absolute body weight [g] Study day (after randomization)						
Animal-No	0	3	7	10	14	17	20	[days]	0	3	7	10	14	17	20
1258 r	47.6	92.3	215.1	279.3	458.8	653.0	854.4	>20	25.4	25.6	25.9	25.5	25.1	24.8	25.4
1258 l	100.9	210.8	332.8	491.9	819.3	1083.4	1120.0								
1261 r	90.8	140.4	272.8	461.7	538.6	789.0	959.2	>20	28.6	29.4	30.9	30.4	30.7	30.3	31.4
1261 l	111.6	209.5	291.2	420.1	575.5	859.6	973.4								
1262 r	68.8	139.3	258.8	284.6	510.9	853.9	925.7	>20	24.5	24.6	25.2	24.9	24.8	25.1	25.8
1262 l	57.6	90.8	147.9	237.2	401.6	629.7	665.5								
1267 r	62.5	68.8	212.5	326.4	672.9	1197.5	1422.1	>20	24.5	23.8	24.3	24.3	24.6	25.0	26.3
1267 l	90.8	145.4	294.4	510.3	1044.8	1567.4	1661.1								
1269 r	84.7	117.0	197.0	274.4	426.3	790.0	906.6	>20	24.0	23.6	24.4	23.8	24.4	24.7	26.1
1269 l	50.6	98.3	126.0	173.2	312.1	471.5	639.9								
1270 r	210.8	292.8	470.6	701.8	1321.4	1975.9	2047.5	>20	25.1	25.1	25.4	25.5	26.1	26.2	28.3
1270 l	117.2	201.6	344.4	451.3	613.8	974.7	1008.0								
1273 r	139.4	249.4	392.9	546.2	1022.5	1193.5	1366.9	>20	29.8	29.3	29.8	30.0	31.2	31.9	33.2
1273 l	86.2	159.5	180.3	228.1	451.2	643.5	853.1								
1275 r	62.5	98.3	164.8	264.7	635.1	949.9	1064.6	>21	31.5	30.4	30.5	30.6	30.4	31.1	32.4
1275 l	75.7	120.6	169.9	258.8	402.2	520.7	605.0								
1280 r	90.8	109.3	178.9	278.4	320.3	530.5	606.4	>21	25.0	25.4	26.3	26.1	26.8	26.4	27.4
1280 l	81.1	120.9	170.6	258.8	432.7	642.0	755.2								
1281 r	117.0	199.6	240.9	465.8	617.9	687.3	931.6	>21	23.6	22.9	22.6	22.0	22.7	22.7	23.1
1281 l	123.0	225.0	285.8	405.0	544.0	661.5	790.1								
n	20	20	20	20	20	20	20		10	10	10	10	10	10	10
Median	88.5	139.8	228.0	305.5	541.3	789.5	928.6		25.1	25.3	25.7	25.5	25.6	25.7	26.9
MIN	47.6	68.8	126.0	173.2	312.1	471.5	605.0		23.6	22.9	22.6	22.0	22.7	22.7	23.1
MAX	210.8	292.8	470.6	701.8	1321.4	1975.9	2047.5		31.5	30.4	30.9	30.6	31.2	31.9	33.2
95% MIN	81.1	139.3	212.5	326.4	510.9	789.0	853.1		24.5	24.6	25.2	24.3	24.6	24.7	25.8
95% MAX	100.9	159.5	285.8	420.1	672.9	974.7	1120.0		25.4	25.6	26.3	26.1	26.8	26.4	28.3

	Relative tumor volume [%]								Relative body weight [%]						
Animal-No	0	3	7	10	14	17	20		0	3	7	10	14	17	20
1258 r	100.0	193.9	452.1	586.9	964.1	1372.2	1795.5	>20	100.0	100.8	102.0	100.4	98.8	97.6	100.0
1258 l	100.0	208.9	329.8	487.4	811.9	1073.6	1109.8								
1261 r	100.0	154.7	300.6	508.8	593.5	869.4	1056.9	>20	100.0	102.8	108.0	106.3	107.3	105.9	109.8
1261 l	100.0	187.7	260.9	376.4	515.7	770.2	872.2								
1262 r	100.0	202.6	376.4	414.0	743.1	1242.1	1346.4	>20	100.0	100.4	102.9	101.6	101.2	102.4	105.3
1262 l	100.0	157.6	256.7	411.7	697.3	1093.2	1155.4								
1267 r	100.0	110.0	340.1	522.2	1076.6	1915.9	2275.4	>20	100.0	97.1	99.2	99.2	100.4	102.0	107.3
1267 l	100.0	160.2	324.4	562.3	1151.3	1727.1	1830.4								
1269 r	100.0	138.1	232.6	323.9	503.3	932.7	1070.4	>20	100.0	98.3	101.7	99.2	101.7	102.9	108.8
1269 l	100.0	194.2	248.9	342.2	616.5	931.4	1264.0								
1270 r	100.0	138.9	223.2	332.9	626.8	937.2	971.2	>20	100.0	100.0	101.2	101.6	104.0	104.4	112.7
1270 l	100.0	172.0	293.8	385.0	523.7	831.6	860.0								
1273 r	100.0	178.9	281.8	391.8	733.4	856.0	980.4	>20	100.0	98.3	100.0	100.7	104.7	107.0	111.4
1273 l	100.0	185.0	209.2	264.6	523.3	746.4	989.5								
1275 r	100.0	157.3	263.6	423.4	1016.2	1519.8	1703.4	>21	100.0	96.5	96.8	97.1	96.5	98.7	102.9
1275 l	100.0	159.3	224.4	341.8	531.2	687.7	799.1								
1280 r	100.0	120.5	197.1	306.8	353.0	584.5	668.2	>21	100.0	101.6	105.2	104.4	107.2	105.6	109.6
1280 l	100.0	149.1	210.3	319.0	533.4	791.4	931.0								
1281 r	100.0	170.6	205.9	398.1	528.1	587.5	796.3	>21	100.0	97.0	95.8	93.2	96.2	96.2	97.9
1281 l	100.0	182.9	232.3	329.2	442.2	537.8	642.3								
n	20	20	20	20	20	20	20		10	10	10	10	10	10	10
Median	100.0	165.4	258.8	388.4	605.0	900.4	1023.2		100.0	99.2	101.4	100.5	101.4	102.7	108.0
MIN	100.0	110.0	197.1	264.6	353.0	537.8	642.3		100.0	96.5	95.8	93.2	96.2	96.2	97.9
MAX	100.0	208.9	452.1	586.9	1151.3	1915.9	2275.4		100.0	102.8	108.0	106.3	107.3	107.0	112.7
95% MIN	100.0	154.7	248.9	376.4	593.5	831.6	971.2		100.0	98.3	99.2	99.2	100.4	102.0	105.3
95% MAX	100.0	172.0	300.6	423.4	743.1	1093.2	1346.4		100.0	100.8	102.9	101.6	104.0	104.4	109.8

Oncotest Exp-Nr.	H327-3
Tumortyp Nr./Pass	LXFA 1041/9N4
Tumor Model	Lung, adeno

Implant / Rando / Induction time	18 days
End result / last study day	20
Therapy	None

	Absolute tumor volume: (a*b*b)/2 [mm3] Study day (after randomization)	Survival time	Absolute body weight [g] Study day (after randomization)
Animal-No	0	[days]	0
1251 r	77.2	>0	26.2
1254 r	95.8	>0	20.8
1264 l	93.8	>0	23.0
n	3		3
Median	93.8		23.0
MIN	77.2		20.8
MAX	95.8		26.2
95% MIN	77.2		20.8
95% MAX	95.8		26.2

	Relative tumor volume [%]		Relative body weight [%]
Animal-No	0		0
1251 r	100.0	>0	100.0
1254 r	100.0	>0	100.0
1264 l	100.0	>0	100.0
n	3		3
Median	100.0		100.0
MIN	100.0		100.0
MAX	100.0		100.0
95% MIN	100.0		100.0
95% MAX	100.0		100.0

Oncotest Exp-Nr.	H327-4
Tumortyp Nr./Pass	LXFA 1041/9N4
Tumor Model	Lung, adeno

Implant / Rando / Induction time	18 days
End result / last study day	20
Therapy	Vehicle control; 10 ml/kg; Day 0

	Absolute tumor volume: (a*b*b)/2 [mm3]		Absolute body weight [g]
	Study day (after randomization)	Survival time	Study day (after randomization)
Animal-No	0	[days]	0
1239 r	344.6	>0	27.9
1246 l	287.6	>0	24.0
1255 r	216.3	>0	24.7
1260 r	425.3	>0	27.2
1266 l	183.8	>0	27.5
1268 r	200.9	>0	26.0
n	6		6
Median	251.9		26.6
MIN	183.8		24.0
MAX	425.3		27.9
95% MIN	183.8		24.7
95% MAX	344.6		27.5

	Relative tumor volume [%]		Relative body weight [%]
Animal-No	0		0
1239 r	100.0	>0	100.0
1246 l	100.0	>0	100.0
1255 r	100.0	>0	100.0
1260 r	100.0	>0	100.0
1266 l	100.0	>0	100.0
1268 r	100.0	>0	100.0
n	6		6
Median	100.0		100.0
MIN	100.0		100.0
MAX	100.0		100.0
95% MIN	100.0		100.0
95% MAX	100.0		100.0

Oncotest Exp-Nr.	H327-5
Tumortyp Nr./Pass	LXFA 1041/9N4
Tumor Model	Lung, adeno

Implant / Rando / Induction time	18 days
End result / last study day	20
Therapy	G-026887; 100 mg/kg/day po; Day 0

	Absolute tumor volume: (a*b*b)/2 [mm3]		Absolute body weight [g]
	Study day (after randomization)	Survival time	Study day (after randomization)
Animal-No	0	[days]	0
1257 r	256.0	>0	26.3
1263 l	219.0	>0	23.5
1271 l	423.2	>0	24.9
1274 r	441.1	>0	31.1
1276 r	166.5	>0	20.8
1277 r	240.1	>0	27.4
n	6		6
Median	248.1		25.6
MIN	166.5		20.8
MAX	441.1		31.1
95% MIN	219.0		23.5
95% MAX	256.0		27.4

	Relative tumor volume [%]		Relative body weight [%]
Animal-No	0		0
1257 r	100.0	>0	100.0
1263 l	100.0	>0	100.0
1271 l	100.0	>0	100.0
1274 r	100.0	>0	100.0
1276 r	100.0	>0	100.0
1277 r	100.0	>0	100.0
n	6		6
Median	100.0		100.0
MIN	100.0		100.0
MAX	100.0		100.0
95% MIN	100.0		100.0
95% MAX	100.0		100.0

Oncotest Exp-Nr.	H327-6	Implant / Rando / Induction time	18 days
Tumortyp Nr./Pass	LXFA 1041/9N4	End result / last study day	20
Tumor Model	Lung, adeno	Therapy	G-026887; 100 mg/kg/day po; Day 0

	Absolute tumor volume: (a*b*b)/2 [mm3]		Absolute body weight [g]
	Study day (after randomization)	Survival time	Study day (after randomization)
Animal-No	0	[days]	0
1240 r	409.1	>0	27.9
1241 r	250.5	>0	27.2
1256 l	267.7	>0	25.2
n	3		3
Median	267.7		27.2
MIN	250.5		25.2
MAX	409.1		27.9
95% MIN	250.5		25.2
95% MAX	409.1		27.9

	Relative tumor volume [%]		Relative body weight [%]
Animal-No	0		0
1240 r	100.0	>0	100.0
1241 r	100.0	>0	100.0
1256 l	100.0	>0	100.0
n	3		3
Median	100.0		100.0
MIN	100.0		100.0
MAX	100.0		100.0
95% MIN	100.0		100.0
95% MAX	100.0		100.0

WHAT IS CLAIMED IS:

1. A method of identifying a patient nonresponsive to treatment with a B-Raf inhibitor, comprising determining the presence or absence of a K-ras mutation, whereby the presence of a K-ras mutation indicates a patient will not respond to said B-Raf inhibitor treatment.

2. A method of determining whether a tumor will respond to treatment with a B-Raf inhibitor, comprising determining in a sample of said tumor the presence of a mutant K-ras protein or gene whereby the presence of a mutant K-ras protein or gene indicates that the tumor will not respond to treatment with a B-Raf inhibitor.

3. The method of claim 1 wherein said K-ras mutation is an activating mutation.

4. The method of claim 1 wherein said K-ras mutation is at least one of G12C; G12A;G12D; G12R; G12S; G12V; G13C; and G13D.

5. The method of claim 1 wherein said B-Raf inhibitor is a specific B-Raf kinase inhibitor.

6. The method of claim 1 wherein the presence of a K-ras mutation is determined by amplifying K-ras nucleic acid from said tumor, or a fragment thereof suspected of containing a mutation, and sequencing said amplified nucleic acid.

7. The method of claim 1 wherein the presence of a K-ras mutation is determined by amplifying K-RAS nucleic acid from said tumor, or a fragment thereof suspected of containing a mutation, and comparing the electrophoretic mobility of the amplified nucleic acid to the electrophoretic mobility of corresponding wild-type K-ras nucleic acid or fragment.

8. A method of predicting whether a patient will be nonresponsive to treatment with a specific B-Raf inhibitor, comprising determining the presence or absence of a K-ras mutation in a tumor of the patient, wherein the K-ras mutation is in codon 12 or codon 13; and wherein if a K-ras mutation is present, the patient is predicted to be nonresponsive to treatment with a specific B-Raf inhibitor.

9. The method of claim 1, wherein the determining the presence or absence of a K-ras mutation in a tumor comprises amplifying a K-ras nucleic acid from the tumor and sequencing the amplified nucleic acid.

10. The method of claim 1, wherein the determining the presence or absence of a K-ras mutation in a tumor comprises detecting a mutant K-ras polypeptide in a sample of the tumor using a specific binding agent to a mutant K-ras polypeptide.

11. The method of claim 1, wherein the K-ras mutation is selected from G12S, G12V, G12D, G12A, G12C, G13A, and G13D.

12. A kit useable for the method of claim 1, comprising material specific for detecting a K-ras mutant gene or protein, and material specific for detecting a B-Raf mutant gene or protein.

13. The kit of claim 12, further comprising instructions for use in identifying a patient nonresponsive to treatment with a B-Raf inhibitor.

14. The kit of claim 13, wherein said patient has lung or colorectal cancer.

15. A method of classifying a breast, lung, colon, ovarian, thyroid, melanoma or pancreatic tumor comprising the steps of: obtaining a tumor sample; detecting expression or activity of a (i) a gene encoding the B-Raf V600E mutant and (ii) a gene encoding a k-Ras mutant in the sample.

16. The method of claim 15, further comprising classifying the tumor as belonging to a tumor subclass based on the results of the detecting step; and selecting a treatment based on the classifying step, wherein said treatment is other than a B-Raf V600E specific inhibitor if said k-RAS mutant is overexpressed in said tumor sample.

17. A method of identifying a tumor nonresponsive to treatment with a B-Raf inhibitor, comprising determining the expression level of a receptor tyrosine kinase (RTK), whereby aberrant expression or induction of said RTK indicates said patient will not respond to said B-Raf inhibitor treatment, and wherein said tumor expresses B-Raf V600E.

18. The method of claim 17, wherein said RTK is EGFR or cMET.

19. The method of claim 18, further comprising treating said tumor by administering an effective amount of an inhibitor of said EGFR or cMET in combination with a B-Raf inhibitor.

20. The method of claim 19, wherein said EGFR inhibitor is erlotinib.

21. The method of claim 20, wherein said combination is administered in a synergistic amount.

22. The method of claim 17, wherein said tumor type is colon or melanoma.

BIOCHEMICAL ENZYME ASSAY DATA

Compound	B-RafV600E $K_{m,ATP}$=65 uM		WT B-Raf $K_{m,ATP}$= 5 uM		WT c-Raf $K_{m,ATP}$= 3 uM	
	$K_{i(app)}$	Adj. IC50 @ 1mM ATP	$K_{i(app)}$	Adj. IC50 @ 1mM ATP	$K_{i(app)}$	Adj. IC50 @ 1mM ATP
GDC-0879[1]	0.2	3.0	0.2	37	0.54	179
PLX-4720[2]	4.6	75	2.8	562	3.3	1091
	<100 nM		>100 nM		>500 nM	

[1]*Cancer Res.69(7):3042-*, [2]*Proc Natl Acad Sci U S A. 105(8):3041-6*

Figure 1. Biochemical enzyme assay data. The enzymatic activity of full length human V600E B-Raf, WT B-Raf, and WT c-Raf was quantified as incorporation of radiolabel from [γ-^{33}P]ATP (4 µM /33 µCi/mL) into FSBA-wtMEK. IC_{50} values were calculated by fitting a standard 4-parameter logistic model to the curve plotted as % of control versus [compound]. K_i's calculated from the estimated IC_{50}'s using a transformation of the Cheng-Prusoff equation were used to predict IC_{50}'s at 1 mM ATP from the equation $IC_{50} = K_i (1 + S/K_m)$.

