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(54) Title: METHODS AND COMPOSITIONS FOR TREATING NON-ERK MAPK PATHWAY INHIBITOR-RESISTANT CANCERS

(57) Abstract: The present invention provides, *inter alia*, methods, pharmaceutical compositions, and kits for treating or ameliorating the effects of a cancer in a subject, which cancer is refractory or resistant to non-ERK MAPK pathway inhibitor therapy. Also provided are methods for identifying a subject having cancer who would benefit from therapy with an ERK inhibitor and methods for inhibiting phosphorylation of RSK in a cancer cell that is refractory or resistant to a non-ERK MAPK pathway inhibitor.

METHODS AND COMPOSITIONS FOR TREATING NON-ERK MAPK PATHWAY INHIBITOR-RESISTANT CANCERS

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims benefit of U.S. Patent Application Serial No. 61/919,551, filed on December 20, 2013 which application is incorporated by reference herein in its entirety.

FIELD OF INVENTION

[0002] The present invention provides, *inter alia*, methods, pharmaceutical compositions, and kits for treating or ameliorating the effects of a cancer in a subject, which cancer is refractory or resistant to non-ERK MAPK pathway inhibitor therapy.

INCORPORATION BY REFERENCE OF SEQUENCE LISTING

[0003] This application contains references to amino acids and/or nucleic acid sequences that have been filed concurrently herewith as sequence listing text file "0375608.txt", file size of 356 KB, created on December 18, 2014. The aforementioned sequence listing is hereby incorporated by reference in its entirety pursuant to 37 C.F.R. § 1.52(e)(5).

BACKGROUND OF THE INVENTION

[0004] Drug inhibitors that target components of the mitogen-activated protein kinases (MAPK) signaling pathway show clinical efficacy in a variety of cancers, particularly those bearing mutations in the BRAF protein kinase. Both RAF and MEK inhibitors are approved for single-agent use in advanced

metastatic BRAF mutant melanoma. Either alone or in combination, BRAF and MEK inhibitor activity is unpredictable in other cancers, with promising efficacy in BRAF mutant thyroid and lung cancer, but only marginal activity in BRAF mutant colorectal cancer.

[0005] As with other targeted therapies, patterns of disease response to RAF and MEK inhibitors appear to be influenced by the intrinsic genetic heterogeneity present in the cancers where the drugs are used. For instance, it has been shown that certain genetic alterations, including PTEN and other changes that activate the PI3K cell growth signaling pathway, may predict a poor initial response, and/or relatively rapid progression, in BRAF mutant melanoma treated with the RAF inhibitor vemurafenib. Likewise, direct mutations in MEK gene loci appear to emerge in tumors that have progressed following either BRAF, MEK, or combined drug treatment. Several additional examples, from RAS and RAF gene amplification and splicing mutations, suggest that acquired drug resistance is produced when oncogenic pleiotropy encounters the selective pressure of targeted drug treatment.

[0006] In view of the foregoing, there is a need for novel targeted agents that would ideally inhibit diverse nodes of oncogenic pathways, and also be effective in combinations by inducing a burden of selective pressures that exceeds the adaptive capacity of diverse cancer genomes. The present application is directed to meeting these and other needs.

SUMMARY OF THE INVENTION

[0007] One embodiment of the present invention is a method for treating or ameliorating the effects of a cancer in a subject, which cancer is

refractory or resistant to non-ERK MAPK pathway inhibitor therapy. The method comprises administering to the subject an effective amount of BVD-523 or a pharmaceutically acceptable salt thereof.

[0008] Another embodiment of the present invention is a method for treating or ameliorating the effects of a cancer in a subject. The method comprises:

(a) identifying a subject with cancer that has become refractory or resistant to BRAF inhibitor therapy, MEK inhibitor therapy, or BRAF and MEK inhibitor therapy; and

(b) administering to the subject with said refractory or resistant cancer an effective amount of an ERK inhibitor, which is BVD-523 or a pharmaceutically acceptable salt thereof.

[0009] A further embodiment of the present invention is a method for treating or ameliorating the effects of cancer in a subject, which cancer is refractory or resistant to BRAF inhibitor therapy, MEK inhibitor therapy, or both. The method comprises administering to the subject an effective amount of BVD-523 or a pharmaceutically acceptable salt thereof.

[0010] Another embodiment of the present invention is a method for identifying a subject having cancer who would benefit from therapy with an ERK inhibitor. The method comprises:

(a) obtaining a biological sample from the subject; and

(b) screening the sample to determine whether the subject has one or more of the following markers:

(i) a switch between RAF isoforms,

(ii) upregulation of receptor tyrosine kinase (RTK) or NRAS signaling,

(iii) reactivation of mitogen activated protein kinase (MAPK) signaling,

(iv) the presence of a MEK activating mutation,

(v) amplification of mutant BRAF,

(vi) STAT3 upregulation,

(vii) mutations in the allosteric pocket of MEK that directly block binding of inhibitors to MEK or lead to constitutive MEK activity,

wherein the presence of one or more of the markers confirms that the subject's cancer is refractory or resistant to BRAF and/or MEK inhibitor therapy and that the subject would benefit from therapy with an ERK inhibitor, which is BVD-523 or a pharmaceutically acceptable salt thereof.

[0011] A further embodiment of the present invention is a pharmaceutical composition for treating or ameliorating the effects of a cancer in a subject, which cancer is refractory or resistant to non-ERK MAPK pathway therapy. The composition comprises a pharmaceutically acceptable carrier or diluent and an effective amount of BVD-523 or a pharmaceutically acceptable salt thereof.

[0012] Another embodiment of the present invention is a kit for treating or ameliorating the effects of a cancer in a subject, which cancer is refractory or resistant to non-ERK MAPK pathway therapy. The kit comprises any of the pharmaceutical compositions according to the present invention packaged together with instructions for its use.

[0013] Another embodiment of the present invention is a method for inhibiting phosphorylation of RSK in a cancer cell that is refractory or resistant to a non-ERK MAPK pathway inhibitor. The method comprises contacting the cancer cell with an effective amount of BVD-523 or a pharmaceutically acceptable salt thereof for a period of time sufficient for phosphorylation of RSK in the cancer cell to be inhibited.

BRIEF DESCRIPTION OF THE DRAWINGS

[0014] The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

[0015] FIGS. 1A-C show the progress of a dose escalation study in a human malignant melanoma cell line (A375 cells) for month 1. Various treatments (trametinib (a type 2 MEK inhibitor), dabrafenib (a BRAF inhibitor), and BVD-523 (an ERK1/2 inhibior)) are as labeled.

[0016] FIGS. 2A-H show the results of a proliferation assay that tracks changes in sensitivity to the escalated agent(s) at month 1. Various treatments (trametinib, dabrafenib, BVD-523, and pacitaxel) are as labeled on the top of the graph. The caption to the right of the graph shows the various types of cells generated from the dose escalation study. For example, "dabrafenib" refers to the cells that have been treated with the highest dose of dabrafenib from month 1 of the dose escalation study. Parental refers to the control cells that have not been treated with drugs. FIGS. 2A-2C and 2G are normalized to control, whereas FIGS. 2D-2F and 2H show the raw data.

[0017] FIGS. 3A-3D show the progress of a dose escalation study in A375 cells for month 2. Various treatments (trametinib, dabrafenib, and BVD-523) are as labeled.

[0018] FIGS. 4A-H show the results of a proliferation assay that tracks changes in sensitivity to the escalated agent(s) at month 2. Various treatments (trametinib, dabrafenib, BVD-523, and pacitaxel) are as labeled on the top of the graph. The caption to the right of the graph shows the various types of cells generated from the dose escalation study. For example, "dabrafenib" refers to the cells that have been treated with the highest dose of dabrafenib from month 2 of the dose escalation study. Parental refers to the control cells that have not been treated with drugs. FIGS. 4A-4C and 4G are normalized to control, whereas FIGS. 4D-4F and 4H show the raw data.

[0019] FIGS. 5A-H show only the parental and BVD-523 cell line data from FIG. 4. Various treatments (trametinib, dabrafenib, BVD-523, and pacitaxel) are as labeled. FIGS. 5A-5C and 5G are normalized to control, whereas FIGS. 5D-5F and 5H show the raw data.

[0020] FIGS. 6A-D show the progress of the dose escalation study in a human malignant cell line (A375 cells) for month 3. Various treatments (trametinib, dabrafenib, and BVD-523) are as labeled.

[0021] FIG. 7 is a histogram showing the results of a proliferation assay as applied to cells grown in the DMSO control wells from the dose escalation assay.

[0022] FIGS. 8A-D are a set of line graphs showing proliferation assays for month 3 of the study. Various treatments (trametinib, dabrafenib, BVD-523, and pacitaxel) are as labeled on the top of the graph. The caption to the

right of the graph shows the various types of cells generated from the dose escalation study. For example, "dabrafenib" refers to the cells that have been treated with the highest dose of dabrafenib from month 3 of the dose escalation study. Parental refers to the control cells that have not been treated with drugs.

[0023] FIGS. 9A-D show only the parental, dabrafenib, and BVD-523 cell line data from FIG. 8.

[0024] FIG. 10A is a dose matrix showing % inhibition of the trametinib/dabrafenib combination in A375 cells using the Alamar Blue cell viability assay. FIG. 10B is a dose matrix showing excess over Bliss for the trametinib/dabrafenib combination. FIGS. 10C and 10D show % viability relative to DMSO only treated controls for dabrafenib and trametinib single agent treatments in A375 cells using the Alamar Blue cell viability assay. FIG. 10E shows % viability relative to DMSO only treated controls for dabrafenib and trametinib combination treatments in A375 cells using the Alamar Blue cell viability assay.

[0025] FIG. 11A is a dose matrix showing % inhibition of the trametinib/dabrafenib combination in A375 cells using the CellTiter-Glo cell viability assay. FIG. 11B is a dose matrix showing excess over Bliss for the trametinib/dabrafenib combination. FIGS. 11C and 11D show % viability relative to DMSO only treated controls for dabrafenib and trametinib single agent treatments in A375 cells using the CellTiter-Glo cell viability assay. FIG. 11E shows % viability relative to DMSO only treated controls for dabrafenib and trametinib combination treatments in A375 cells using the CellTiter-Glo cell viability assay.

[0026] FIG. 12A is a dose matrix showing % inhibition of the BVD-523/dabrafenib combination in A375 cells using the Alamar Blue cell viability assay. FIG. 12B is a dose matrix showing excess over Bliss for the BVD-523/dabrafenib combination. FIGS. 12C and 12D show % viability relative to DMSO only treated controls for dabrafenib and BVD-523 single agent treatments in A375 cells using the Alamar Blue cell viability assay. FIG. 12E shows % viability relative to DMSO only treated controls for dabrafenib and BVD-523 combination treatments in A375 cells using the Alamar Blue cell viability assay.