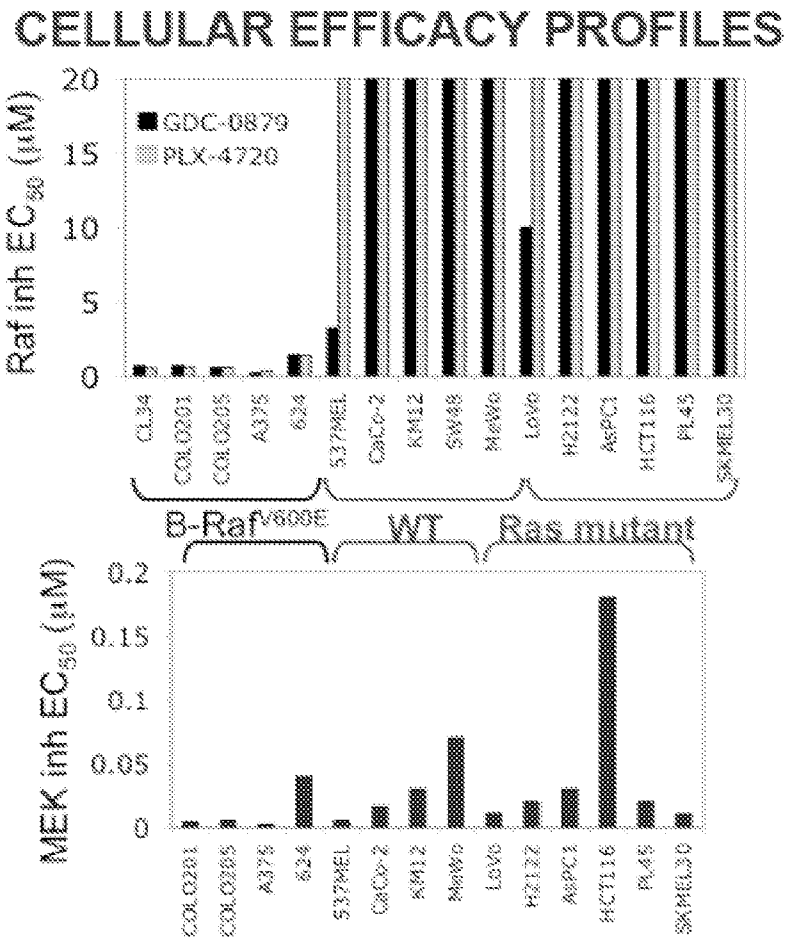


Figure 2. Viability assays in tumor lines of different Raf/Ras mutational status. Compound EC_{50} values measured using B-Raf^{V600E} and WT tumor lines in 4-day viability/proliferation assays using the CellTiter-Glo reagent (Promega)

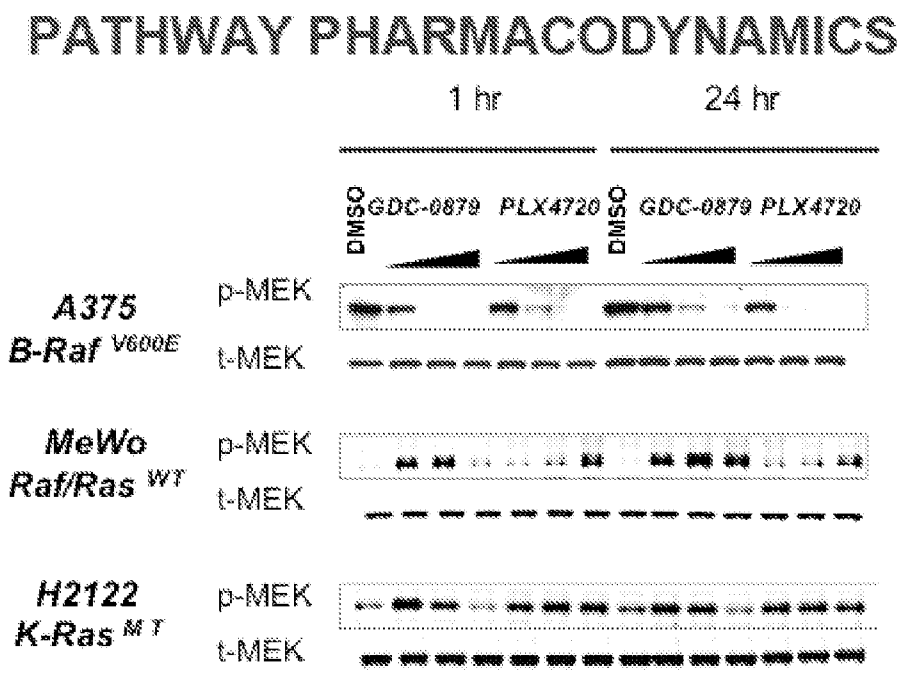


Figure 3. Western blot analysis of pMEK levels in B-Raf^{V600E}, Raf/Ras^{WT} and K-Ras^{MT} lines.
Cells were treated with increasing doses of GDC-0879 and PLX4720 (0.1, 1, 10 μM) for the indicated times.

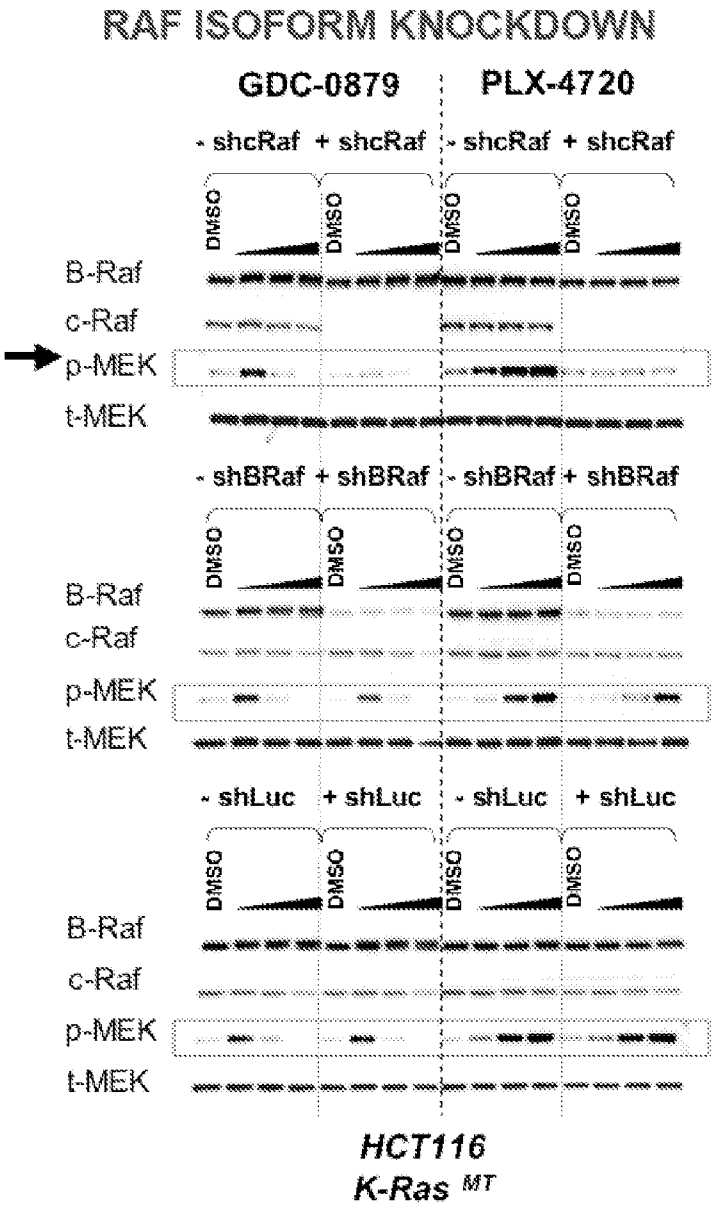


Figure 4. Western blot analysis of pMEK levels upon inducible c- and B-Raf knockdown in HCT116 Ras^{MT} cells. Stable clones were incubated with doxycycline for 24 hours to induce shRNA expression and were subsequently treated for 1 hr with increasing amounts of inhibitors (0.1, 1, 10 μM).

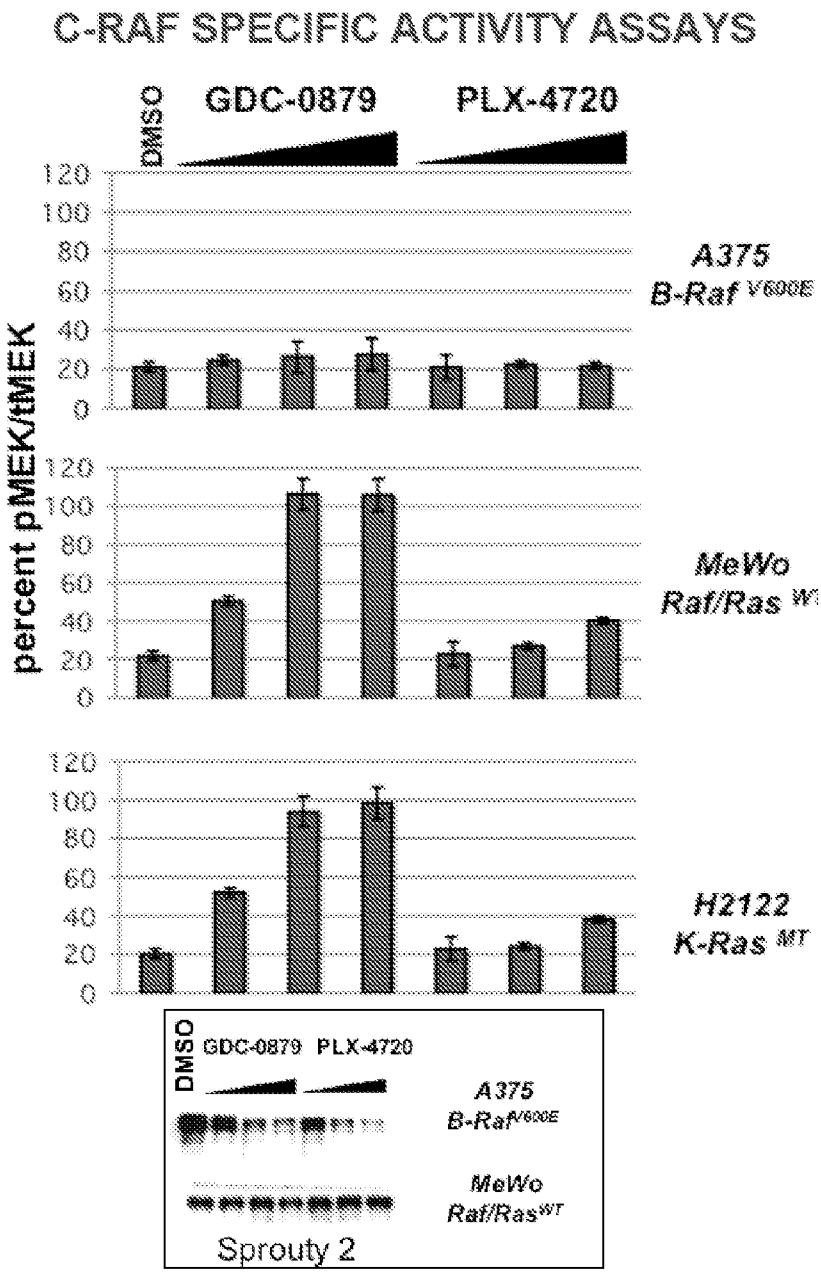
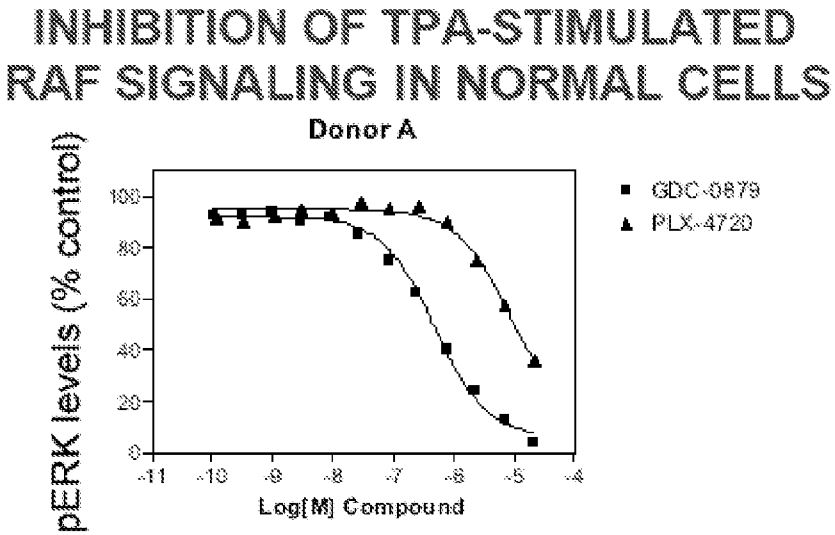


Figure 5. c-Raf specific activity assays after short-term treatment with inhibitors. Cells were treated for 1hr with inhibitors (0.1, 1, 10 μM), washed and lysed. c-Raf was immunoprecipitated and used in an in vitro kinase assay with MEK as substrate.



	Donor A IC50 (nM)	Donor B IC50 (nM)
GDC-0879	454	420
PLX-4720	9043	7672

Figure 6. Inhibition of TPA-stimulated pERK levels in peripheral blood mononuclear cells (PBMC). Human PBMCs were incubated with compounds for 1 hour, then stimulated for 10 minutes with 1 μg/mL TPA. Cells were fixed in formaldehyde, permeabilized in methanol, stained for phospho-ERK (pERK) and analyzed by flow cytometry.

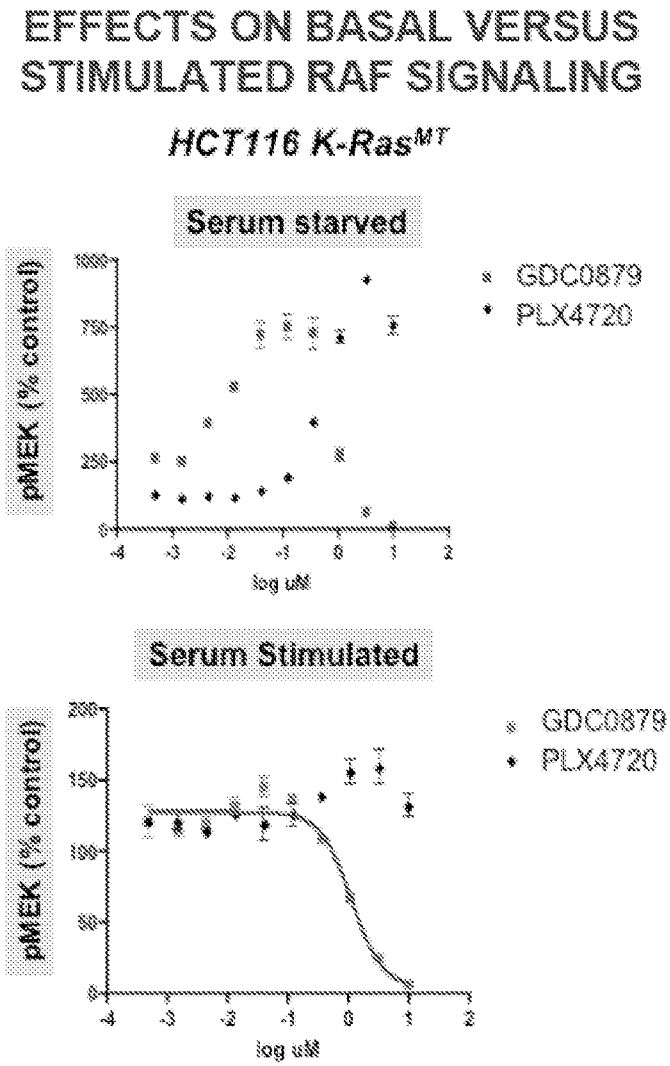


Figure 7. Effects on basal vs. serum stimulated pMEK levels in HCT116 cells. Cells were serum starved overnight and incubated with compounds for 1 hour, then stimulated for 10 minutes with 10% FCS in triplicate for bottom panel. Phospho- and total MEK levels were determined by MSD. pMEK levels were normalized to total MEK and plotted as percent of DMSO control.