[0027] FIG. 13A is a dose matrix showing % inhibition of the BVD-523/dabrafenib combination in A375 cells using the CellTiter-Glo cell viability assay. FIG. 13B is a dose matrix showing excess over Bliss for the BVD-523/dabrafenib combination. FIGS. 13C and 13D show % viability relative to DMSO only treated controls for dabrafenib and BVD-523 single agent treatments in A375 cells using the CellTiter-Glo cell viability assay. FIG. 13E shows % viability relative to DMSO only treated controls for dabrafenib and BVD-523 combination treatments in A375 cells using the CellTiter-Glo cell viability assay.

[0028] FIG. 14A is a dose matrix showing % inhibition of the trametinib/BVD-523 combination in A375 cells using the Alamar Blue cell viability assay. FIG. 14B is a dose matrix showing excess over Bliss for the trametinib/BVD-523 combination. FIGS. 14C and 14D show % viability relative to DMSO only treated controls for BVD-523 and trametinib single agent treatments in A375 cells using the Alamar Blue cell viability assay. FIG. 14E shows % viability relative to DMSO only treated controls for BVD-523 and

trametinib combination treatments in A375 cells using the Alamar Blue cell viability assay.

[0029] FIG. 15A is a dose matrix showing % inhibition of the trametinib/BVD-523 combination in A375 cells using the CellTiter-Glo cell viability assay. FIG. 15B is a dose matrix showing excess over Bliss for the trametinib/BVD-523 combination. FIGS. 15C and 15D show % viability relative to DMSO only treated controls for BVD-523 and trametinib single agent treatments in A375 cells using the CellTiter-Glo cell viability assay. FIG. 15E shows % viability relative to DMSO only treated controls for BVD-523 and trametinib combination treatments in A375 cells using the CellTiter-Glo cell viability assay.

[0030] FIGS. 16A-D are a set of images showing Western blot analysis of MAPK signaling in A375 cells after a 4 hour treatment with various concentrations (in nM) of BVD-523, dabrafenib (Dab), and Trametinib (Tram). 40 µg of total protein was loaded in each lane except where indicated otherwise. In this experiment, duplicate samples were collected. FIGS. 16A and 16B show results from duplicate samples. Similarly, FIGS. 16C and 16D also show results from duplicate samples. In FIGS. 16A and 16B, pRSK1 had a relatively weak signal in A375 cells compared to other markers. A different pRSK1-S380 antibody from Cell Signaling (cat. #11989) was tested but did not give a detectable signal (data not shown). In FIGS. 16C and 16D, pCRAF-338 gave a minimal signal.

[0031] FIGS. 17A-D are a set of images showing Western blot analysis of MAPK signaling in a human colorectal carcinoma cell line (HCT116 cells) after a 4 hour treatment with various concentrations (in nM) of BVD-523,

dabrafenib (Dab), and Trametinib (Tram). 40 µg of total protein was loaded in each lane except where indicated otherwise. In this experiment, duplicate samples were collected. FIGS. 17A and 17B show results from duplicate samples. Similarly, FIGS. 17C and 17D also show results from duplicate samples. In FIGS. 17A-17B, pRSK1 levels appear to be very low in HCT116 cells, and in FIGS. 17C and 17D, pCRAF-338 signal was also very weak.

[0032] FIGS. 18A-D are a set of images showing Western blot analysis of cell cycle and apoptosis signaling in A375 melanoma cells after a 24 hour treatment with various concentrations (in nM) of BVD-523 ("BVD523"), trametinib ("tram") and/or dabrafenib ("Dab") as labelled. 50 µg of total protein was loaded in each lane except where indicated otherwise. In this experiment, duplicate samples were collected. FIGS. 18A and 18B show results from duplicate samples. Similarly, FIGS. 18C and 18D also show results from duplicate samples. In FIGS. 18A and 18B, no band of a size corresponding to cleaved PARP (89 kDa) was apparent.

[0033] FIG. 19 shows that BVD-523 can treat acquired resistance to targeted drugs *in-vivo*. A patient-derived line, ST052C, was isolated from a BRAFV600E melanoma patient that progressed following 10 months of therapy with MAPK-pathway directed therapies. Treated *ex vivo*, ST052C exhibited acquired cross-resistance to dabrafenib at 50 mg/kg BID. Meanwhile, BVD-523 was effective in ST052C as a single-agent at 100 mg/kg BID.

[0034] FIG. 20 is a flowchart showing the dose escalation protocol used herein.

[0035] FIG. 21 shows a schematic of the mitogen-activated protein kinases (MAPK) pathway.

[0036] FIG. 22 shows the results of single agent proliferation assays. Proliferation results are shown for treatment with BVD-523 (FIG. 22A), SCH772984 (FIG. 22B), Dabrafenib (FIG. 22C), Trametinib (FIG. 22D), and Paclitaxel (FIG. 22E).

[0037] FIG. 23 shows the results of the combination of BVD-523 and Dabrafenib. FIG. 23A shows a dose matrix showing inhibition (%) for the combination in RKO parental cells. FIG. 23B – FIG. 23C show the results of single agent proliferation assays for the combination in 23A. FIG. 23D shows Loewe excess for the combination in 23A and FIG. 23E shows Bliss excess for the combination in 23A. FIG. 23F shows a dose matrix showing inhibition (%) for the combination in RKO MEK1 (Q56P/+) – clone 1 cells. FIG. 23G – FIG. 23H show the results of single agent proliferation assays for the combination in 23F. FIG. 23I shows Loewe excess for the combination in 23F and FIG. 23J shows Bliss excess for the combination in 23F. FIG. 23K shows a dose matrix showing inhibition (%) for the combination in RKO MEK1 (Q56P/+) – clone 2 cells. FIG. 23L – FIG. 23M show the results of single agent proliferation assays for the combination in 23K. FIG. 23N shows Loewe excess for the combination in 23K and FIG. 23O shows Bliss excess for the combination in 23K.

[0038] FIG. 24 shows the results of the combination of SCH772984 and Dabrafenib. FIG. 24A shows a dose matrix showing inhibition (%) for the combination in RKO parental cells. FIG. 24B – FIG. 24C show the results of single agent proliferation assays for the combination in 24A. FIG. 24D shows

Loewe excess for the combination in 24A and FIG. 24E shows Bliss excess for the combination in 24A. FIG. 24F shows a dose matrix showing inhibition (%) for the combination in RKO MEK1 (Q56P/+) – clone 1 cells. FIG. 24G – FIG. 24H show the results of single agent proliferation assays for the combination in 24F. FIG. 24I shows Loewe excess for the combination in 24F and FIG. 24J shows Bliss excess for the combination in 24F. FIG. 24K shows a dose matrix showing inhibition (%) for the combination in RKO MEK1 (Q56P/+) – clone 2 cells. FIG. 24L – FIG. 24M show the results of single agent proliferation assays for the combination in 24K. FIG. 24N shows Loewe excess for the combination in 24K and FIG. 24O shows Bliss excess for the combination in 24K.

[0039] FIG. 25 shows the results of the combination of Trametinib and Dabrafenib. FIG. 25A shows a dose matrix showing inhibition (%) for the combination in RKO parental cells. FIG. 25B – FIG. 25C show the results of single agent proliferation assays for the combination in 25A. FIG. 25D shows Loewe excess for the combination in 25A and FIG. 25E shows Bliss excess for the combination in 25A. FIG. 25F shows a dose matrix showing inhibition (%) for the combination in RKO MEK1 (Q56P/+) – clone 1 cells. FIG. 25G – FIG. 25H show the results of single agent proliferation assays for the combination in 25F. FIG. 25I shows Loewe excess for the combination in 25F and FIG. 25J shows Bliss excess for the combination in 25F. FIG. 25K shows a dose matrix showing inhibition (%) for the combination in RKO MEK1 (Q56P/+) – clone 2 cells. FIG. 25L – FIG. 25M show the results of single agent proliferation assays for the combination in 25K. FIG. 25N shows Loewe

excess for the combination in 25K and FIG. 25O shows Bliss excess for the combination in 25K.

[0040] FIG. 26A shows Lowe Volumes for the combinations tested. FIG. 26B shows Bliss Volumes for the combinations tested. FIG. 26C shows Synergy Scores for the combinations tested.

[0041] FIG. 27 shows the changes in MAPK and Effector Pathway Signaling in MEK acquired resistance. Isogenic RKO parental and MEK1 (Q56P/+) cells were treated with compound for 4 or 24h and then immuno-blotted with the indicated antibodies. Dabrafenib was the BRAF inhibitor and trametinib was the MEK inhibitor. FIG. 27A shows increased signaling in RKO MEK1 (Q56P/+) cells. FIG 27B – FIG. 27C show the results of a 4 hour treatment in Experiment 1 (See, Example 7) in RKO Parental (27B) and RKO MEK1 (Q56P/+) (27C) cells. FIG. 27D – FIG. 27E show the results of a 4 hour treatment in Experiment 2 (See, Example 7) in RKO Parental (27D) and RKO MEK1 (Q56P/+) (27E) cells. FIG. 27F – FIG. 27G show the results of a 4 hour treatment in Experiment 2 (See, Example 7) in RKO Parental (27F) and RKO MEK1 (Q56P/+) (27G) cells. FIG. 27H – FIG. 27I show a summary of results in RKO Parental (27H) and RKO MEK1 (Q56P/+) (27I) cells.

[0042] FIG. 28 shows the results of the combination of BVD-523 and SCH772984. FIG. 28A shows a dose matrix showing inhibition (%) for the combination in A375 cells. FIG. 28B – FIG. 28C show the results of single agent proliferation assays for the combination in 28A. FIG. 28D shows Loewe excess for the combination in 28A and FIG. 28E shows Bliss excess for the combination in 28A.

DETAILED DESCRIPTION OF THE INVENTION

[0043] One embodiment of the present invention is a method for treating or ameliorating the effects of a cancer in a subject, which cancer is refractory or resistant to non-ERK MAPK pathway inhibitor therapy. The method comprises administering to the subject an effective amount of BVD-523 or a pharmaceutically acceptable salt thereof.

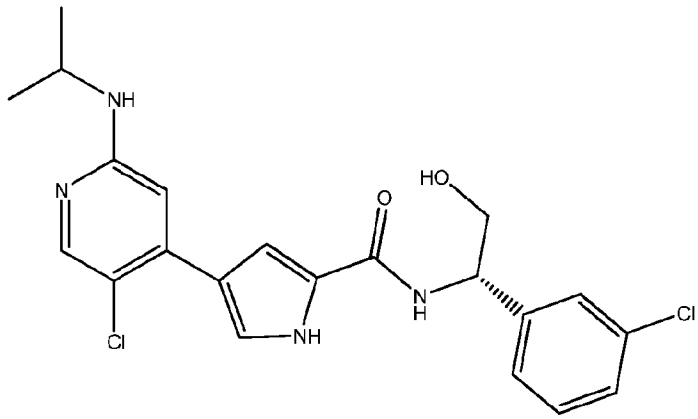
[0044] As used herein, the terms "treat," "treating," "treatment" and grammatical variations thereof mean subjecting an individual subject to a protocol, regimen, process or remedy, in which it is desired to obtain a physiologic response or outcome in that subject, e.g., a patient. In particular, the methods and compositions of the present invention may be used to slow the development of disease symptoms or delay the onset of the disease or condition, or halt the progression of disease development. However, because every treated subject may not respond to a particular treatment protocol, regimen, process or remedy, treating does not require that the desired physiologic response or outcome be achieved in each and every subject or subject population, e.g., patient population. Accordingly, a given subject or subject population, e.g., patient population may fail to respond or respond inadequately to treatment.

[0045] As used herein, the terms “ameliorate”, "ameliorating" and grammatical variations thereof mean to decrease the severity of the symptoms of a disease in a subject.

[0046] As used herein, a “subject” is a mammal, preferably, a human. In addition to humans, categories of mammals within the scope of the present invention include, for example, farm animals, domestic animals, laboratory

animals, etc. Some examples of farm animals include cows, pigs, horses, goats, etc. Some examples of domestic animals include dogs, cats, etc. Some examples of laboratory animals include primates, rats, mice, rabbits, guinea pigs, etc.

[0047] In the present invention, BVD-523 corresponds to a compound according to formula (I):



and pharmaceutically acceptable salts thereof. BVD-523 may be synthesized according to the methods disclosed, *e.g.*, in U.S. Patent No. 7,354,939. Enantiomers and racemic mixtures of both enantiomers of BVD-523 are also contemplated within the scope of the present invention. BVD-523 is an ERK1/2 inhibitor with a mechanism of action that is believed to be, e.g., unique and distinct from certain other ERK1/2 inhibitors, such as SCH772984 and the pyrimidinal structure used by Hatzivassiliou et al. (2012). For example, other ERK1/2 inhibitors, such as SCH772984, inhibit autophosphorylation of ERK (Morris et al., 2013), whereas BVD-523 allows for the autophosphorylation of ERK while still inhibiting ERK. (See, *e.g.*, FIG. 18).

[0048] As used herein, the words "resistant" and "refractory" are used interchangeably. Being "resistant" to non-ERK MAPK pathway inhibitor therapy treatments means that non-ERK MAPK inhibitors have reduced efficacy in treating cancer.

[0049] As used herein, a "non-ERK MAPK inhibitor" means any substance that reduces the activity, expression or phosphorylation of proteins or other members of the MAPK pathway that results in a reduction of cell growth or an increase in cell death, with the exception of ERK1/2 inhibitors. As used herein, an "ERK1/2 inhibitor" means those substances that (i) directly interact with ERK1 and/or ERK2, *e.g.*, by binding to ERK1/2 and (ii) decrease the expression or the activity of ERK1 and/or ERK2 protein kinases. Therefore, inhibitors that act upstream of ERK1/2, such as MEK inhibitors and RAF inhibitors, are not ERK1/2 inhibitors according to the present invention (but they are non-ERK MAPK inhibitors). Non-limiting examples of ERK1/2 inhibitors according to the present invention include AEZS-131 (Aeterna Zentaris), AEZS-136 (Aeterna Zentaris), BVD-523 (BioMed Valley Discoveries, Inc.), SCH-722984 (Merck & Co.), SCH-772984 (Merck & Co.), SCH-900353 (MK-8353) (Merck & Co.), pharmaceutically acceptable salts thereof, and combinations thereof.

[0050] An overview of the mammalian MAPK cascades is shown in FIG. 21. The MAPK pathway is reviewed in *e.g.*, Akinleye et al., 2013. Briefly, with respect to the ERK1/2 module in FIG. 21 (light purple box), the MAPK 1/2 signaling cascade is activated by ligand binding to receptor tyrosine kinases (RTK). The activated receptors recruit and phosphorylate adaptor proteins Grb2 and SOS, which then interact with membrane-bound

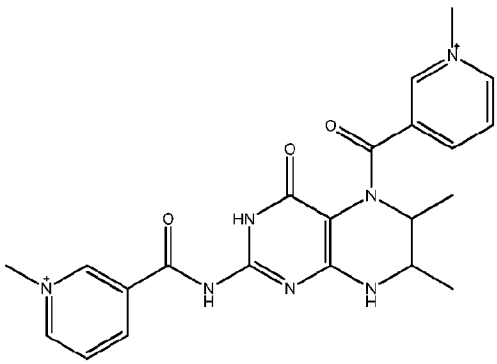
GTPase Ras and cause its activation. In its activated GTP-bound form, Ras recruits and activates RAF kinases (A-RAF, B-RAF, and C-RAF/RAF-1). The activated RAF kinases activate MAPK 1/2 (MKK1/2), which in turn catalyzes the phosphorylation of threonine and tyrosine residues in the activation sequence Thr-Glu-Tyr of ERK1/2. With respect to the JNK/p38 module (yellow box in FIG. 21), upstream kinases, MAP3Ks, such as MEKK1/4, ASK1/2, and MLK1/2/3, activate MAP2K3/6 (MKK3/6), MAP2K4 (MKK4), and MAP2K7 (MKK7). These MAP2K's then activate JNK protein kinases, including JNK1, JNK2, and JNK3, as well as p38 α/β/γ/δ. To execute their functions, JNKs activate several transcription factors, including c-Jun, ATF-2, NF-ATc1, HSF-1 and STAT3. With respect to the ERK5 module (blue box in FIG. 21), the kinases upstream of MAP2K5 (MKK5) are MEKK2 and MEKK3. The best characterized downstream target of MEK5 is ERK5, also known as big MAP kinase 1 (BMK1) because it is twice the size of other MAPKs.

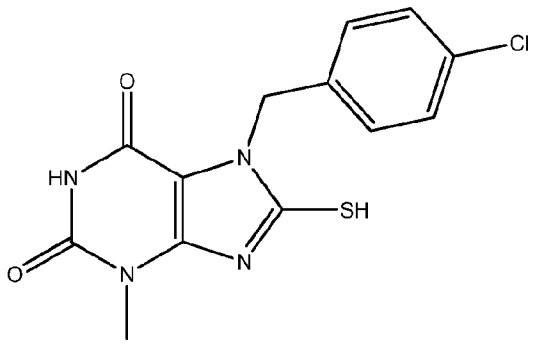
[0051] Non-limiting examples of non-ERK MAPK pathway inhibitors according to the present invention include RAS inhibitors, RAF inhibitors (such as, *e.g.*, inhibitors of A-RAF, B-RAF, C-RAF (RAF-1)), MEK inhibitors, and combinations thereof. Preferably, the non-ERK MAPK pathway inhibitors are BRAF inhibitors, MEK inhibitors, and combinations thereof.

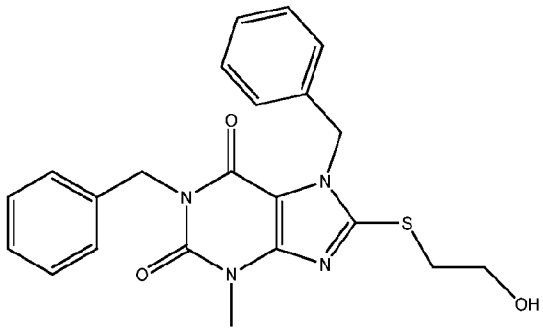
[0052] As used herein, a "RAS inhibitor" means those substances that (i) directly interact with RAS, *e.g.*, by binding to RAS and (ii) decrease the expression or the activity of RAS. Non-limiting exemplary RAS inhibitors include, but are not limited to, farnesyl transferase inhibitors (such as, *e.g.*, tipifarnib and lonafarnib), farnesyl group-containing small molecules (such as, *e.g.*, salirasib and TLN-4601), DCAI, as disclosed by Maurer (Maurer *et al.*,

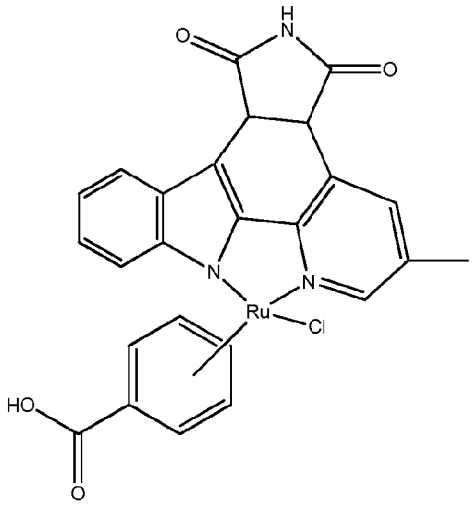
2012), Kobe0065 and and Kobe2602, as disclosed by Shima (Shima *et al.*, 2013), HBS 3 (Patgiri *et al.*, 2011), and AIK-4 (Allinky).