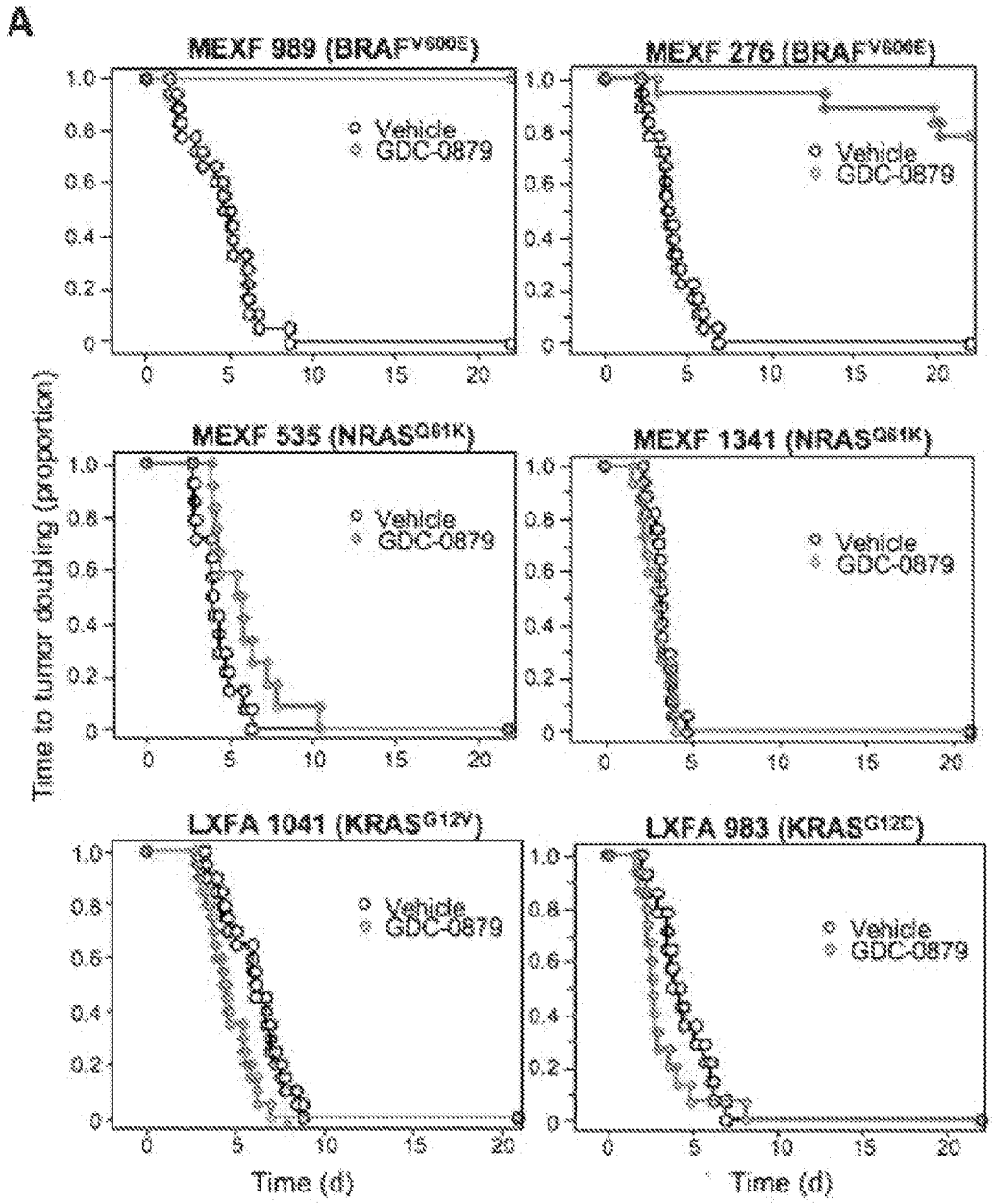

A
MEXF 989 (BRAF V600E)
MEXF 276 (BRAF V600E)
MEXF 535 (NRAS Q61K)
MEXF 1341 (NRAS Q61K)
LXFA 1041 (KRAS G12V)
LXFA 983 (KRAS G12C)
Vehicle
GDC-0879
Time to tumor doubling (proportion)
Time (d)

Fig. 8A

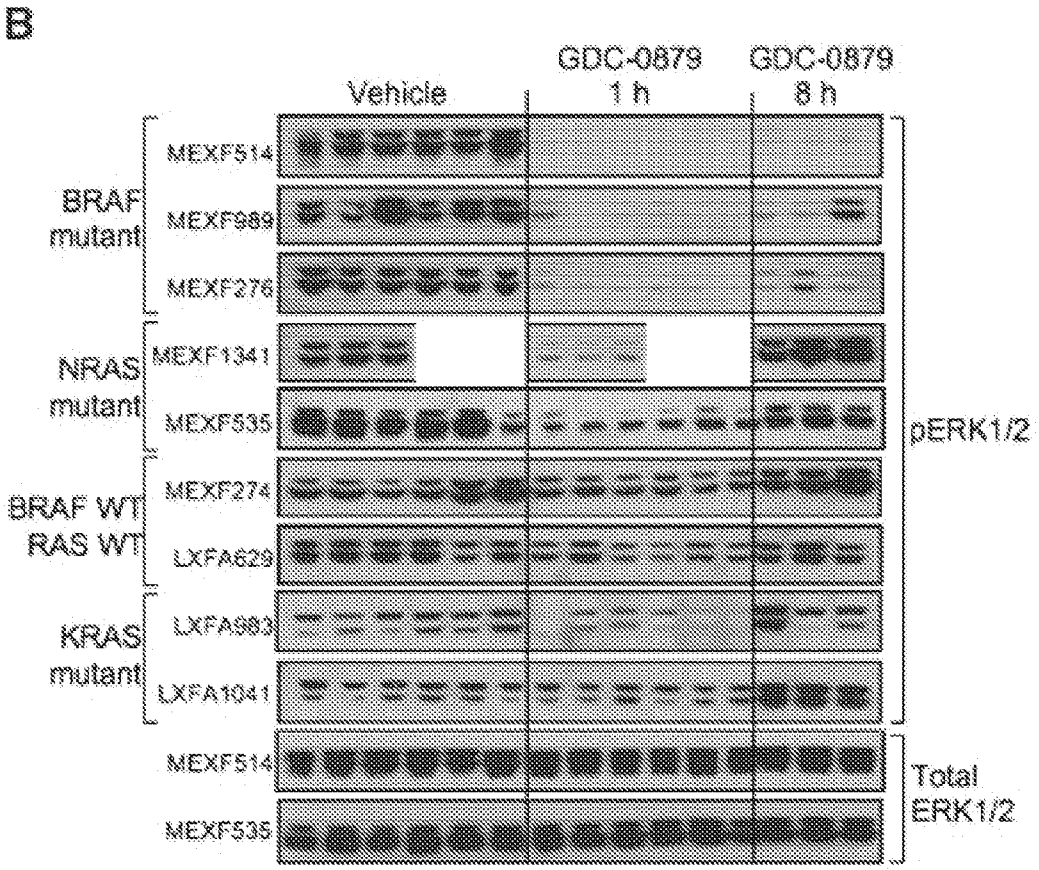

B
Vehicle
GDC-0879 1 h
GDC-0879 8 h
BRAF mutant
NRAS mutant
BRAF WT RAS WT
KRAS mutant
MEXF514
MEXF989
MEXF276
MEXF1341
MEXF535
MEXF274
LXFA629
LXFA983
LXFA1041
MEXF514
MEXF535
pERK1/2
Total ERK1/2

Fig. 8B

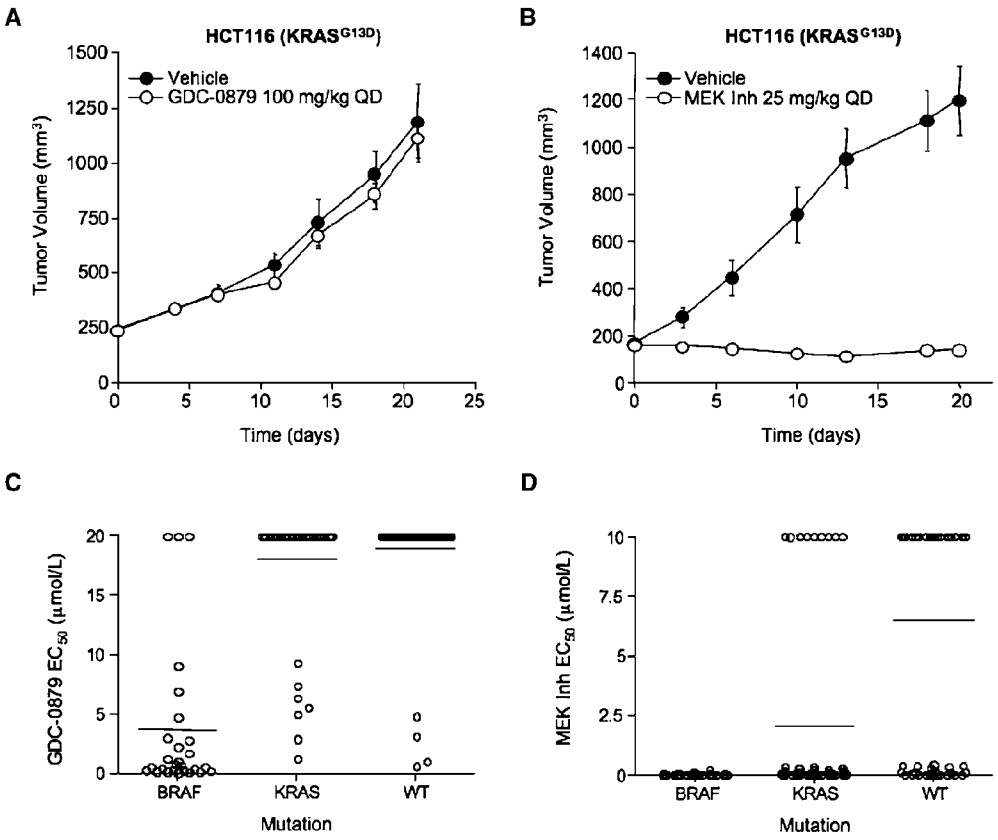

A
HCT116 (KRASG13D)
Vehicle
GDC-0879 100 mg/kg QD
Tumor Volume (mm3)
Time (days)
B
HCT116 (KRASG13D)
Vehicle
MEK Inh 25 mg/kg QD
Tumor Volume (mm3)
Time (days)
C
GDC-0879 EC50 (µmol/L)
BRAF
KRAS
WT
Mutation
D
MEK Inh EC50 (µmol/L)
BRAF
KRAS
WT
Mutation

Fig. 9A, B, C & D

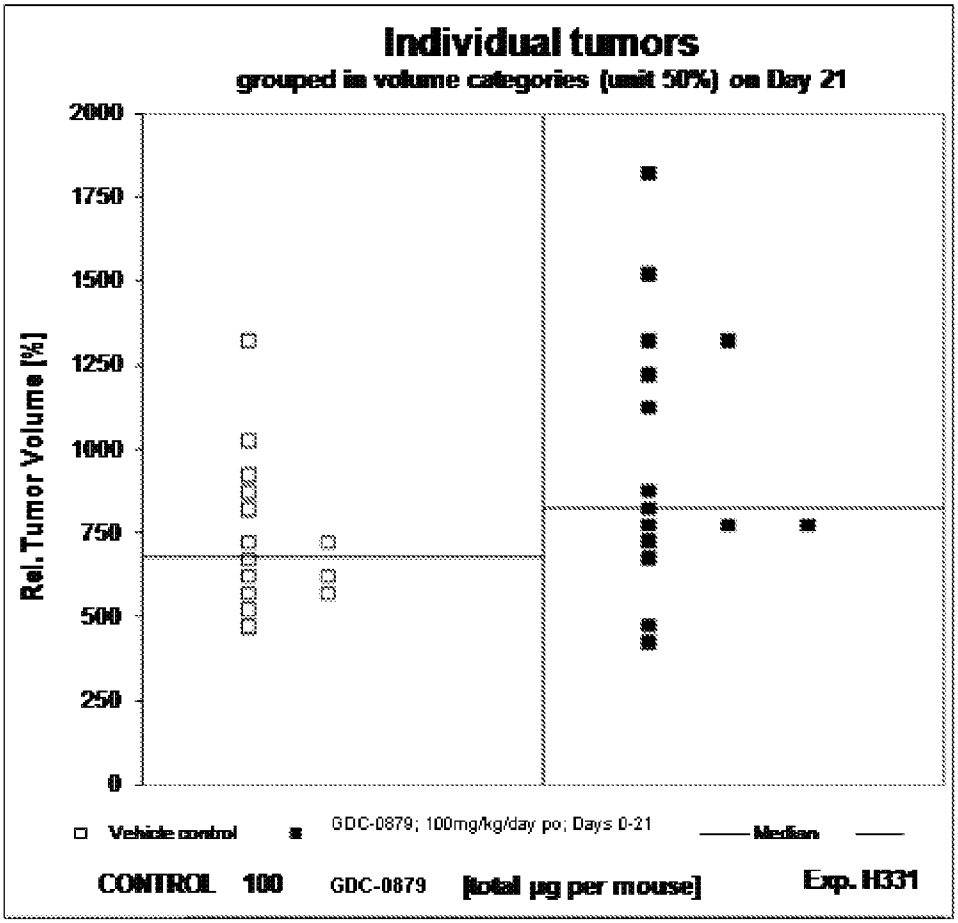

Individual tumors
grouped in volume categories (unit 50%) on Day 21
Rel. Tumor Volume [%]
2000
1750
1500
1250
1000
750
500
250
0
Vehicle control
GDC-0879; 100mg/kg/day po; Days 0-21
Median
CONTROL 100 GDC-0879 [total µg per mouse]
Exp. H331

Fig. 10

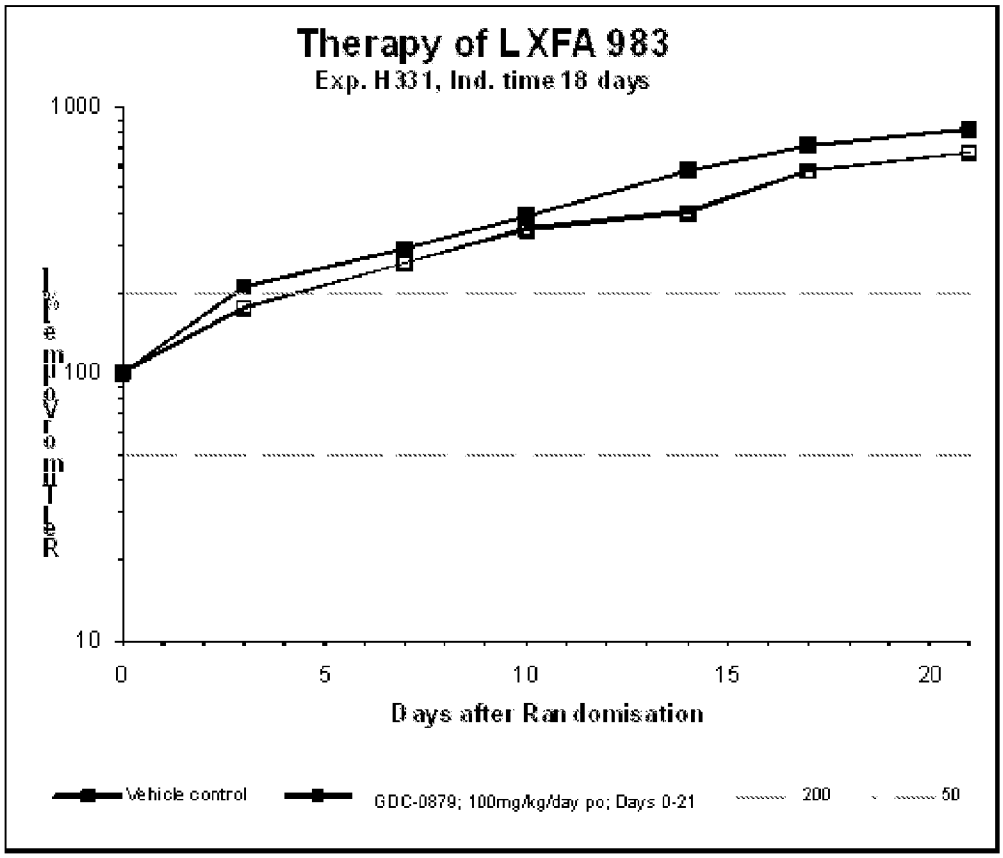

Therapy of LXFA 983
Exp. H331, Ind. time 18 days
1000
100
10
Tumor Volume
0
5
10
15
20
Days after Randomisation
Vehicle control
GDC-0879; 100mg/kg/day po; Days 0-21
200
50

Fig. 11

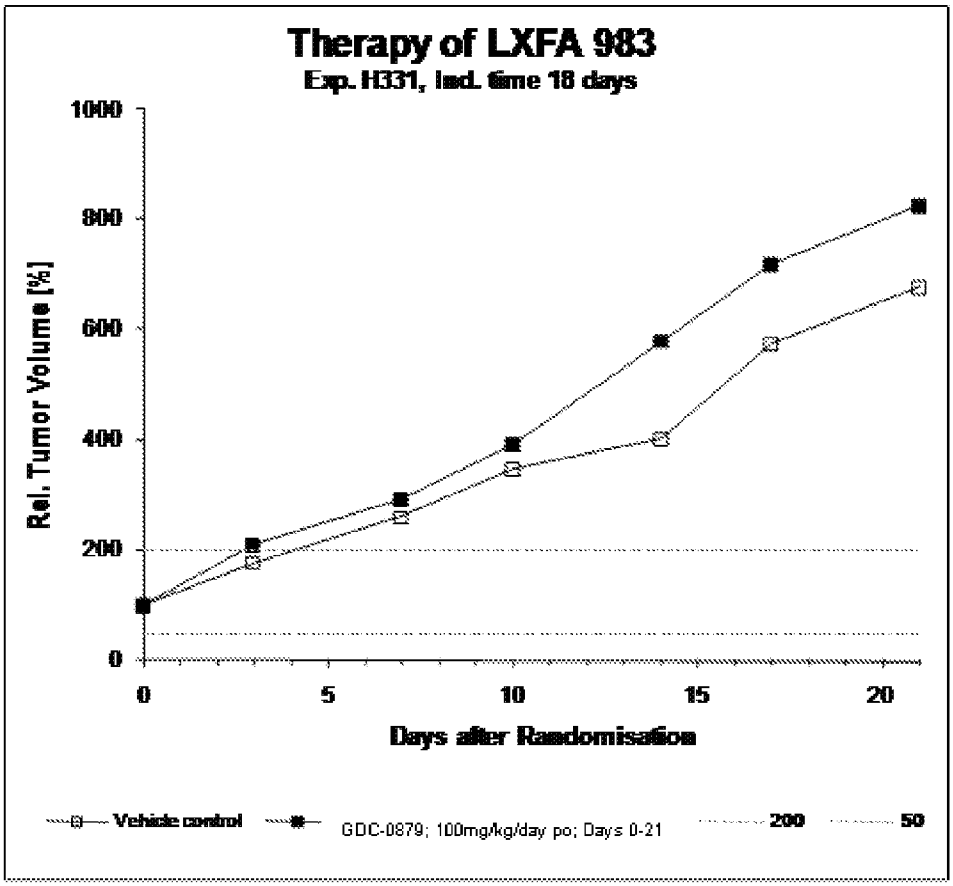

Therapy of LXFA 983
Exp. H331, Ind. time 18 days
Rel. Tumor Volume [%]
1000
800
600
400
200
0
0
5
10
15
20
Days after Randomisation
Vehicle control
GDC-0879; 100mg/kg/day po; Days 0-21
200
50

Fig. 12

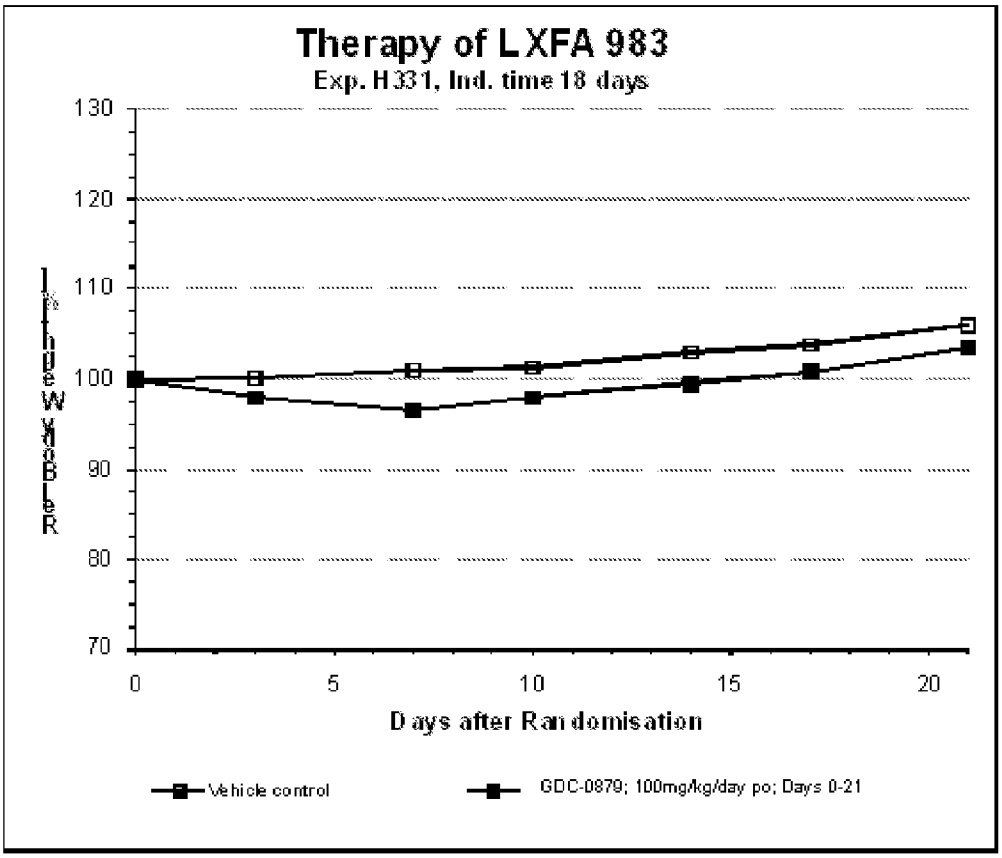

Therapy of LXFA 983
Exp. H331, Ind. time 18 days
130
120
110
100
90
80
70
0
5
10
15
20
Days after Randomisation
Vehicle control
GDC-0879; 100mg/kg/day po; Days 0-21

Fig. 13

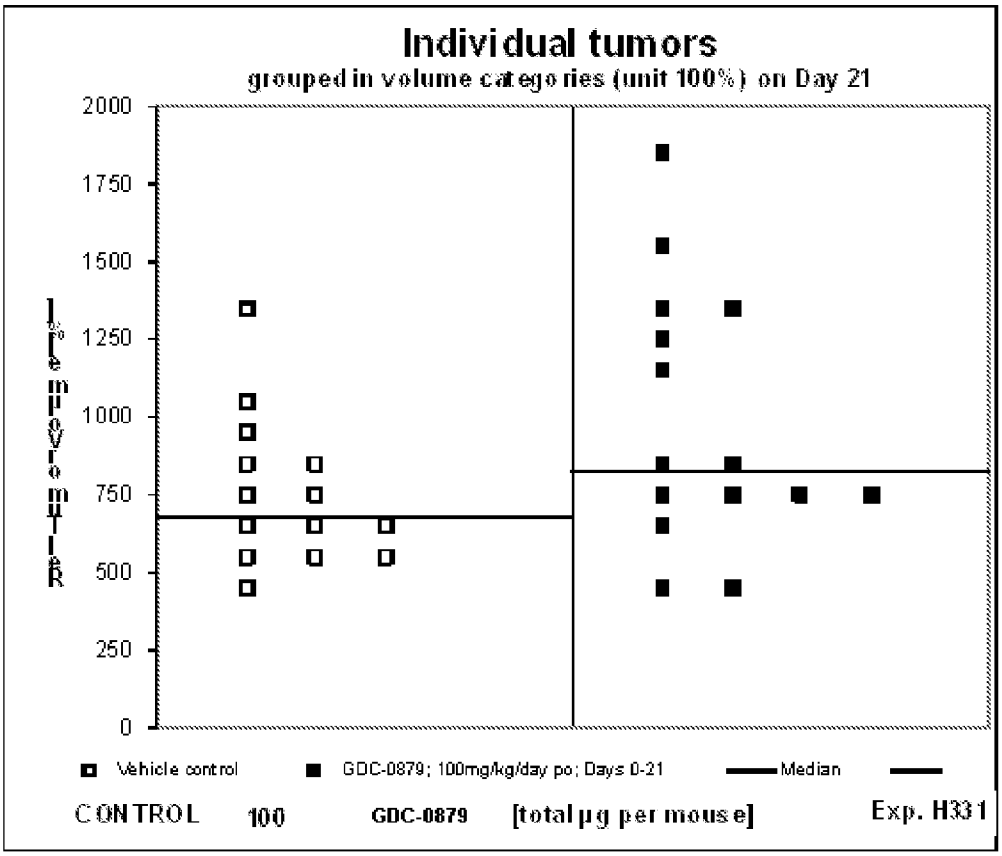

Individual tumors
grouped in volume categories (unit 100%) on Day 21
2000
1750
1500
1250
1000
750
500
250
0
Vehicle control
GDC-0879; 100mg/kg/day po; Days 0-21
Median
CONTROL
100
GDC-0879
[total µg per mouse]
Exp. H331

Fig. 14

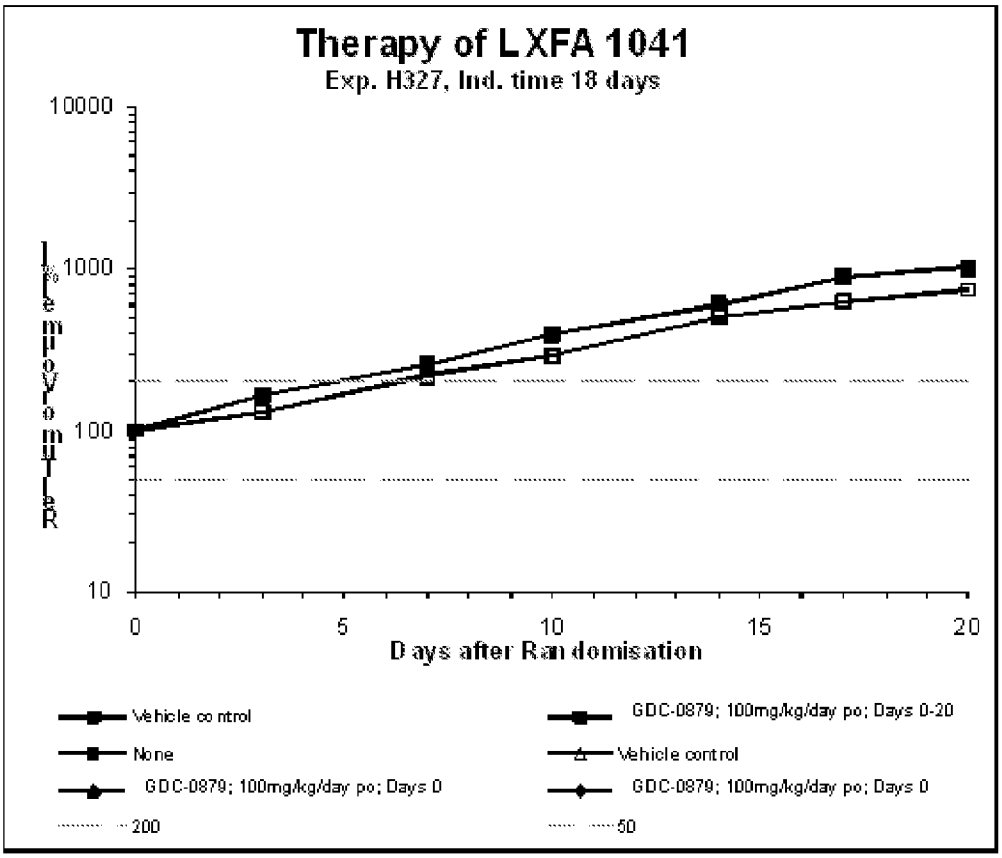

Therapy of LXFA 1041
Exp. H327, Ind. time 18 days
Tumor volume [%]
10000
1000
100
10
0
5
10
15
20
Days after Randomisation
Vehicle control
None
GDC-0879; 100mg/kg/day po; Days 0
200
GDC-0879; 100mg/kg/day po; Days 0-20
Vehicle control
GDC-0879; 100mg/kg/day po; Days 0
50

Fig. 15

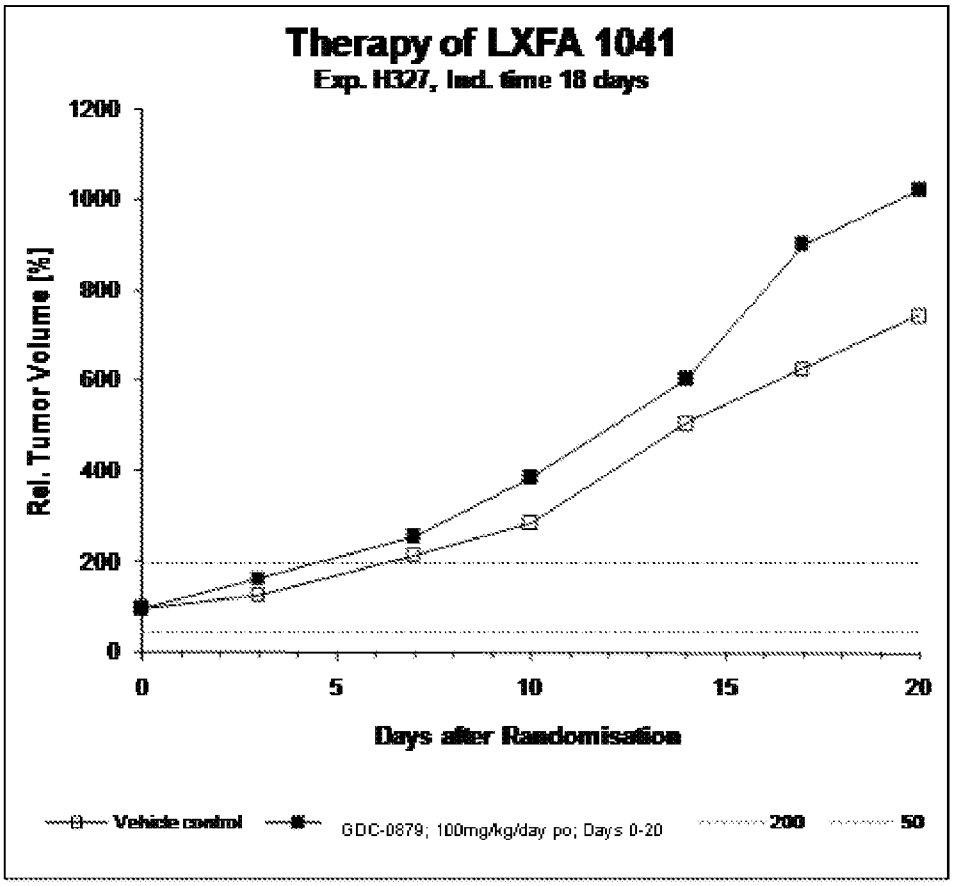

Therapy of LXFA 1041
Exp. H327, Incl. time 18 days
Rel. Tumor Volume [%]
1200
1000
800
600
400
200
0
0
5
10
15
20
Days after Randomisation
Vehicle control
GDC-0879; 100mg/kg/day po; Days 0-20
200
50

Fig. 16

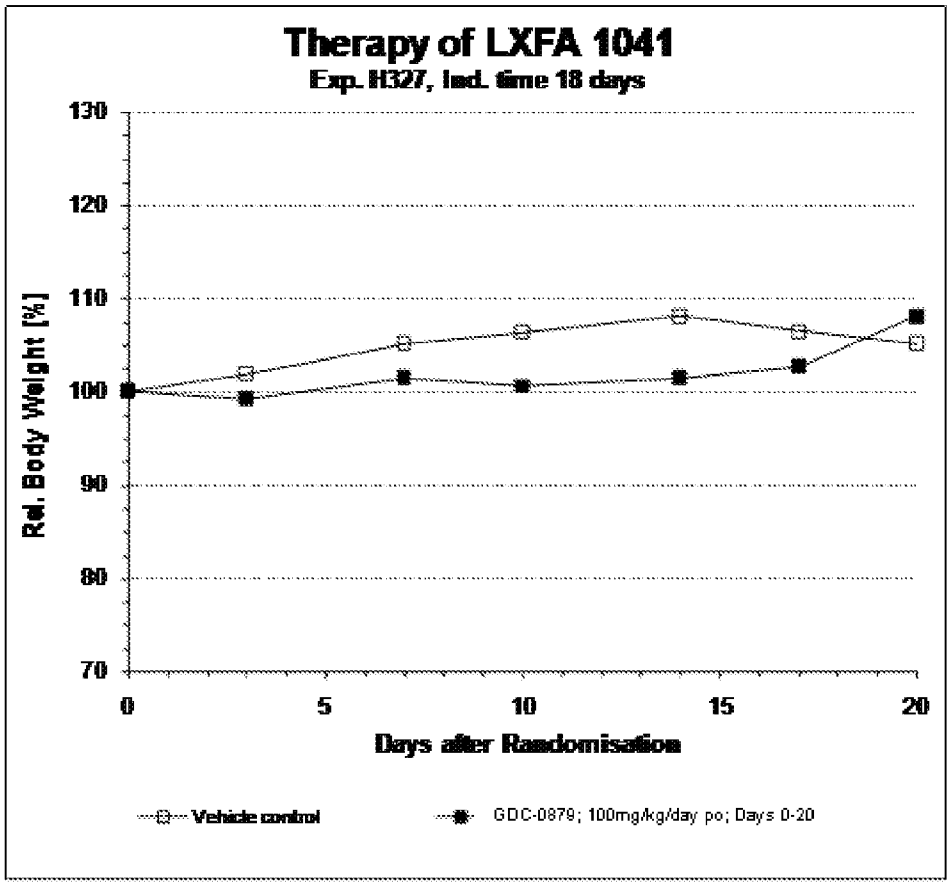

Therapy of LXFA 1041
Exp. H327, Ind. time 18 days
Rel. Body Weight [%]
130
120
110
100
90
80
70
0
5
10
15
20
Days after Randomisation
Vehicle control
GDC-0879; 100mg/kg/day po; Days 0-20