[0053] As used herein, a "RAF inhibitor" means those substances that (i) directly interact with RAF, *e.g.*, by binding to RAF and (ii) decrease the expression or the activity of RAF, such as, *e.g.*, A-RAF, B-RAF, and C-RAF (RAF-1). Non-limiting exemplary RAF inhibitors, including BRAF inhibitors, include:

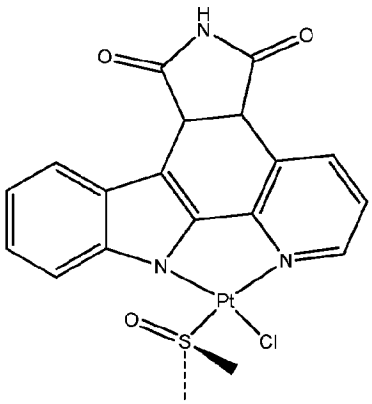
Compound 7  (Li *et al.*),

Compound 9  (*Id.*),

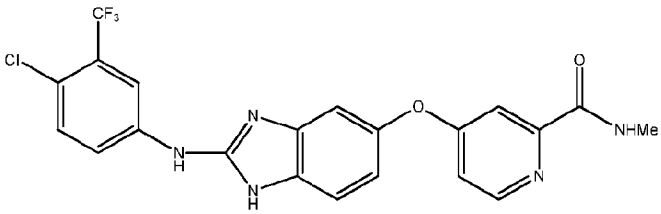
Compound 10  (*Id.*),



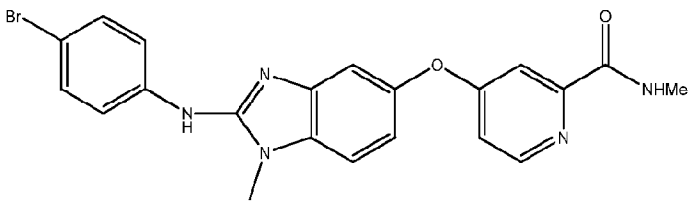
Compound 13 (*Id.*),



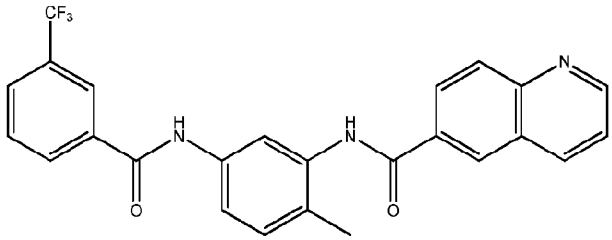
Compound 14 (*Id.*),

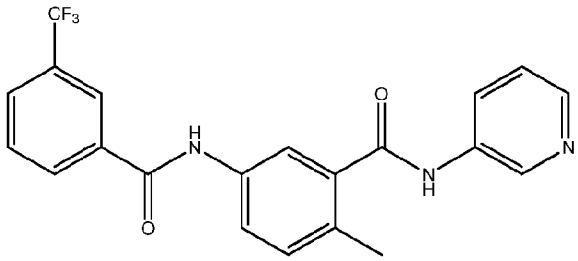


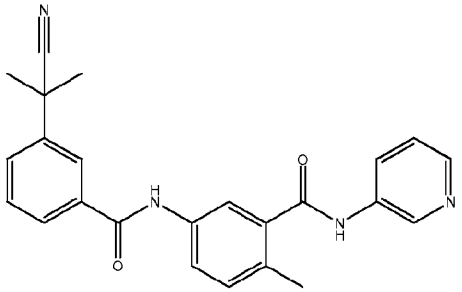
Compound 15 (*Id.*),

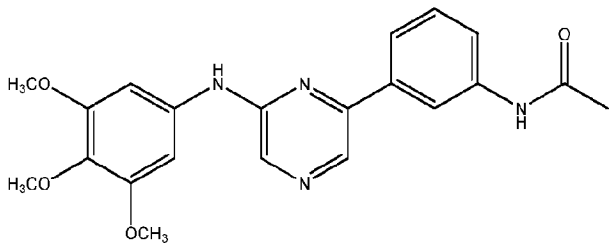


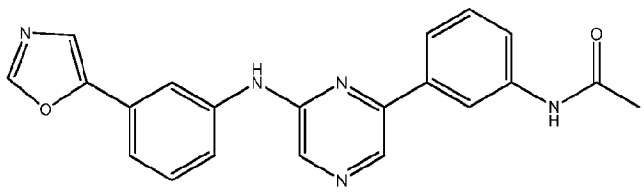
Compound 16 (*Id.*),

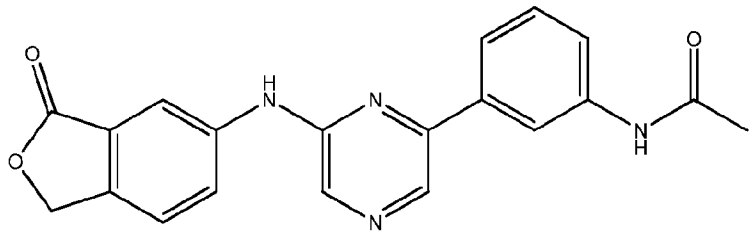
Compound 18  (*Id.*),

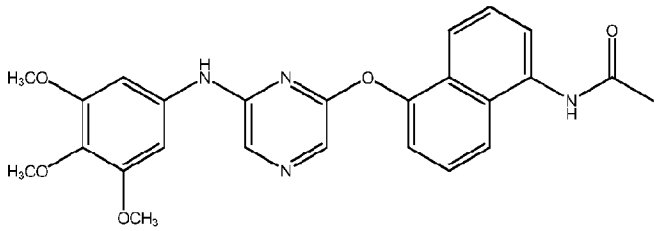
Compound 19  (*Id.*),

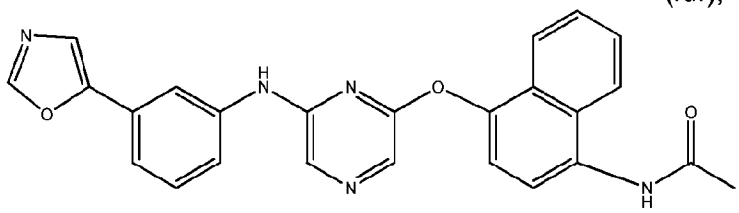
Compound 20  (*Id.*),

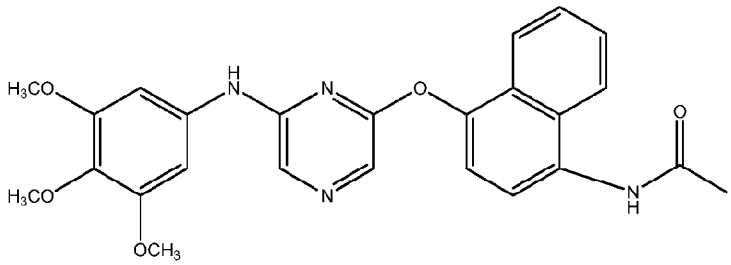
Compound 21  (*Id.*),

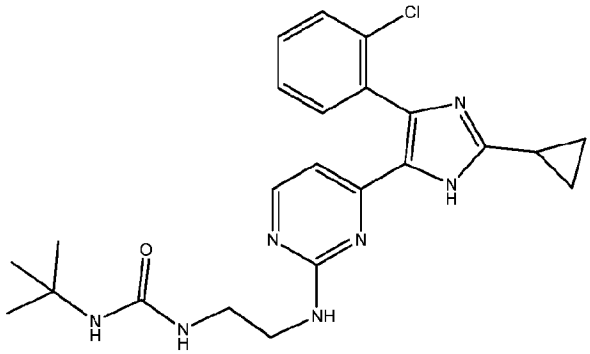
Compound 22  (*Id.*),

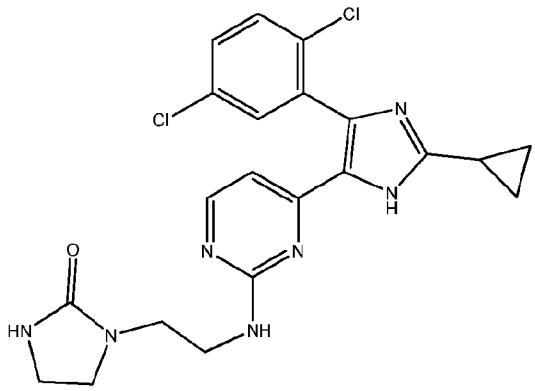
Compound 23 
(*Id.*),

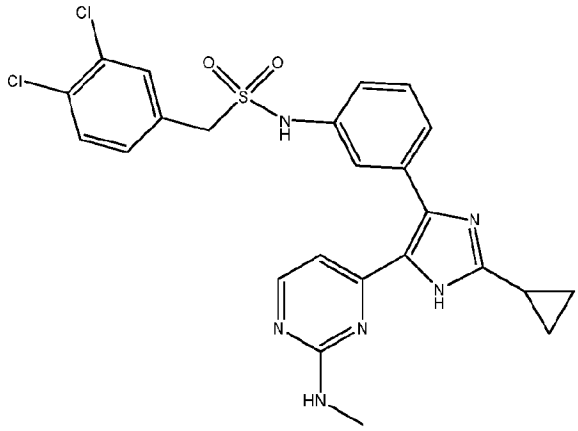
Compound 24  (*Id.*),

Compound 25  (*Id.*),

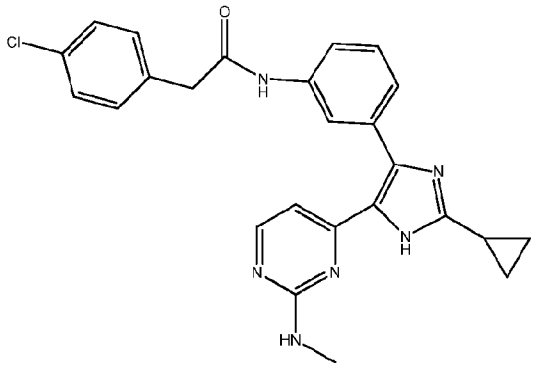
Compound 26  (*Id.*),

Compound 27  (*Id.*),

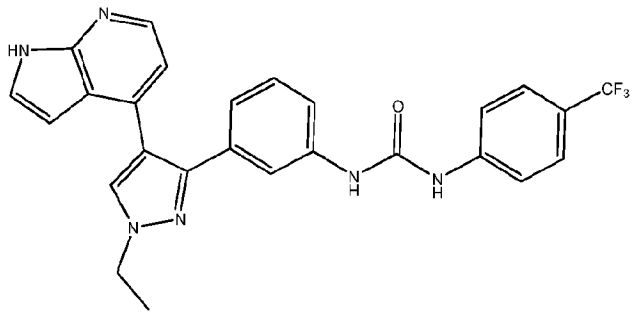
Compound 28  (*Id.*),



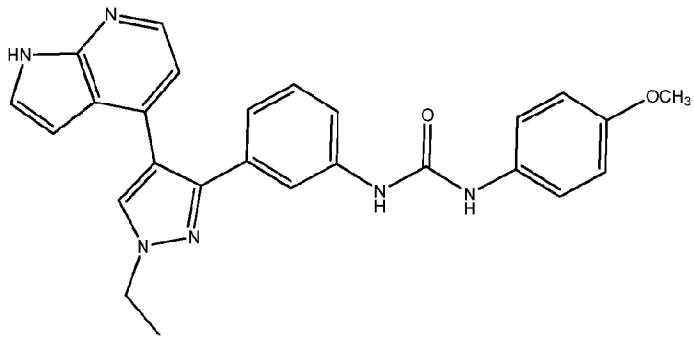
Compound 30 (*Id.*),



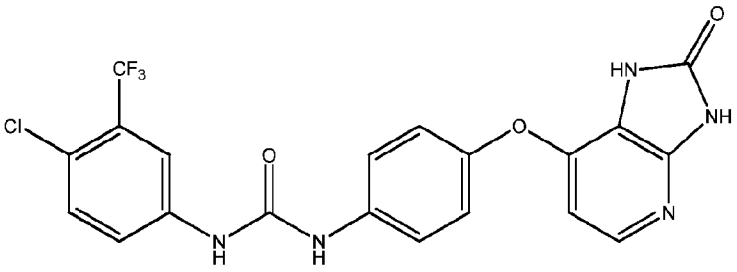
Compound 31 (*Id.*),

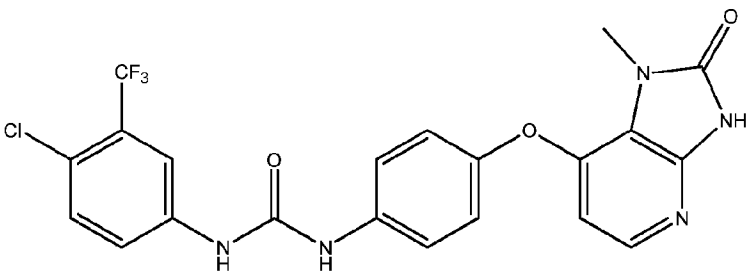


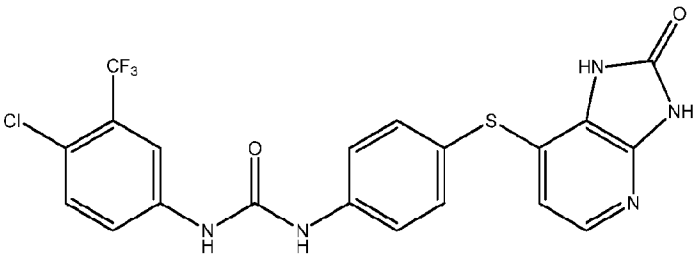
Compound 32 (*Id.*),

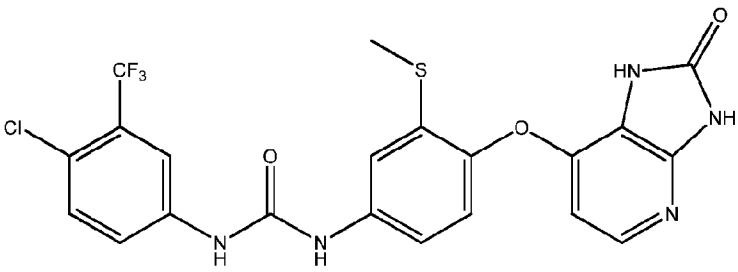


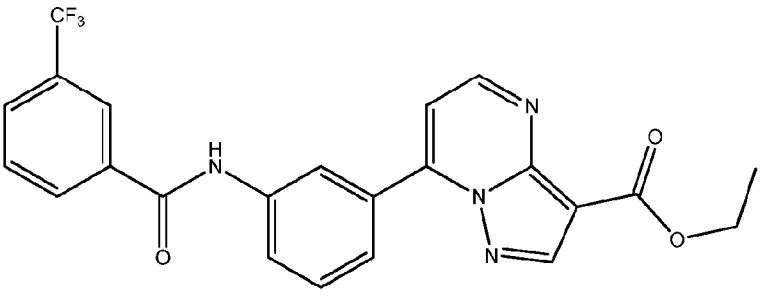
Compound 33 (*Id.*),

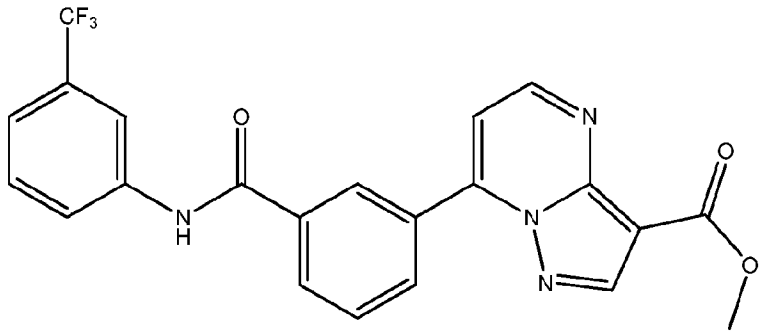
Compound 34  (*Id.*),

Compound 35 
(*Id.*),

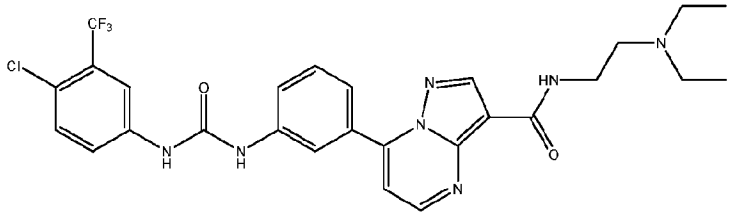
Compound 36  (*Id.*),

Compound 37  (*Id.*),

Compound 38 
(*Id.*),



Compound 39 (*Id.*),



Compound 40 (*Id.*),

AAL881 (Novartis); AB-024 (Ambit Biosciences), ARQ-736 (ArQule), ARQ-761 (ArQule), AZ628 (Axon Medchem BV), BeiGene-283 (BeiGene), BIIB-024 (MLN 2480) (Sunesis & Takeda), b-raf inhibitor (Sareum), BRAF kinase inhibitor (Selexagen Therapeutics), BRAF siRNA 313 (tacaccagcaagctagatgca) and 523 (cctatcgttagagtcttcctg) (Liu *et al.*, 2007), CTT239065 (Institute of Cancer Research), dabrafenib (GSK2118436), DP-4978 (Deciphera Pharmaceuticals), HM-95573 (Hanmi), GDC-0879 (Genentech), GW-5074 (Sigma Aldrich), ISIS 5132 (Novartis), L779450 (Merck), LBT613 (Novartis), LErafAON (NeoPharm, Inc.), LGX-818 (Novartis), pazopanib (GlaxoSmithKline), PLX3202 (Plexxikon), PLX4720 (Plexxikon), PLX5568 (Plexxikon), RAF-265 (Novartis), RAF-365 (Novartis), regorafenib (Bayer Healthcare Pharmaceuticals, Inc.), RO 5126766 (Hoffmann-La Roche), SB-590885 (GlaxoSmithKline), SB699393 (GlaxoSmithKline), sorafenib (Onyx Pharmaceuticals), TAK 632 (Takeda), TL-241 (Teligene), vemurafenib (RG7204 or PLX4032) (Daiichi Sankyo), XL-281 (Exelixis), ZM-

336372 (AstraZeneca), pharmaceutically acceptable salts thereof, and combinations thereof.