Fig. 17

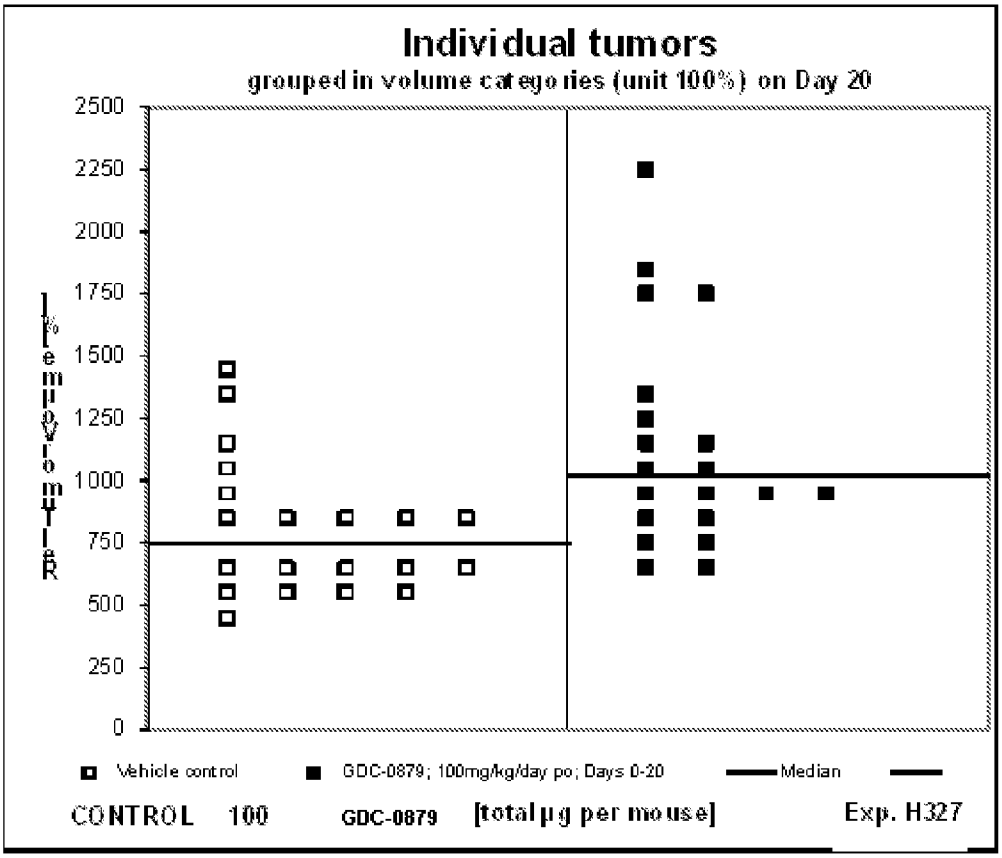

Individual tumors
grouped in volume categories (unit 100%) on Day 20
Tumor volume
2500
2250
2000
1750
1500
1250
1000
750
500
250
0
Vehicle control
GDC-0879; 100mg/kg/day po; Days 0-20
Median
CONTROL 100
GDC-0879
[total µg per mouse]
Exp. H327

Fig. 18

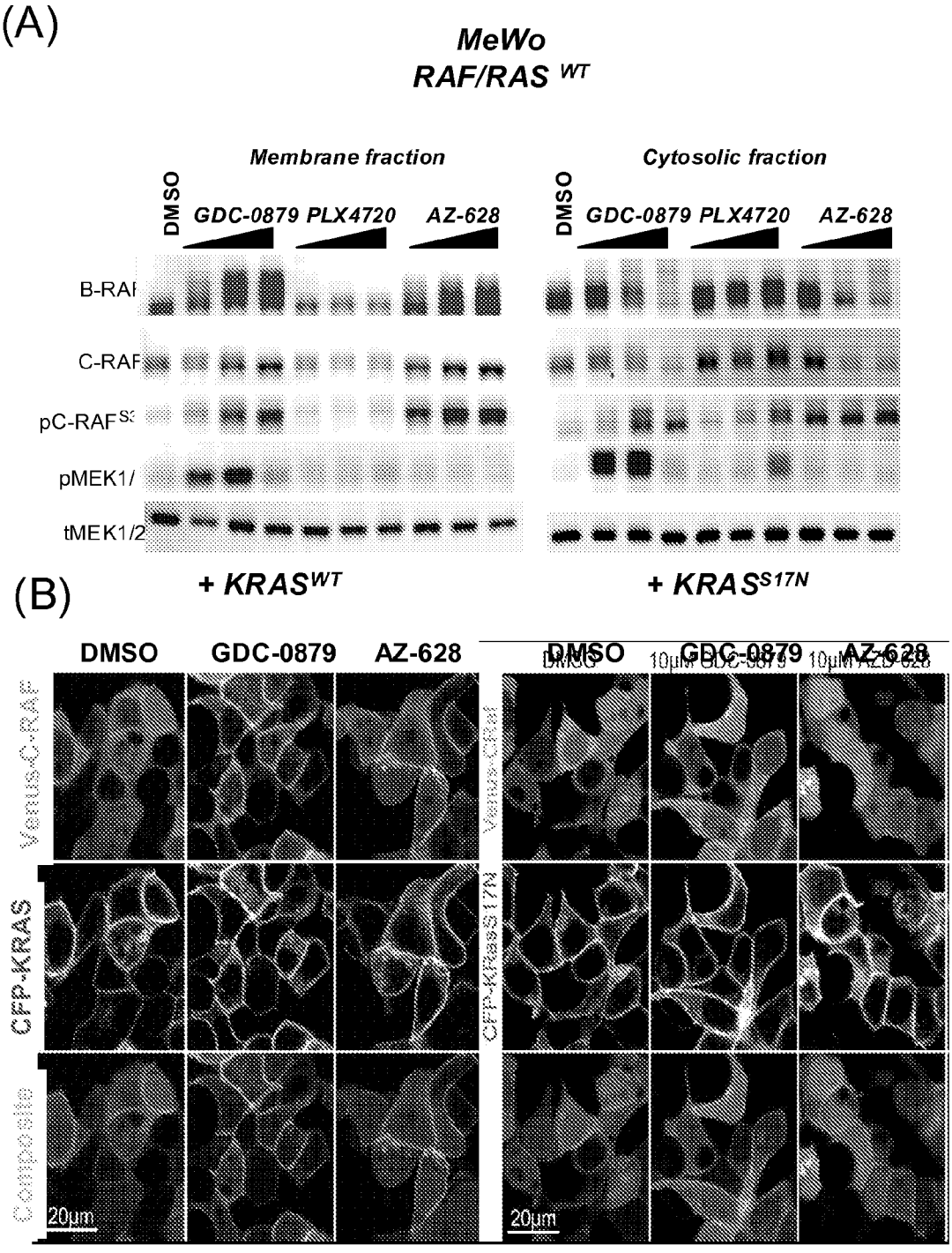
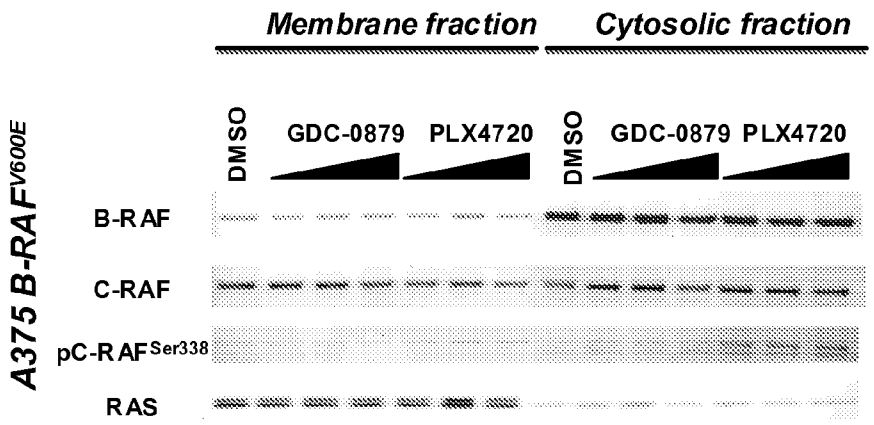
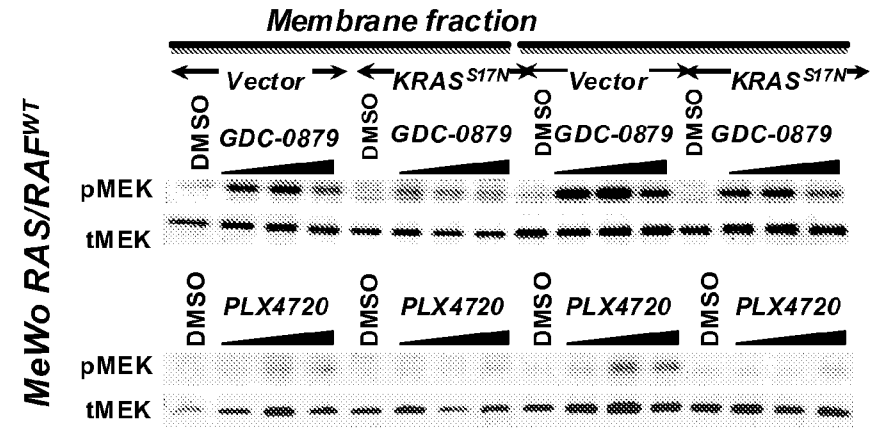

(A)
MeWo
RAF/RAS WT
Membrane fraction
Cytosolic fraction
DMSO
GDC-0879
PLX4720
AZ-628
B-RAF
C-RAF
pC-RAF
pMEK1/
tMEK1/2
+ KRASWT
+ KRASS17N
(B)
DMSO
GDC-0879
AZ-628
Venus-C-RAF
CFP-KRAS
Composite
20μm

Fig. 19A & B

(A)

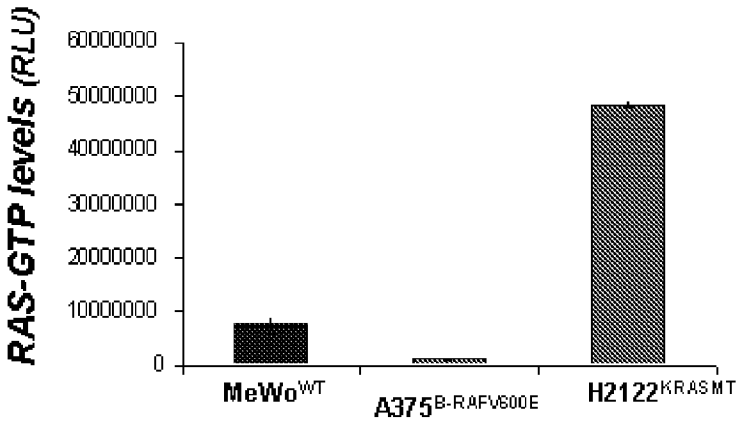

Membrane fraction
Cytosolic fraction
DMSO
GDC-0879
PLX4720
DMSO
GDC-0879
PLX4720
A375 B-RAF V600E
B-RAF
C-RAF
pC-RAF Ser338
RAS

(B)

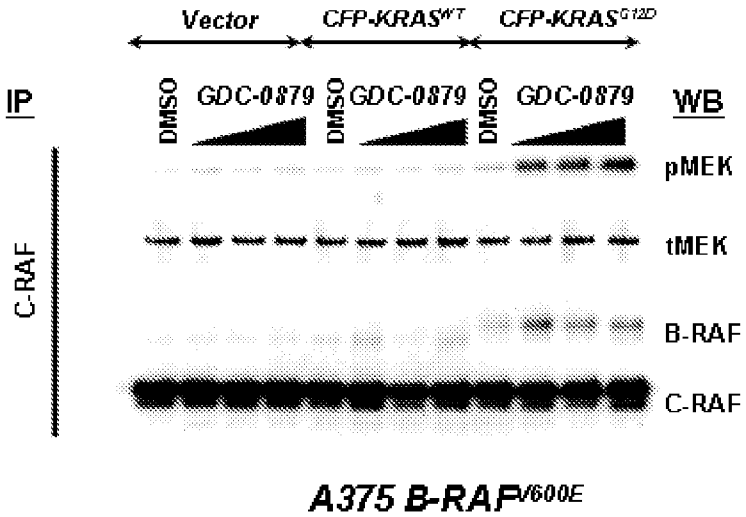

Membrane fraction
Vector
KRAS S17N
Vector
KRAS S17N
DMSO
GDC-0879
DMSO
GDC-0879
DMSO
GDC-0879
DMSO
GDC-0879
pMEK
tMEK
DMSO
PLX4720
DMSO
PLX4720
DMSO
PLX4720
DMSO
PLX4720
pMEK
tMEK
MeWo RAS/RAF WT

Figs. 20A & B

(C)


RAS-GTP levels (RLU)
60000000
50000000
40000000
30000000
20000000
10000000
0
MeWo WT
A375 B-RAFV600E
H2122 KRASMT

(D)

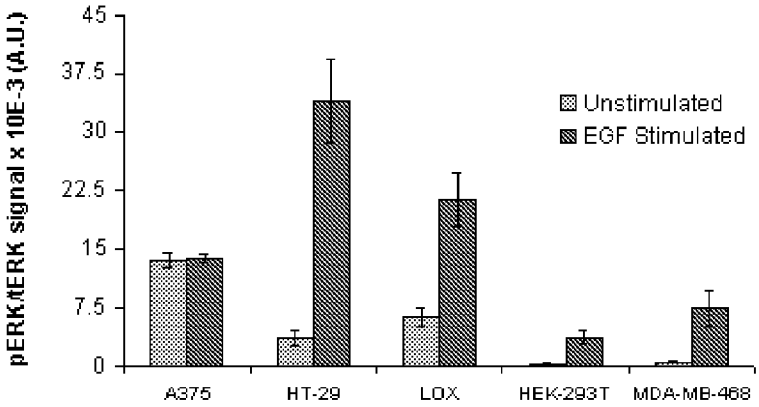

Vector
CFP-KRASWT
CFP-KRASG12D
IP
DMSO
GDC-0879
DMSO
GDC-0879
DMSO
GDC-0879
WB
C-RAF
pMEK
tMEK
B-RAF
C-RAF
A375 B-RAFV600E

Figs. 20C & D

(A)

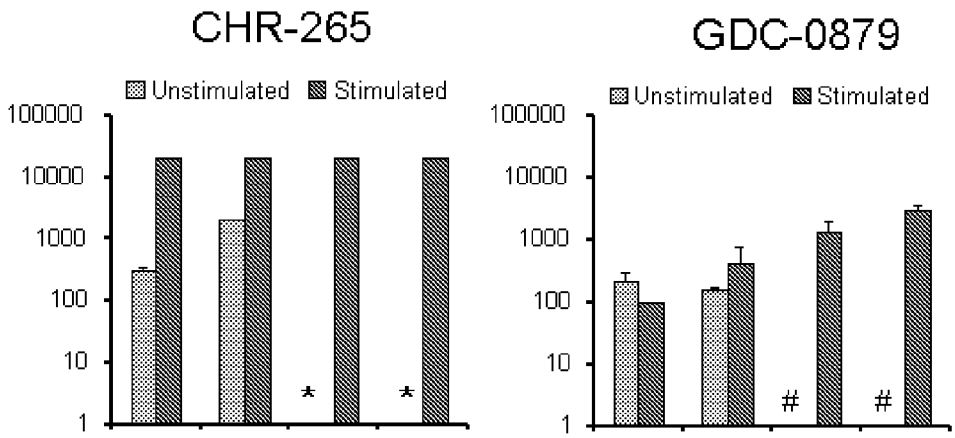
Cell Line	Tissue type	B-Raf status	Surface EGFR Staining
A375	Melanoma	V600E mutant	57
HT29	Colon	V600E mutant	81
LOX IMVI	Melanoma	V600E mutant	168
HEK-293T	Kidney	Wildtype	ND
MDA-MD-468	Breast	Wildtype	ND