[0054] As used herein, a "MEK inhibitor" means those substances that (i) directly interact with MEK, *e.g.*, by binding to MEK and (ii) decrease the expression or the activity of MEK. Thus, inhibitors that act upstream of MEK, such as RAS inhibitors and RAF inhibitors, are not MEF inhibitors according to the present invention. Non-limiting examples of MEK inhibitors include anthrax toxin, antroquinonol (Golden Biotechnology), ARRY-142886 (6-(4-bromo-2-chloro-phenylamino)-7-fluoro-3-methyl-3H-benzoimidazole-5-carboxylic acid (2-hydroxy-ethoxy)-amide) (Array BioPharma), ARRY-438162 (Array BioPharma), AS-1940477 (Astellas), AS-703988 (Merck KGaA), bentamapimod (Merck KGaA), BI-847325 (Boehringer Ingelheim), E-6201 (Eisai), GDC-0623 (Hoffmann-La Roche), GDC-0973 (cobimetinib) (Hoffmann-La Roche), L783277 (Merck), lethal factor portion of anthrax toxin, MEK162 (Array BioPharma), PD 098059 (2-(2'-amino-3'-methoxyphenyl)-oxanaphthalen-4-one) (Pfizer), PD 184352 (CI-1040) (Pfizer), PD-0325901 (Pfizer), pimasertib (Santhera Pharmaceuticals), RDEA119 (Ardea Biosciences/Bayer), refametinib (AstraZeneca), RG422 (Chugai Pharmaceutical Co.), RO092210 (Roche), RO4987655 (Hoffmann-La Roche), RO5126766 (Hoffmann-La Roche), selumetinib (AZD6244) (AstraZeneca), SL327 (Sigma), TAK-733 (Takeda), trametinib (Japan Tobacco), U0126 (1,4-diamino-2,3-dicyano-1,4-bis(2-aminophenylthio)butadiene) (Sigma), WX-554 (Wilex), YopJ polypeptide (Mittal *et al.*, 2010), pharmaceutically acceptable salts thereof, and combinations thereof.

[0055] In one aspect of this embodiment, substantially all phosphorylation of ribosomal s6 kinase (RSK) is inhibited after administration of BVD-523 or a pharmaceutically acceptable salt thereof. As used herein in the context of RSK phosphorylation, "substantially all" means a reduction of greater than 50% reduction, preferably greater than 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% reduction.

[0056] In another aspect of this embodiment, the cancer has MAPK activity. As used herein, having "MAPK activity" means that proteins downstream of ERK are still active, even if proteins upstream of ERK may not be active. Such a cancer may be a solid tumor cancer or a hematologic cancer.

[0057] In the present invention, cancers include both solid and hemotologic cancers. Non-limiting examples of solid cancers include adrenocortical carcinoma, anal cancer, bladder cancer, bone cancer (such as osteosarcoma), brain cancer, breast cancer, carcinoid cancer, carcinoma, cervical cancer, colon cancer, endometrial cancer, esophageal cancer, extrahepatic bile duct cancer, Ewing family of cancers, extracranial germ cell cancer, eye cancer, gallbladder cancer, gastric cancer, germ cell tumor, gestational trophoblastic tumor, head and neck cancer, hypopharyngeal cancer, islet cell carcinoma, kidney cancer, large intestine cancer, laryngeal cancer, leukemia, lip and oral cavity cancer, liver tumor/cancer, lung tumor/cancer, lymphoma, malignant mesothelioma, Merkel cell carcinoma, mycosis fungoides, myelodysplastic syndrome, myeloproliferative disorders, nasopharyngeal cancer, neuroblastoma, oral cancer, oropharyngeal cancer,

osteosarcoma, ovarian epithelial cancer, ovarian germ cell cancer, pancreatic cancer, paranasal sinus and nasal cavity cancer, parathyroid cancer, penile cancer, pituitary cancer, plasma cell neoplasm, prostate cancer, rhabdomyosarcoma, rectal cancer, renal cell cancer, transitional cell cancer of the renal pelvis and ureter, salivary gland cancer, Sezary syndrome, skin cancers (such as cutaneous t-cell lymphoma, Kaposi's sarcoma, mast cell tumor, and melanoma), small intestine cancer, soft tissue sarcoma, stomach cancer, testicular cancer, thymoma, thyroid cancer, urethral cancer, uterine cancer, vaginal cancer, vulvar cancer, and Wilms' tumor.

[0058] Examples of hematologic cancers include, but are not limited to, leukemias, such as adult/childhood acute lymphoblastic leukemia, adult/childhood acute myeloid leukemia, chronic lymphocytic leukemia, chronic myelogenous leukemia, and hairy cell leukemia, lymphomas, such as AIDS-related lymphoma, cutaneous T-cell lymphoma, adult/childhood Hodgkin lymphoma, mycosis fungoides, adult/childhood non-Hodgkin lymphoma, primary central nervous system lymphoma, Sézary syndrome, cutaneous T-cell lymphoma, and Waldenstrom macroglobulinemia, as well as other proliferative disorders such as chronic myeloproliferative disorders, Langerhans cell histiocytosis, multiple myeloma/plasma cell neoplasm, myelodysplastic syndromes, and myelodysplastic/myeloproliferative neoplasms.

[0059] Preferably, the cancer is selected from the group consisting of a cancer of the large intestine, breast cancer, pancreatic cancer, skin cancer, and endometrial cancers. More preferably, the cancer is melanoma.

[0060] In another aspect of this embodiment, the method further comprises administering to the subject at least one additional therapeutic agent effective for treating or ameliorating the effects of the cancer. The additional therapeutic agent may be selected from the group consisting of an antibody or fragment thereof, a cytotoxic agent, a toxin, a radionuclide, an immunomodulator, a photoactive therapeutic agent, a radiosensitizing agent, a hormone, an anti-angiogenesis agent, and combinations thereof.

[0061] As used herein, an "antibody" encompasses naturally occurring immunoglobulins as well as non-naturally occurring immunoglobulins, including, for example, single chain antibodies, chimeric antibodies (*e.g.*, humanized murine antibodies), and heteroconjugate antibodies (*e.g.*, bispecific antibodies). Fragments of antibodies include those that bind antigen, (*e.g.*, Fab', $F(ab')_2$, Fab, Fv, and rIgG). See also, *e.g.*, Pierce Catalog and Handbook, 1994-1995 (Pierce Chemical Co., Rockford, Ill.); Kuby, J., Immunology, 3rd Ed., W.H. Freeman & Co., New York (1998). The term antibody also includes bivalent or bispecific molecules, diabodies, triabodies, and tetrabodies. The term "antibody" further includes both polyclonal and monoclonal antibodies.

[0062] Examples of therapeutic antibodies that may be used in the present invention include rituximab (Rituxan), Cetuximab (Erbitux), bevacizumab (Avastin), and Ibritumomab (Zevalin).

[0063] Cytotoxic agents according to the present invention include DNA damaging agents, antimetabolites, anti-microtubule agents, antibiotic agents, etc. DNA damaging agents include alkylating agents, platinum-based agents, intercalating agents, and inhibitors of DNA replication. Non-limiting examples

of DNA alkylating agents include cyclophosphamide, mechlorethamine, uramustine, melphalan, chlorambucil, ifosfamide, carmustine, lomustine, streptozocin, busulfan, temozolomide, pharmaceutically acceptable salts thereof, prodrugs, and combinations thereof. Non-limiting examples of platinum-based agents include cisplatin, carboplatin, oxaliplatin, nedaplatin, satraplatin, triplatin tetranitrate, pharmaceutically acceptable salts thereof, prodrugs, and combinations thereof. Non-limiting examples of intercalating agents include doxorubicin, daunorubicin, idarubicin, mitoxantrone, pharmaceutically acceptable salts thereof, prodrugs, and combinations thereof. Non-limiting examples of inhibitors of DNA replication include irinotecan, topotecan, amsacrine, etoposide, etoposide phosphate, teniposide, pharmaceutically acceptable salts thereof, prodrugs, and combinations thereof. Antimetabolites include folate antagonists such as methotrexate and premetrexed, purine antagonists such as 6-mercaptopurine, dacarbazine, and fludarabine, and pyrimidine antagonists such as 5-fluorouracil, arabinosylcytosine, capecitabine, gemcitabine, decitabine, pharmaceutically acceptable salts thereof, prodrugs, and combinations thereof. Anti-microtubule agents include without limitation vinca alkaloids, paclitaxel (Taxol®), docetaxel (Taxotere®), and ixabepilone (Ixempra®). Antibiotic agents include without limitation actinomycin, anthracyclines, valrubicin, epirubicin, bleomycin, plicamycin, mitomycin, pharmaceutically acceptable salts thereof, prodrugs, and combinations thereof.

[0064] Cytotoxic agents according to the present invention also include an inhibitor of the PI3K/Akt pathway. Non-limiting examples of an inhibitor of the PI3K/Akt pathway include A-674563 (CAS # 552325-73-2), AGL 2263,

AMG-319 (Amgen, Thousand Oaks, CA), AS-041164 (5-benzo[1,3]dioxol-5-ylmethylene-thiazolidine-2,4-dione), AS-604850 (5-(2,2-Difluoro-benzo[1,3]dioxol-5-ylmethylene)-thiazolidine-2,4-dione), AS-605240 (5-quinoxilin-6-methylene-1,3-thiazolidine-2,4-dione), AT7867 (CAS # 857531-00-1), benzimidazole series, Genentech (Roche Holdings Inc., South San Francisco, CA), BML-257 (CAS # 32387-96-5), CAL-120 (Gilead Sciences, Foster City, CA), CAL-129 (Gilead Sciences), CAL-130 (Gilead Sciences), CAL-253 (Gilead Sciences), CAL-263 (Gilead Sciences), CAS # 612847-09-3, CAS # 681281-88-9, CAS # 75747-14-7, CAS # 925681-41-0, CAS # 98510-80-6, CCT128930 (CAS # 885499-61-6), CH5132799 (CAS # 1007207-67-1), CHR-4432 (Chroma Therapeutics, Ltd., Abingdon, UK), FPA 124 (CAS # 902779-59-3), GS-1101 (CAL-101) (Gilead Sciences), GSK 690693 (CAS # 937174-76-0), H-89 (CAS # 127243-85-0), Honokiol, IC87114 (Gilead Science), IPI-145 (Intellikine Inc.), KAR-4139 (Karus Therapeutics, Chilworth, UK), KAR-4141 (Karus Therapeutics), KIN-1 (Karus Therapeutics), KT 5720 (CAS # 108068-98-0), Miltefosine, MK-2206 dihydrochloride (CAS # 1032350-13-2), ML-9 (CAS # 105637-50-1), Naltrindole Hydrochloride, OXY-111A (NormOxys Inc., Brighton, MA), perifosine, PHT-427 (CAS # 1191951-57-1), PI3 kinase delta inhibitor, Merck KGaA (Merck & Co., Whitehouse Station, NJ), PI3 kinase delta inhibitors, Genentech (Roche Holdings Inc.), PI3 kinase delta inhibitors, Incozen (Incozen Therapeutics, Pvt. Ltd., Hydrabad, India), PI3 kinase delta inhibitors-2, Incozen (Incozen Therapeutics), PI3 kinase inhibitor, Roche-4 (Roche Holdings Inc.), PI3 kinase inhibitors, Roche (Roche Holdings Inc.), PI3 kinase inhibitors, Roche-5 (Roche Holdings Inc.), PI3-alpha/delta inhibitors, Pathway Therapeutics (Pathway Therapeutics Ltd.,

South San Francisco, CA), PI3-delta inhibitors, Cellzome (Cellzome AG, Heidelberg, Germany), PI3-delta inhibitors, Intellikine (Intellikine Inc., La Jolla, CA), PI3-delta inhibitors, Pathway Therapeutics-1 (Pathway Therapeutics Ltd.), PI3-delta inhibitors, Pathway Therapeutics-2 (Pathway Therapeutics Ltd.), PI3-delta/gamma inhibitors, Cellzome (Cellzome AG), PI3-delta/gamma inhibitors, Cellzome (Cellzome AG), PI3-delta/gamma inhibitors, Intellikine (Intellikine Inc.), PI3-delta/gamma inhibitors, Intellikine (Intellikine Inc.), PI3-delta/gamma inhibitors, Pathway Therapeutics (Pathway Therapeutics Ltd.), PI3-delta/gamma inhibitors, Pathway Therapeutics (Pathway Therapeutics Ltd.), PI3-gamma inhibitor Evotec (Evotec), PI3-gamma inhibitor, Cellzome (Cellzome AG), PI3-gamma inhibitors, Pathway Therapeutics (Pathway Therapeutics Ltd.), PI3K delta/gamma inhibitors, Intellikine-1 (Intellikine Inc.), PI3K delta/gamma inhibitors, Intellikine-1 (Intellikine Inc.), pictilisib (Roche Holdings Inc.), PIK-90 (CAS # 677338-12-4), SC-103980 (Pfizer, New York, NY), SF-1126 (Semafore Pharmaceuticals, Indianapolis, IN), SH-5, SH-6, Tetrahydro Curcumin, TG100-115 (Targegen Inc., San Diego, CA), Triciribine, X-339 (Xcovery, West Palm Beach, FL), XL-499 (Evotech, Hamburg, Germany), pharmaceutically acceptable salts thereof, and combinations thereof.

[0065] In the present invention, the term “toxin” means an antigenic poison or venom of plant or animal origin. An example is diphtheria toxin or portions thereof.

[0066] In the present invention, the term “radionuclide” means a radioactive substance administered to the patient, *e.g.*, intravenously or orally, after which it penetrates via the patient’s normal metabolism into the target

organ or tissue, where it delivers local radiation for a short time. Examples of radionuclides include, but are not limited to, I-125, At-211, Lu-177, Cu-67, I-131, Sm-153, Re-186, P-32, Re-188, In-114m, and Y-90.

[0067] In the present invention, the term "immunomodulator" means a substance that alters the immune response by augmenting or reducing the ability of the immune system to produce antibodies or sensitized cells that recognize and react with the antigen that initiated their production. Immunomodulators may be recombinant, synthetic, or natural preparations and include cytokines, corticosteroids, cytotoxic agents, thymosin, and immunoglobulins. Some immunomodulators are naturally present in the body, and certain of these are available in pharmacologic preparations. Examples of immunomodulators include, but are not limited to, granulocyte colony-stimulating factor (G-CSF), interferons, imiquimod and cellular membrane fractions from bacteria, IL-2, IL-7, IL-12, CCL3, CCL26, CXCL7, and synthetic cytosine phosphate-guanosine (CpG).