(B)

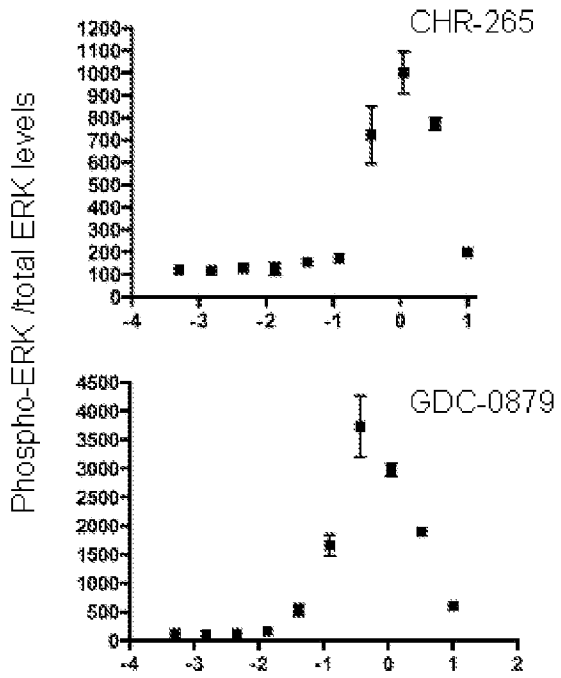


Figs. 21 A & B

(C)


CHR-265
GDC-0879
Unstimulated
Stimulated
100000
10000
1000
100
10
1
*
*
#
#

(D)

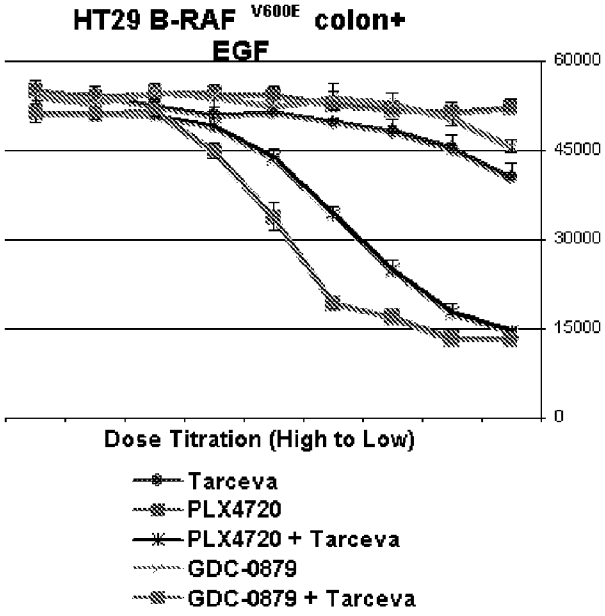

CHR-265
GDC-0879
Phospho-ERK /total ERK levels

Figs. 21C & D

(A)

Compound	LOX (B-Raf^{V600E}) pMEK IC_{50} (nM)		HT-29 (B-Raf^{V600E}) pMEK IC_{50} (nM)	
	-EGF	+EGF	-EGF	+EGF
GDC-0879 Expt 1 Expt 2	 25 14	 27 17	 10 19	 25 20
PLX-4720 Expt 1 Expt 2	 16 14	 >1000 >1000	 34 34	 >1000 >1000

(B)



Figs. 22A & B

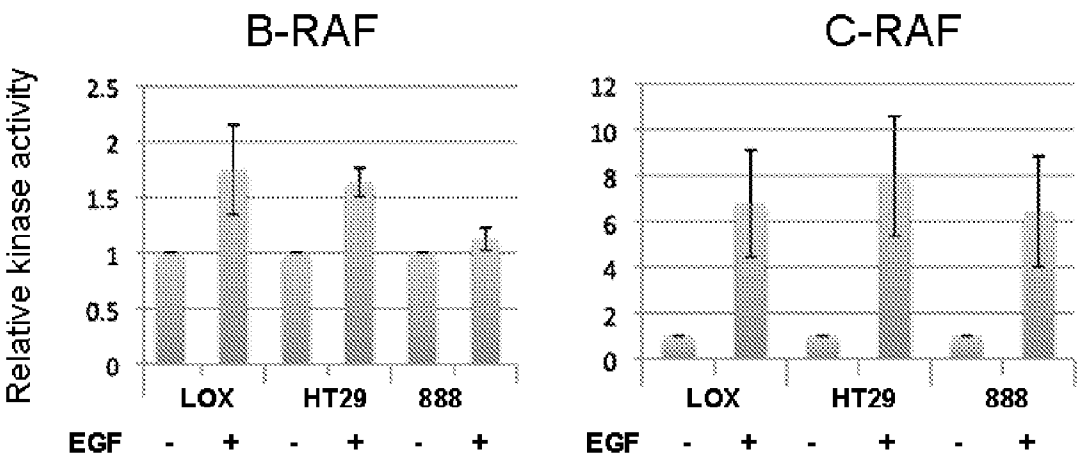

B-RAF
C-RAF
Relative kinase activity
2.5
2
1.5
1
0.5
0
12
10
8
6
4
2
0
LOX
HT29
888
EGF - + - + - +

Fig. 23

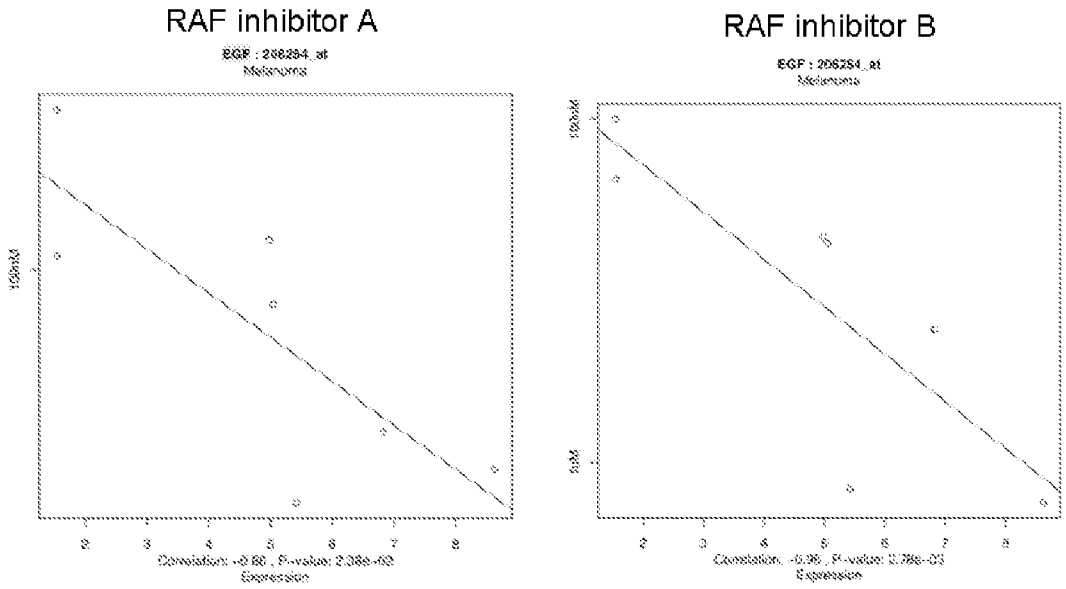


Fig. 24

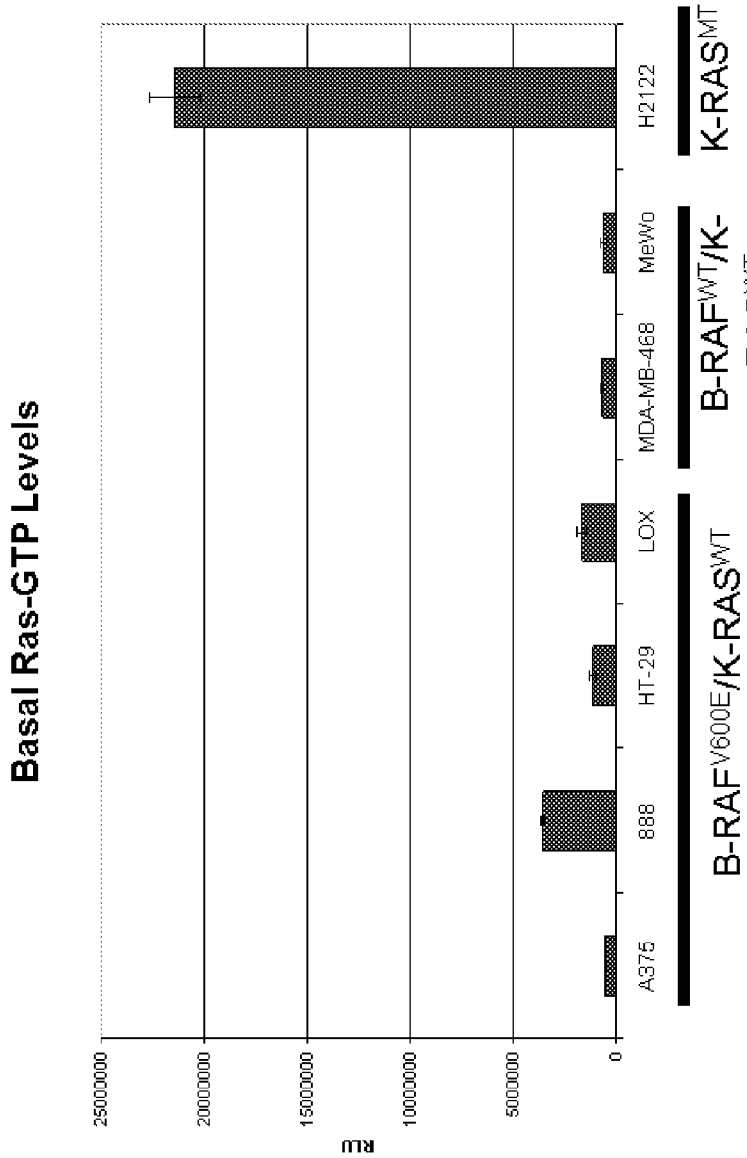

Basal Ras-GTP Levels
RLU
25000000
20000000
15000000
10000000
5000000
0
A375
888
HT-29
LOX
MDA-MB-468
MeWo
H2122
B-RAF^{V600E}/K-RAS^{WT}
B-RAF^{WT}/K-RAS^{WT}
K-RAS^{MT}

Fig. 25

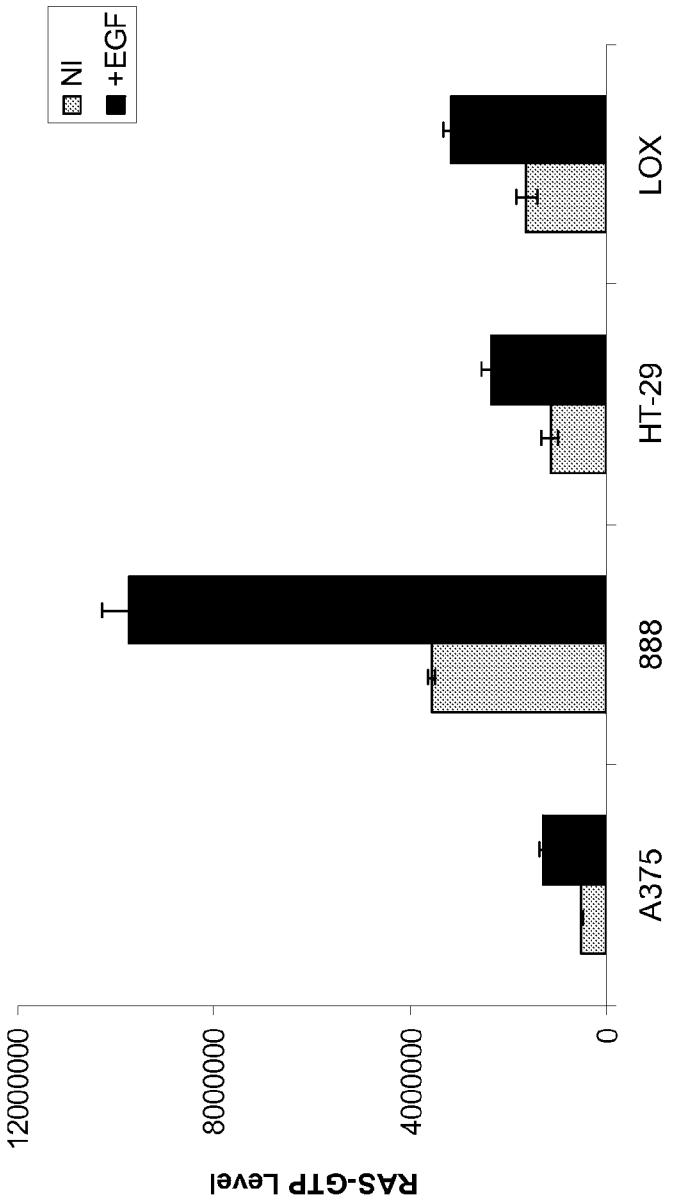

NI
+EGF
RAS-GTP Level
12000000
8000000
4000000
0
A375
888
HT-29
LOX

Fig. 26

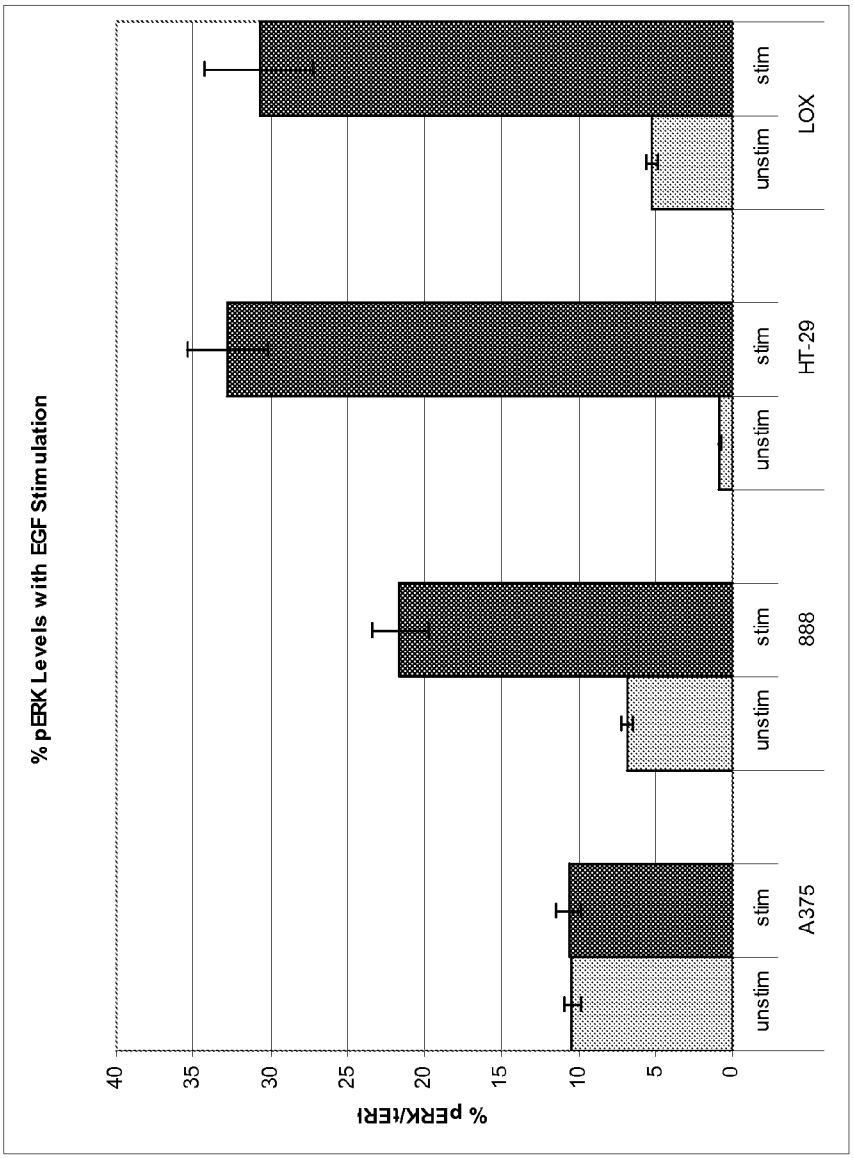

% pERK Levels with EGF Stimulation
% pERK/tERK
0
5
10
15
20
25
30
35
40
unstim
stim
A375
unstim
stim
888
unstim
stim
HT-29
unstim
stim
LOX