[0068] In the present invention, the term "photoactive therapeutic agent" means compounds and compositions that become active upon exposure to light. Certain examples of photoactive therapeutic agents are disclosed, e.g., in U.S. Patent Application Serial No. 2011/0152230 A1, "Photoactive Metal Nitrosyls For Blood Pressure Regulation And Cancer Therapy."

[0069] In the present invention, the term "radiosensitizing agent" means a compound that makes tumor cells more sensitive to radiation therapy. Examples of radiosensitizing agents include misonidazole, metronidazole, tirapazamine, and trans sodium crocetinate.

[0070] In the present invention, the term "hormone" means a substance released by cells in one part of a body that affects cells in another part of the body. Examples of hormones include, but are not limited to, prostaglandins, leukotrienes, prostacyclin, thromboxane, amylin, antimullerian hormone, adiponectin, adrenocorticotropic hormone, angiotensinogen, angiotensin, vasopressin, atriopeptin, brain natriuretic peptide, calcitonin, cholecystokinin, corticotropin-releasing hormone, encephalin, endothelin, erythropoietin, follicle-stimulating hormone, galanin, gastrin, ghrelin, glucagon, gonadotropin-releasing hormone, growth hormone-releasing hormone, human chorionic gonadotropin, human placental lactogen, growth hormone, inhibin, insulin, somatomedin, leptin, liptropin, luteinizing hormone, melanocyte stimulating hormone, motilin, orexin, oxytocin, pancreatic polypeptide, parathyroid hormone, prolactin, prolactin releasing hormone, relaxin, renin, secretin, somatostain, thrombopoietin, thyroid-stimulating hormone, testosterone, dehydroepiandrosterone, androstenedione, dihydrotestosterone, aldosterone, estradiol, estrone, estriol, cortisol, progesterone, calcitriol, and calcidiol.

[0071] Some compounds interfere with the activity of certain hormones or stop the production of certain hormones. These hormone-interfering compounds include, but are not limited to, tamoxifen (Nolvadex®), anastrozole (Arimidex®), letrozole (Femara®), and fulvestrant (Faslodex®). Such compounds are also within the meaning of hormone in the present invention.

[0072] As used herein, an "anti-angiogenesis" agent means a substance that reduces or inhibits the growth of new blood vessels, such as, *e.g.*, an inhibitor of vascular endothelial growth factor (VEGF) and an inhibitor

of endothelial cell migration. Anti-angiogenesis agents include without limitation 2-methoxyestradiol, angiostatin, bevacizumab, cartilage-derived angiogenesis inhibitory factor, endostatin, IFN-α, IL-12, itraconazole, linomide, platelet factor-4, prolactin, SU5416, suramin, tasquinimod, tecogalan, tetrathiomolybdate, thalidomide, thrombospondin, thrombospondin, TNP-470, ziv-aflibercept, pharmaceutically acceptable salts thereof, prodrugs, and combinations thereof.

[0073] Another embodiment of the present invention is a method for treating or ameliorating the effects of a cancer in a subject. The method comprises:

(a) identifying a subject with cancer that has become refractory or resistant to BRAF inhibitor therapy, MEK inhibitor therapy, or BRAF and MEK inhibitor therapy; and

(b) administering to the subject with said refractory or resistant cancer an effective amount of an ERK inhibitor, which is BVD-523 or a pharmaceutically acceptable salt thereof.

[0074] Suitable and preferred subjects are as disclosed herein. In this embodiment, the methods may be used to treat the cancers disclosed above. In accordance with the present invention, the cancer may have MAPK activity.

[0075] In one aspect of this embodiment, identifying a subject with cancer that is refractory or resistant to BRAF and/or MEK inhibitor therapy comprises:

(a) obtaining a biological sample from the subject; and

(b) screening the sample to determine whether the subject has become resistant to an inhibitor therapy selected from the group consisting of BRAF inhibitor therapy, MEK inhibitor therapy, and combinations thereof.

[0076] In the present invention, biological samples include, but are not limited to, blood, plasma, urine, skin, saliva, and biopsies. Biological samples are obtained from a subject by routine procedures and methods which are known in the art.

[0077] Preferably, screening for a cancer that is refractory or resistant to BRAF inhibitor therapy may comprise, *e.g.*, identifying (i) a switch between RAF isoforms, (ii) upregulation of RTK or NRAS signaling, (iii) reactivation of mitogen activated protein kinase (MAPK) signaling, (iv) the presence of a MEK activating mutation, and combinations thereof.

[0078] A switch between RAF isoforms may occur in subjects having acquired resistance to BRAF inhibitor therapy. To detect such a switch, BRAF inhibitor-resistant tumor cells may be retrieved from a patient and analyzed via Western blotting for ERK and phospho-ERK levels in the presence of a BRAF inhibitor. Comparison with BRAF inhibitor-sensitive cells treated with a BRAF inhibitor may reveal higher levels of phospho-ERK in BRAF inhibitor-resistant tumor cells, implying that a switch has taken place in which another RAF isoform phosphorylates ERK in place of BRAF. Confirmation of which RAF isoform has taken over may involve sh/siRNA-mediated knockdown of ARAF and CRAF individually in BRAF inhibitor-resistant cells exposed to a BRAF inhibitor, followed by subsequent Western blotting for ERK and phospho-ERK levels. If, for example, ARAF knockdown

in BRAF inhibitor-resistant cells exposed to a BRAF inhibitor still results in high levels of phospho-ERK, it would indicate that CRAF has taken over phosphorylating ERK. Likewise, if CRAF was knocked down in BRAF inhibitor-resistant cells exposed to BRAF inhibitor and ERK was still highly phosphorylated, it would mean that ARAF has taken over ERK phosphorylation. RAF isoform switching may also involve simultaneous knockdown of ARAF and CRAF in BRAF inhibitor-resistant cells in the presence of BRAF inhibitor, effectively blocking all RAF-mediated phosphorylation. A resulting decrease in ERK phosphorylation would indicate that the BRAF inhibitor-resistant cells have the capacity to switch between RAF isoforms in order to phosphorylate ERK (Villanueva, et al., 2010).

[0079] Upregulation of RTK or NRAS signaling may also be a cause of BRAF inhibitor resistance. Detection may, *e.g.*, first involve using Western blotting protocols with phospho-specific antibodies to analyze the activation of the downstream RAF effectors MEK1/2 and ERK1/2. If BRAF inhibitor-resistant cells show high activation levels of these proteins in the presence of a BRAF inhibitor, RTK or NRAS upregulation may be the cause. Gene expression profiling (or other related methods) of BRAF inhibitor-resistant cells in the presence of a BRAF inhibitor may reveal higher expression levels of KIT, MET, EGFR, and PDGFRβ RTKs as compared to BRAF inhibitor-sensitive cells. Real-time quantitative polymerase chain reaction experiments, or other similar procedures, focusing on any of these genes may confirm higher expression levels while phospho-RTK arrays (R&D Systems, Minneapolis, MN) may show elevated activation-associated tyrosine phosphorylation. Alternatively, NRAS activation may be detected by various

gene sequencing protocols. Activating mutations in NRAS, particularly Q61K, may indicate that B-RAF signaling has been bypassed. In melanoma cells, activated NRAS uses C-RAF to signal to MEK-ERK. Thus, activated NRAS may enable a similar bypass pathway in BRAF inhibitor-resistant cells exposed to BRAF inhibitor. Further confirmation of these mechanisms in a given BRAF inhibitor-resistant sample may be accomplished, for example, using sh/siRNA-mediated knockdown of upregulated RTKs or activated NRAS in the presence of BRAF inhibitor. Any significant levels of growth inhibition may indicate that upregulation of RTK or NRAS signaling is the cause of BRAF inhibition in that particular sample (Nazarian, et al., 2010).

[0080] Detecting reactivation of MAPK signaling in BRAF inhibitor-resistant cells may indicate another bypass mechanism for BRAF inhibitor resistance. COT and C-RAF have been shown to be upregulated in a BRAF V600E background exposed to BRAF inhibitor. Quantiative real-time RT-PCR, *e.g.*, may reveal increased COT expression in BRAF inhibitor-resistant cells in the presence of BRAF inhibitor. Furthermore, sh/siRNA-mediated knockdown of COT in BRAF inhibitor-resistant cells in the presence of BRAF inhibitor may reduce the viability of BRAF inhibitor-resistant cells, indicating that these particular cells may be sensitive to COT inhibition and/or combination BRAF inhibitor/MEK inhibitor treatments (Johannessen, et al., 2010).

[0081] Reactivation of MAPK signaling may also be accomplished in a BRAF inhibitor-resistant background by activating mutations in MEK1. Targeted, massively parallel sequencing of genomic DNA from a BRAF inhibitor-resistant tumor may reveal activating mutations in MEK1, such as

C121S, G128D, N122D, and Y130, among others. Other, undocumented mutations in MEK1 may be analyzed by, for example, expressing the particular mutation in a BRAF inhibitor-sensitive cell line such as A375. Determining levels of growth inhibition in these cells upon exposure to BRAF inhibitor may indicate if the MEK1 mutation is causing resistance to BRAF inhibitory therapy. To confirm such a finding, Western blotting for elevated levels of phospho-ERK1/2 in cells ectopically expressing the MEK1 mutation may indicate that the MEK1 mutation is allowing the BRAF inhibitor-resistant tumor to bypass BRAF and promote phosphorylation of ERK through MEK1 (Wagle, et al., 2011).

[0082] In accordance with the present invention, screening for a cancer that is refractory or resistant to MEK inhibitor therapy may comprise, *e.g.*, identifying (i) amplification of mutant BRAF, (ii) STAT3 upregulation, (iii) mutations in the allosteric pocket of MEK that directly block binding of inhibitors to MEK or lead to constitutive MEK activity, and combinations thereof.

[0083] Amplification of mutant BRAF may cause MEK inhibitor resistance. MEK inhibitor resistance is typically associated with high levels of phosphorylated ERK and MEK in the presence of a MEK inhibitor, which may be assessed via, for example, Western blotting. Amplification of mutant BRAF in MEK inhibitor-resistant cell lines may be detected by, for example, fluorescence in situ hybridization (FISH) or quantitative PCR from genomic DNA of the resistant cell lines. Confirmation that BRAF amplification is a primary cause of MEK inhibitor resistance may entail using BRAF-targeted sh/siRNAs in resistant cells. If a significant decrease in MEK or ERK

phosphorylation is observed, BRAF amplification may be a suitable target for further therapeutic approaches. (Corcoran, et al., 2010).