Fig. 27

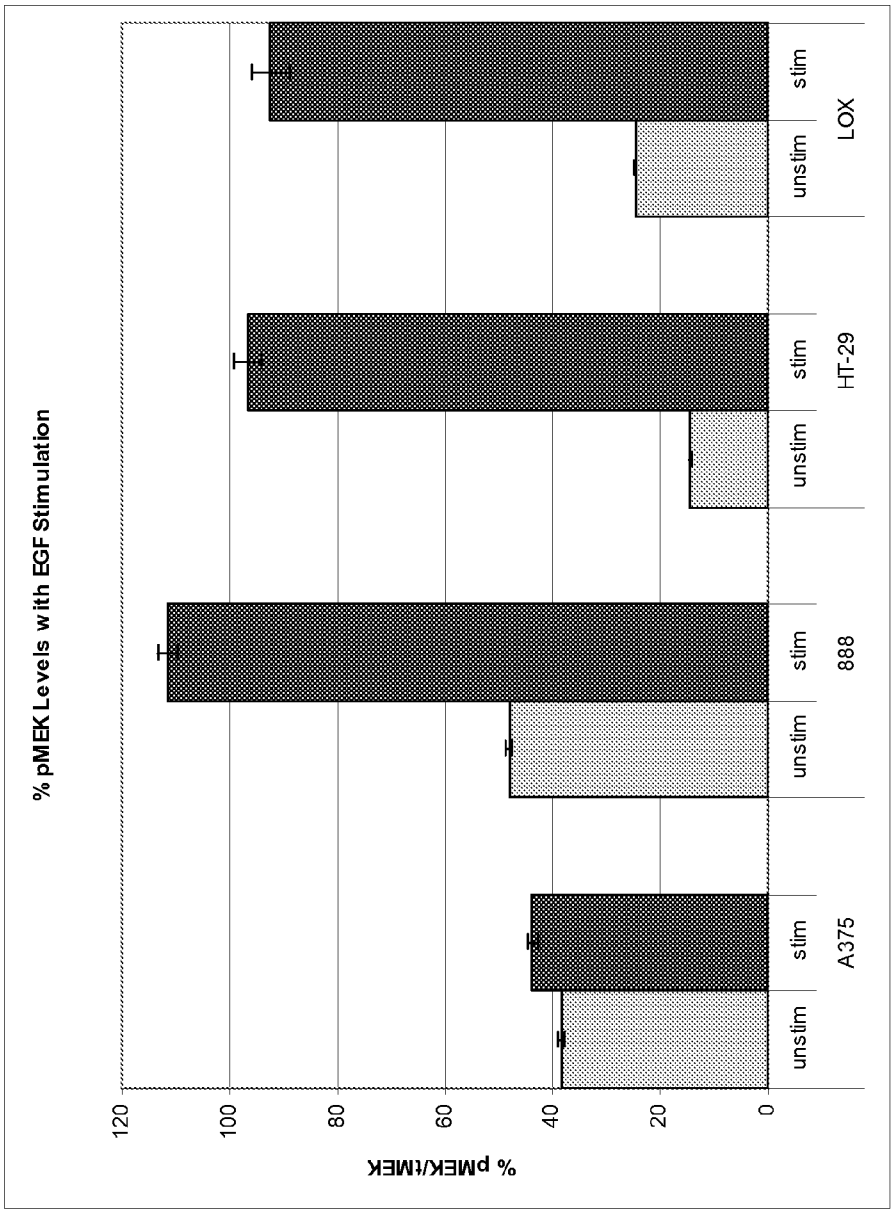

% pMEK Levels with EGF Stimulation
% pMEK/tMEK
120
100
80
60
40
20
0
unstim
stim
A375
888
HT-29
LOX

Fig. 28

pERK IC50 (nM)	888 (B-RAFV600E)		HT-29 (B-RAFV600E)		LOX (B-RAFV600E)	
	-EGF	+EGF	-EGF	+EGF	-EGF	+EGF
GDC-0879	14	599	15	136	75	231
RAF inhibitor a	16	>1000	49	>1000	99	>1000
PLX-4720	10	>1000	6	>1000	28	>1000

pERK IC50 (nM)	624 (B-RAFV600E)	
	-HGF	+HGF
RAF inhibitor a	6.4	>10000

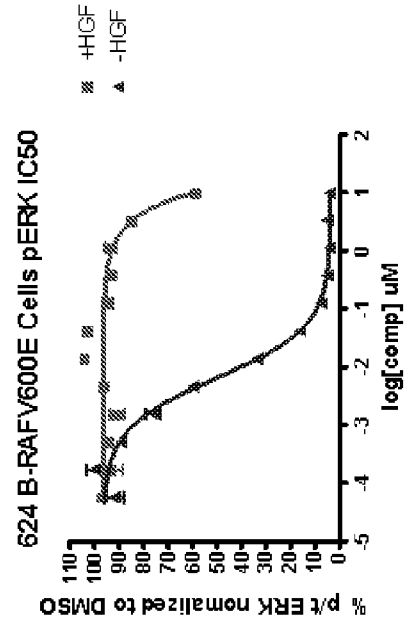


Fig. 29

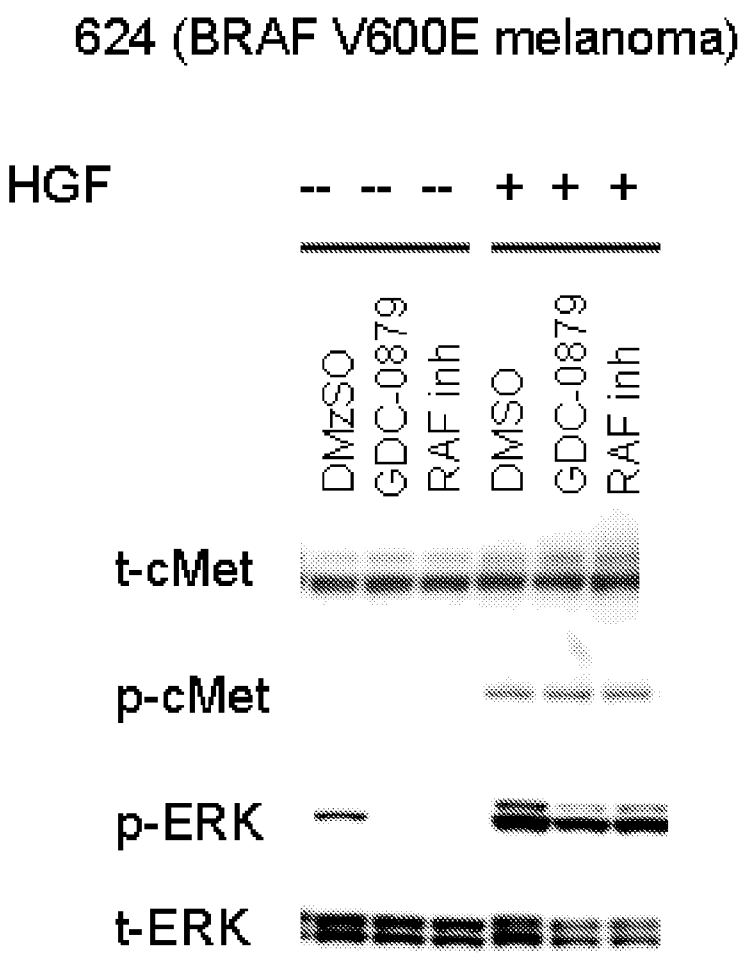

624 (BRAF V600E melanoma)
HGF
-- -- -- + + +
DMSO
GDC-0879
RAF inh
DMSO
GDC-0879
RAF inh
t-cMet
p-cMet
p-ERK
t-ERK

Fig. 30

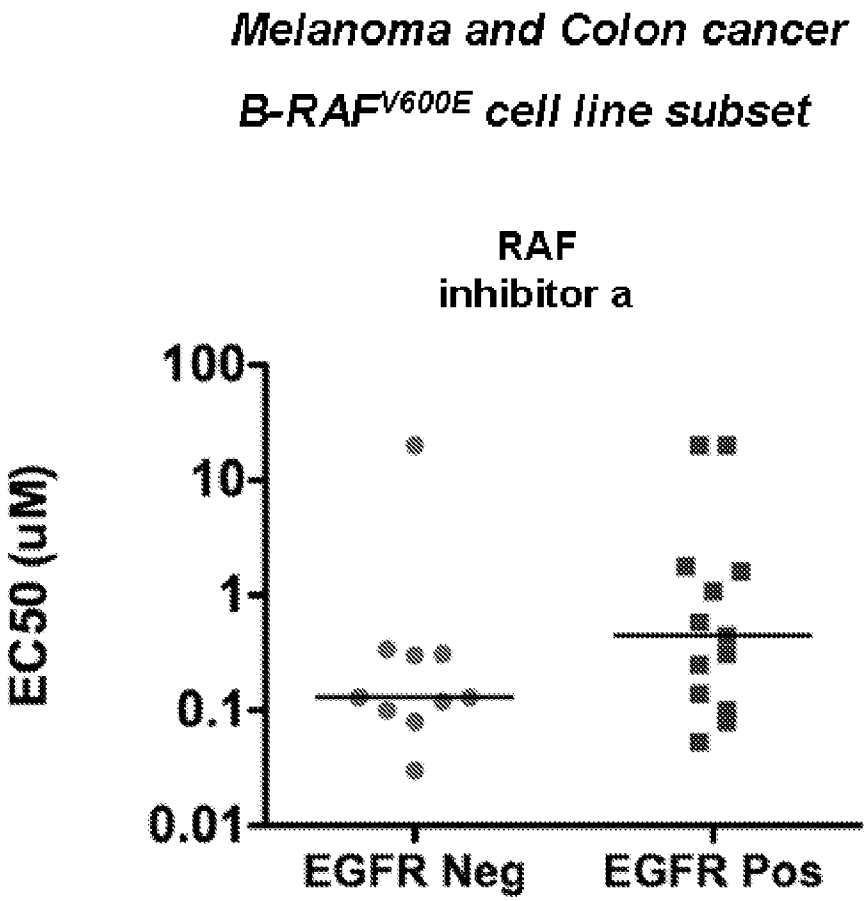
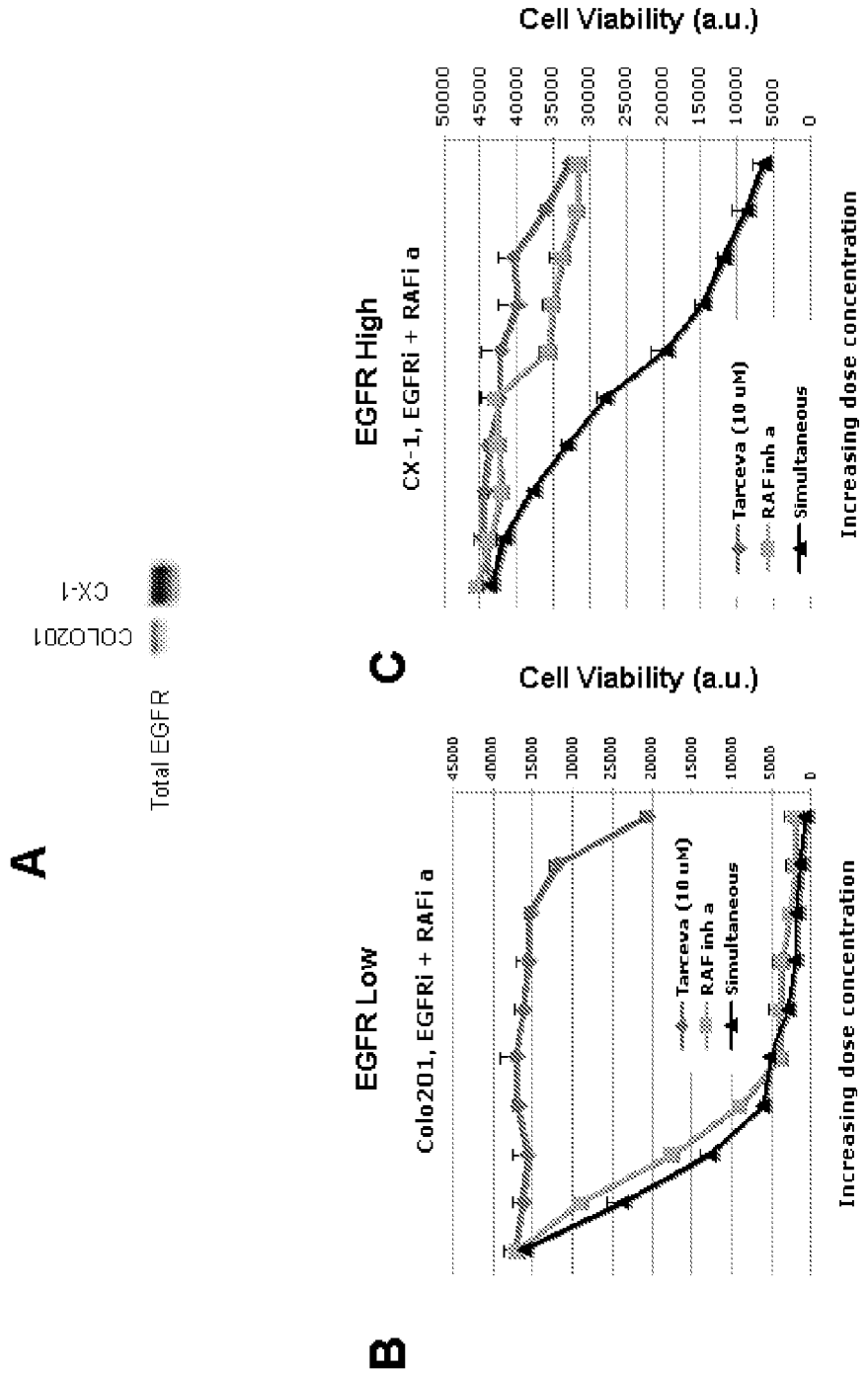

Melanoma and Colon cancer
B-RAF V600E cell line subset
RAF
inhibitor a
EC50 (uM)
100
10
1
0.1
0.01
EGFR Neg
EGFR Pos

Fig. 31


A
Total EGFR
COLO201
CX-1
B
EGFR Low
Colo201, EGFRi + RAFi a
Cell Viability (a.u.)
Increasing dose concentration
Tarceva (10 uM)
RAF inh a
Simultaneous
C
EGFR High
CX-1, EGFRi + RAFi a
Cell Viability (a.u.)
Increasing dose concentration
Tarceva (10 uM)
RAF inh a
Simultaneous

Figs. 32A – C

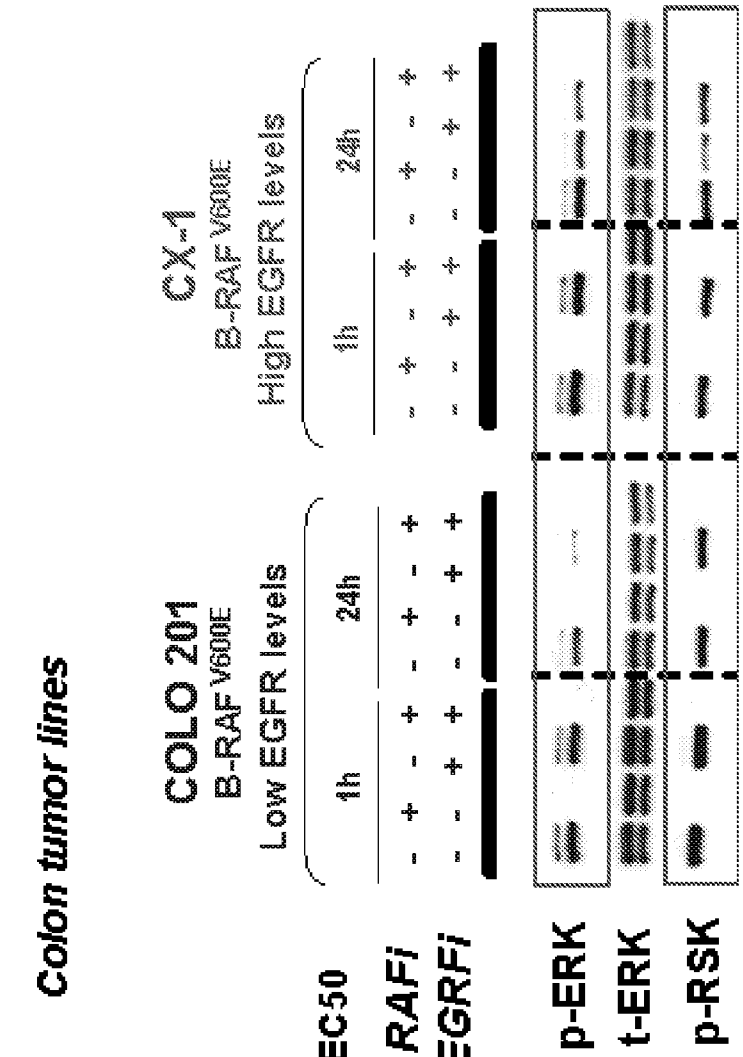

Colon tumor lines
COLO 201
B-RAF V600E
Low EGFR levels
CX-1
B-RAF V600E
High EGFR levels
1h
24h
1XEC50
RAFi
EGRFi
p-ERK
t-ERK
p-RSK

Fig. 33

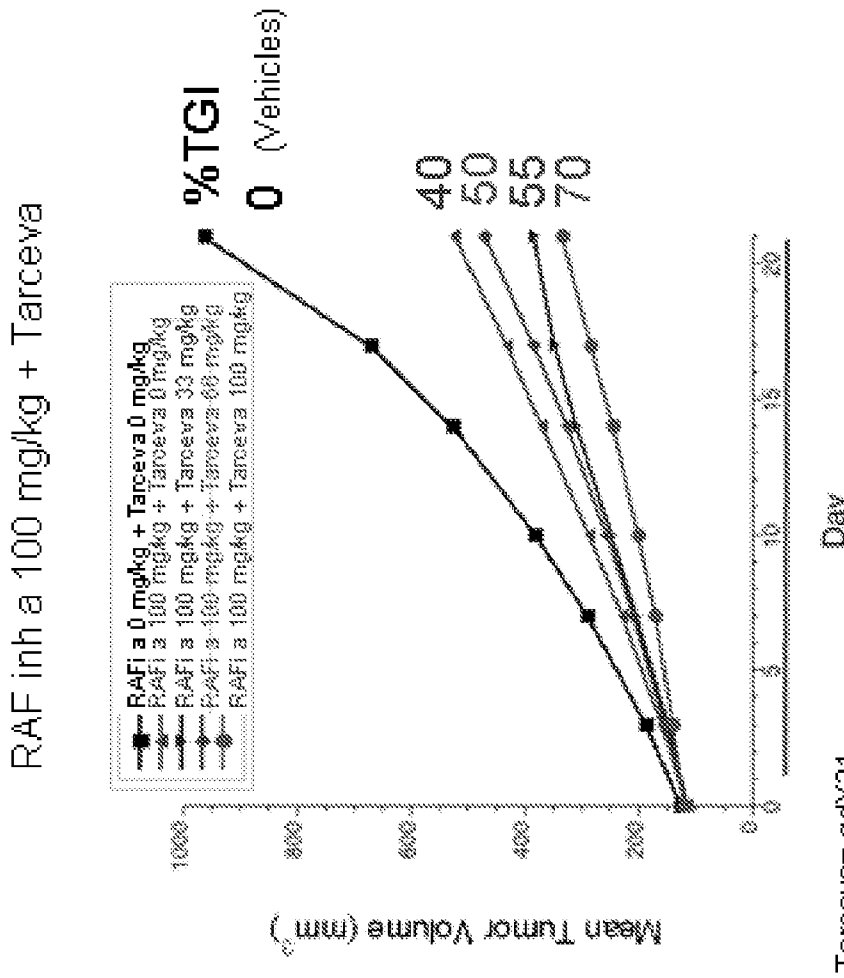

RAF inh a 100 mg/kg + Tarceva
RAFi a 0 mg/kg + Tarceva 0 mg/kg
RAFi a 100 mg/kg + Tarceva 0 mg/kg
RAFi a 100 mg/kg + Tarceva 33 mg/kg
RAFi a 100 mg/kg + Tarceva 66 mg/kg
RAFi a 100 mg/kg + Tarceva 100 mg/kg
%TGI
0 (Vehicles)
40
50
55
70
Mean Tumor Volume (mm³)
Day
Tarceva= qdX21
RAF inh a=qdX21

Fig. 34A

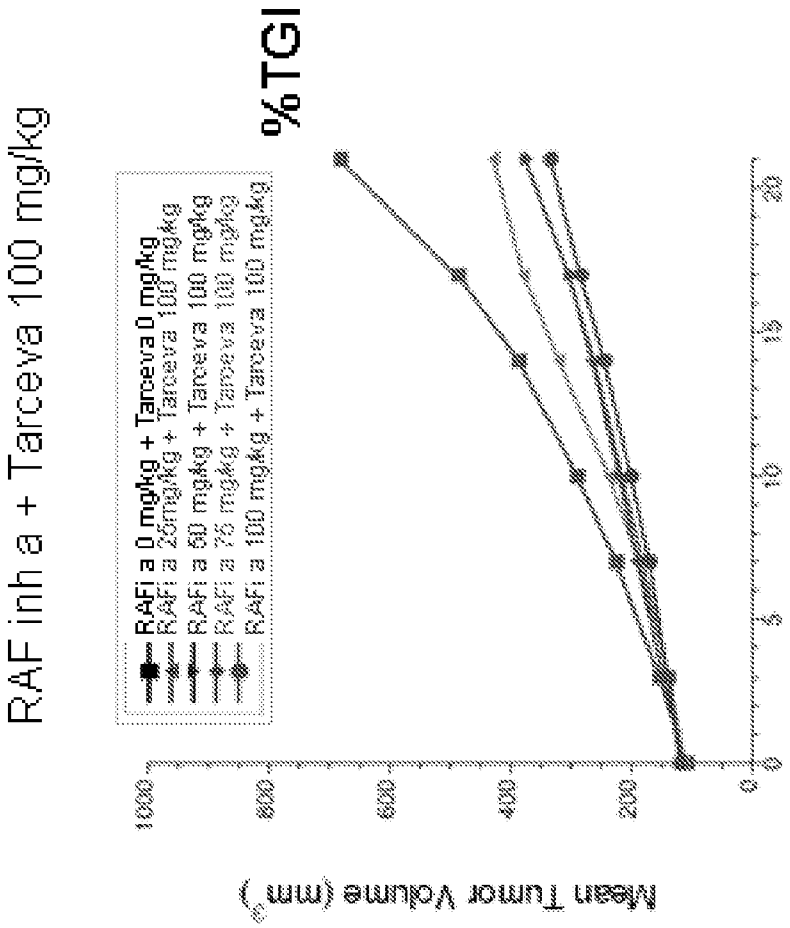

RAF inh a + Tarceva 100 mg/kg
%TGI
RAFi a 0 mg/kg + Tarceva 0 mg/kg
RAFi a 20mg/kg + Tarceva 100 mg/kg
RAFi a 60 mg/kg + Tarceva 100 mg/kg
RAFi a 25 mg/kg + Tarceva 100 mg/kg
RAFi a 100 mg/kg + Tarceva 100 mg/kg
Mean Tumor Volume (mm³)
1000
800
600
400
200
0
0
5
10
15
20
Day

Fig. 34B

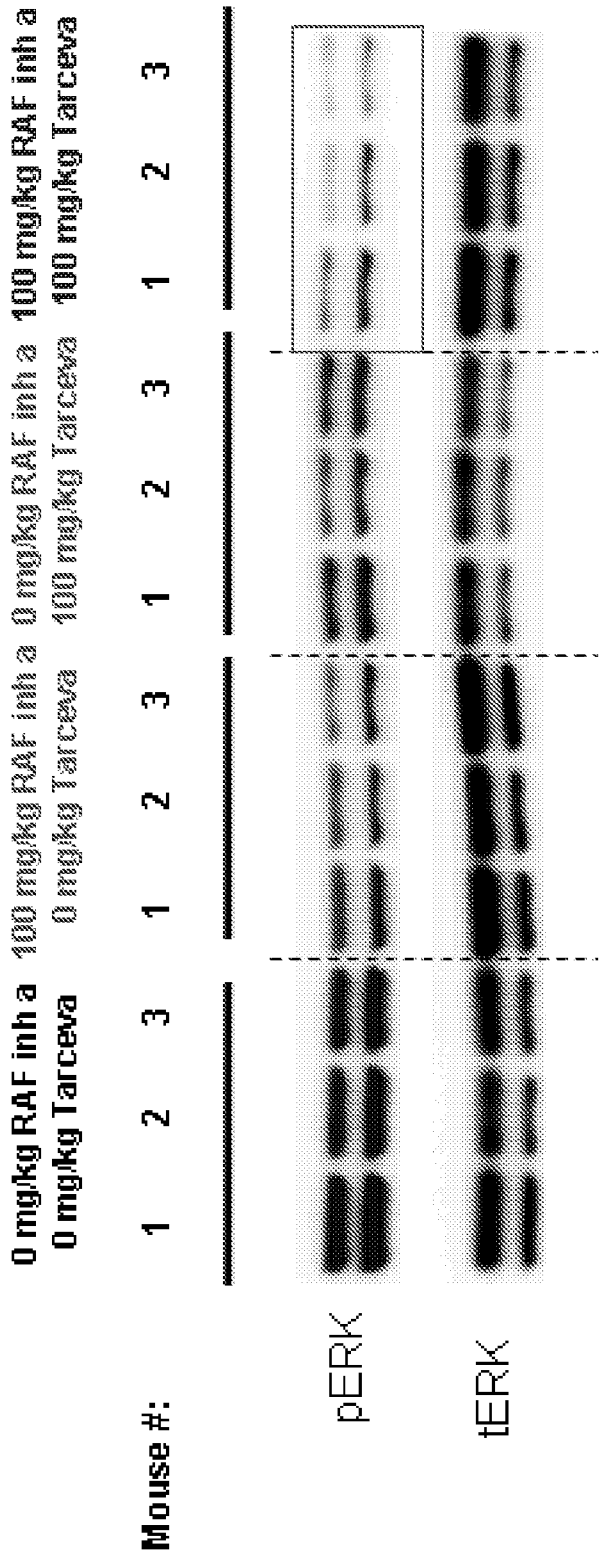

0 mg/kg RAF inh a
0 mg/kg Tarceva
100 mg/kg RAF inh a
0 mg/kg Tarceva
0 mg/kg RAF inh a
100 mg/kg Tarceva
100 mg/kg RAF inh a
100 mg/kg Tarceva
Mouse #:
1 2 3 1 2 3 1 2 3 1 2 3
pERK
tERK

Fig. 34C

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 10/46520

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - G01N 33/48 (2010.01)
USPC - 436/63, 436/64, 436/94
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
USPC: 436/63, 436/64, 436/94

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)
Databases consulted: PubWEST DB=PGPB,USPT,USOC,EPAB,JPAB; PLUR=NO; OP=ADJ; Google Scholar, Goole Patents
Seacrh Terms Used: KRAS mutation, BRAF inhibitor, activating mutation, G12C, G12A, G12D, G12R, G12S, G12V, G13C, G13D, Sorafenib, Nexavar, BAY 43-9006, PLX-4720, PLX-4032, RG7204 or GDC-08

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2009/0075267 A1 (Siena et al.) 19 March 2009 (19.03.2009) esp: abstract, paras [0009]-[0014], [0027], [0034], [0048], [0049], [0070], [0072]-[0074], [0086], [0087], [0089]-[0097], [0099], [0100]-[0102], [0104]-[0106], [0113], [0114], [0116], [0119], [0125], [0130], Fig. 1, Table 1, Table 2.	1-14
Y	Wilhelm et al. "BAY 43-9006 Exhibits Broad Spectrum Oral Antitumor Activity and Targets the RAF/MEK/ERK Pathway and Receptor Tyrosine Kinases Involved in Tumor Progression and Angiogenesis" CANCER RESEARCH, October 1, 2004, v64, pg 7099-7109. esp: abstract, pg 7104 last paragraph last sentence; pg 7106 section entitled "Discussion" first paragraph and second paragraph; pg 7107 second column, last paragraph.	1-14
Y	Wan et al. "Mechanism of Activation of the RAF-ERK Signaling Pathway by Oncogenic Mutations of B-RAF" Cell, March 19, 2004, Vol. 116, 855-867. entire document, esp: abstract, pg 860 section entitled "Overall Structure and B-RAF and BAY43-9006 Interactions"; Figs. 4-6.	1-14
Y	Takezawa et al. "Sorafenib Inhibits Non-Small Cell Lung Cancer Cell Growth by Targeting B-RAF in KRAS Wild-Type Cells and C-RAF in KRAS Mutant Cells" Cancer Res August 15, 2009, v 69: (16) pg 6515-6521. entire document, esp: pg 6514, col 1, para 5; pg 6515, col 2, para 1; pg. 6518, col 1, para 1 and para 2; Fig. 5A and Fig 5B.	1-14
Y	Sharma et al. "Targeting Mitogen-Activated Protein Kinase/Extracellular Signal?Regulated Kinase Kinase in the Mutant (V600E)B-Raf Signaling Cascade Effectively Inhibits Melanoma Lung Metastases" Cancer Res 2006; 66: (16). August 15, 2006. esp: abstract, pg 8202 section entitled "Targeting mutant V600EB-Raf signaling in melanoma cells inhibits development of lung metastases"; pg 8206 section entitled "Discussion".	1-14

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

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
30 November 2010 (30.11.201)	16 DEC 2010
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 10/46520

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

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

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

This International Searching Authority found multiple inventions in this international application, as follows:
This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I: claims 1-14, directed to a method of identifying a patient nonresponsive to treatment with a B-Raf inhibitor, comprising determining the presence or absence of a K-ras mutation, whereby the presence of a K-ras mutation indicates the patient will not respond to said B-raf inhibitor treatment.

Group II: claims 15 and 16, directed to a method of classifying a breast, lung, colon, ovarian, thyroid, melanoma or pancreatic tumor comprising the steps of: obtaining a tumor sample; detecting the expression or activity of i) a gene encoding the B-Raf V600E mutant and ii) a gene encoding a K-ras mutant in the sample.

- Please see extra sheet for continuation -

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-14

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 10/46520

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2009/0202989 A1 (Hillan) 13 August 2009 (13.08.2009) entire document, esp: abstract, paras [0007]-[0012], [0022].	1-14
Y, P	Siena et al. "Biomarkers Predicting Clinical Outcome of Epidermal Growth Factor Receptor ? Targeted Therapy in Metastatic Colorectal Cancer" JNCI Review, Vol. 101, Issue 19, October 7, 2009, pg 1308-1324. entire document, esp: abstract, pg 1310 section entitled KRAS mutations; pg 1314 section entitled "BRAF mutations".	1-14

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 10/46520

Continuation of Box III: Lack of Unity of Invention

Group III: claims 17-22, directed to a method of identifying a tumor nonresponsive to treatment with a B-Raf inhibitor, comprising determining the level of expression of a receptor tyrosine kinase (RTK), whereby aberrant expression of an RTK indicates that the patient will not respond to B-Raf treatment, and wherein said tumor expresses the B-Raf V600E mutation.

The inventions listed as Groups I - III do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The special technical feature of the Group I claims is a method of identifying a patient nonresponsive to treatment with a B-Raf inhibitor, comprising determining the presence or absence of a K-ras mutation, whereby the presence of a K-ras mutation indicates the patient will not respond to said B-raf inhibitor treatment. The special technical feature of the Group II claims is a method of classifying a breast, lung, colon, ovarian, thyroid, melanoma or pancreatic tumor comprising the steps of: obtaining a tumor sample; detecting the expression or activity of i) a gene encoding the B-Raf V600E mutant and ii) a gene encoding a K-ras mutant in the sample. The special technical feature of the Group III claims is a method of identifying a tumor nonresponsive to treatment with a B-Raf inhibitor, comprising determining the level of expression of a receptor tyrosine kinase (RTK), whereby aberrant expression of an RTK indicates that the patient will not respond to B-Raf treatment, and wherein said tumor expresses the B-Raf V600E mutation.

The only common technical element shared by the above groups is that they are related to the association of B-raf or B-raf mutations with cancer. Groups II and III share the additional common technical element wherein the B-raf mutation is a V600E mutation. These common technical elements do not represent an improvement over the prior art of US 2009/0075267 A1 to Siena et al. (see abstract, para [0002], [0009]-[0012], [0016], [0017], [0071]). Groups I and II share the common technical element of non-responsiveness to a B-raf inhibitor being mediated by an additional factor or polypeptide. This common technical element does not improve upon the prior art of the article entitled "Sorafenib Inhibits Non?Small Cell Lung Cancer Cell Growth by Targeting B-RAF in KRAS Wild-Type Cells and C-RAF in KRAS Mutant Cells" by Takezawa et al. (see pg 6514, col 1, para 5; pg 6515, col 2, para 1; pg. 6518, col 1, para 1 and para 2, specifically: "Depletion of B-RAF resulted in significant inhibition of cell proliferation (Fig. 5A) and an increase in the proportion of cells in G1 phase of the cell cycle (Fig. 5B), whereas depletion of C-RAF had no such effects, in NSCLC cells harboring wild-type KRAS. In contrast, depletion of C-RAF induced significant inhibition of cell proliferation (Fig. 5A) and an increase in the proportion of cells in G1 phase (Fig. 5B), whereas depletion of BRAF had only a less pronounced effect on cell proliferation, in NSCLC cells with mutant KRAS"). Therefore, the inventions of Groups I-III lack unity of invention under PCT Rule 13 because they do not share a same or corresponding special technical feature.