[0084] Identifying STAT3 upregulation may indicate that a particular tumor sample is resistant to MEK inhibitor therapy. Genome-wide expression profiling may reveal the STAT3 pathway to be upregulated in a tumor. Other techniques, such as Western blotting for phospho-STAT3 and real-time qPCR for the STAT pathway-associated genes JAK1 and IL6ST may reveal upregulated STAT3. Further confirmation that STAT3 upregulation causes MEK inhibitor resistance in a particular sample may comprise the use of sh/siRNAs against STAT3 in the sample followed by appropriate Western blotting for MEK and ERK activation as well as phospho-STAT3 and total STAT3. Growth inhibition studies may show that STAT3 knockdown sensitizes previously MEK inhibitor-resistant cells to MEK inhibition. A similar effect may be seen if the sample were exposed to a STAT3 inhibitor such as JSI-124. Additional confirmation that STAT3 upregulation is the cause of MEK inhibitor resistance in a particular tumor could arise from Western blotting for BIM expression, including BIM-EL, BIM-L, and BIM-SL. BIM expression leads to MEK inhibitor-induced apoptosis, thus STAT3 upregulation may lower BIM levels. STAT3 is known to regulate the expression of miR 17-92, which suppresses BIM expression. Upregulated STAT3 may lead to higher levels of miR 17-92, which will lower BIM levels and promote resistance to MEK inhibition. Thus, real-time qPCR of miR 17-92 levels may also assist in assessing whether STAT3 upregulation is causing MEK inhibition resistance in a particular sample. (Dai, et al., 2011).

[0085] Mutations in the allosteric pocket of MEK that can directly block binding of inhibitors to MEK or lead to constitutive MEK activity may be detected by methods disclosed below. Such mutations have been identified previously by Emery and colleagues (Emery, et al., 2009) as well as Wang and colleagues (Wang et al., 2011). Other mutations may affect MEK1 codons located within or abutting the N-terminal negative regulatory helix, such as P124L and Q56P. (*Id.*).

[0086] Methods for identifying mutations in nucleic acids, such as the above identified MEK genes, are known in the art. Nucleic acids may be obtained from biological samples. In the present invention, biological samples include, but are not limited to, blood, plasma, urine, skin, saliva, and biopsies. Biological samples are obtained from a subject by routine procedures and methods which are known in the art.

[0087] Non-limiting examples of methods for identifying mutations include PCR, sequencing, hybrid capture, in-solution capture, molecular inversion probes, fluorescent in situ hybridization (FISH) assays, and combinations thereof.

[0088] Various sequencing methods are known in the art. These include, but are not limited to, Sanger sequencing (also referred to as dideoxy sequencing) and various sequencing-by-synthesis (SBS) methods as disclosed in, *e.g.*, Metzker 2005, sequencing by hybridization, by ligation (for example, WO 2005021786), by degradation (for example, U.S. Patent Nos. 5,622,824 and 6,140,053) and nanopore sequencing (which is commercially available from Oxford Nanopore Technologies, UK). In deep sequencing techniques, a given nucleotide in the sequence is read more than once during

the sequencing process. Deep sequencing techniques are disclosed in *e.g.*, U.S. Patent Publication No. 20120264632 and International Patent Publication No. WO2012125848.

[0089] PCR-based methods for detecting mutations are known in the art and employ PCR amplification, where each target sequence in the sample has a corresponding pair of unique, sequence-specific primers. For example, the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method allows for rapid detection of mutations after the genomic sequences are amplified by PCR. The mutation is discriminated by digestion with specific restriction endonucleases and is identified by electrophoresis. *See, e.g.*, Ota et al., 2007. Mutations may also be detected using real time PCR. *See, e.g.*, International Application publication No. WO2012046981.

[0090] Hybrid capture methods are known in the art and are disclosed in *e.g.*, U.S. Patent Publication No. 20130203632 and U.S. Patent Nos. 8,389,219 and 8,288,520. These methods are based on the selective hybridization of the target genomic regions to user-designed oligonucleotides. The hybridization can be to oligonucleotides immobilized on high or low density microarrays (on-array capture), or solution-phase hybridization to oligonucleotides modified with a ligand (e.g. biotin) which can subsequently be immobilized to a solid surface, such as a bead (in-solution capture).

[0091] Molecular Inversion Probe (MIP) techniques are known in the art and are disclosed in *e.g.*, Absalan et al., 2008. This method uses MIP molecules, which are special "padlock" probes (Nilsson et al, 1994) for genotyping. A MIP molecule is a linear oligonucleotide that contains specific regions, universal sequences, restriction sites and a Tag (index) sequence

(16-22 bp). A MIP hybridizes directly around the genetic marker/SNP of interest. The MIP method may also use a number of "padlock" probe sets that hybridize to genomic DNA in parallel (Hardenbol et al., 2003). In case of a perfect match, genomic homology regions are ligated by undergoing an inversion in configuration (as suggested by the name of the technique) and creating a circular molecule. After the first restriction, all molecules are amplified with universal primers. Amplicons are restricted again to ensure short fragments for hybridization on a microarray. Generated short fragments are labeled and, through a Tag sequence, hybridized to a cTag (complementary strand for index) on an array. After the formation of Tag-cTag duplex, a signal is detected.

[0092] The following Tables 1, 2, and 3 show the SEQ ID Nos. of representative nucleic acid and amino acid sequences of wild type BRAF, N-RAS, and MEK1 from various animals in the sequence listing. These sequences may be used in methods for identifying subjects with mutant BRAF, N-RAS, and MEK1 genotypes.

Table 1 – BRAF sequences

SEQ ID NO.	polypeptide or nucleic acid sequence	Organism	Other information
1	nucleic acid	human	
2	polypeptide	human	
3	nucleic acid	rat (Rattus norvegicus)	
4	polypeptide	rat (Rattus norvegicus)	
5	nucleic acid	mouse, Mus musculus	
6	polypeptide	mouse, Mus musculus	
7	nucleic acid	rabbit, Oryctolagus	

SEQ ID NO.	polypeptide or nucleic acid sequence	Organism	Other information
		cuniculus	
8	polypeptide	rabbit, Oryctolagus cuniculus	
9	nucleic acid	guinea pig, Cavia porcellus	
10	polypeptide	guinea pig, Cavia porcellus	
11	nucleic acid	dog, Canis lupus familiaris	variant x1
12	polypeptide	dog, Canis lupus familiaris	variant x1
13	nucleic acid	dog, Canis lupus familiaris	variant x2
14	polypeptide	dog, Canis lupus familiaris	variant x2
15	nucleic acid	cat, Felis catus	
16	polypeptide	cat, Felis catus	
17	nucleic acid	cow, Bos taurus	variant X1
18	polypeptide	cow, Bos taurus	variant X1
19	nucleic acid	cow, Bos taurus	variant X2
20	polypeptide	cow, Bos taurus	variant X2
21	nucleic acid	cow, Bos taurus	variant X3
22	polypeptide	cow, Bos taurus	variant X3
23	nucleic acid	cow, Bos taurus	variant X4
24	polypeptide	cow, Bos taurus	variant X4
25	nucleic acid	cow, Bos taurus	variant X5
26	polypeptide	cow, Bos taurus	variant X5
27	nucleic acid	cow, Bos taurus	variant X6
28	polypeptide	cow, Bos taurus	variant X6
29	nucleic acid	cow, Bos taurus	variant X7
30	polypeptide	cow, Bos taurus	variant X7
31	nucleic acid	cow, Bos taurus	variant X8
32	polypeptide	cow, Bos taurus	variant X8
33	nucleic acid	cow, Bos taurus	variant X9
34	polypeptide	cow, Bos taurus	variant X9
35	nucleic acid	cow, Bos taurus	variant X10
36	polypeptide	cow, Bos taurus	variant X10
37	nucleic acid	cow, Bos taurus	variant X11
38	polypeptide	cow, Bos taurus	variant X11
39	nucleic acid	cow, Bos taurus	variant 2
40	polypeptide	cow, Bos taurus	variant 2
41	nucleic acid	horse, Equus caballus	
42	polypeptide	horse, Equus caballus	

SEQ ID NO.	polypeptide or nucleic acid sequence	Organism	Other information
43	nucleic acid	chicken, Gallus gallus	
44	polypeptide	chicken, Gallus gallus	

Table 2 – N-RAS sequences

SEQ ID NO.	polypeptide or nucleic acid sequence	Organism	Other information
45	nucleic acid	human	
46	polypeptide	human	
47	nucleic acid	rat (Rattus norvegicus)	
48	polypeptide	rat (Rattus norvegicus)	
49	nucleic acid	mouse, Mus musculus	
50	polypeptide	mouse, Mus musculus	
51	nucleic acid	guinea pig, Cavia porcellus	
52	polypeptide	guinea pig, Cavia porcellus	
53	nucleic acid	guinea pig, Cavia porcellus	variant X1
54	polypeptide	guinea pig, Cavia porcellus	variant X1
55	nucleic acid	dog, Canis lupus familiaris	
56	polypeptide	dog, Canis lupus familiaris	
57	nucleic acid	cat, Felis catus	
58	polypeptide	cat, Felis catus	
59	nucleic acid	cow, Bos taurus	
60	polypeptide	cow, Bos taurus	
61	nucleic acid	chicken, Gallus gallus	
62	polypeptide	chicken, Gallus gallus	

Table 3 – MEK1 sequences

SEQ ID NO.	polypeptide or nucleic acid sequence	Organism
63	nucleic acid	human
64	polypeptide	human
65	nucleic acid	rat (Rattus norvegicus)
66	polypeptide	rat (Rattus norvegicus)
67	nucleic acid	mouse, Mus musculus
68	polypeptide	mouse, Mus musculus
69	nucleic acid	rabbit, Oryctolagus cuniculus

SEQ ID NO.	polypeptide or nucleic acid sequence	Organism
70	polypeptide	rabbit, Oryctolagus cuniculus
71	nucleic acid	guinea pig, Cavia porcellus
72	polypeptide	guinea pig, Cavia porcellus
73	nucleic acid	dog, Canis lupus familiaris
74	polypeptide	dog, Canis lupus familiaris
75	nucleic acid	cat, Felis catus
76	polypeptide	cat, Felis catus
77	nucleic acid	cow, Bos taurus
78	polypeptide	cow, Bos taurus
79	nucleic acid	horse, Equus caballus
80	polypeptide	horse, Equus caballus
81	nucleic acid	chicken, Gallus gallus
82	polypeptide	chicken, Gallus gallus

[0093] In another aspect of this embodiment, the method further comprises administering at least one additional therapeutic agent, preferably an inhibitor of the PI3K/Akt pathway, as disclosed herein.

[0094] A further embodiment of the present invention is a method for treating or ameliorating the effects of cancer in a subject, which cancer is refractory or resistant to BRAF inhibitor therapy, MEK inhibitor therapy, or both. The method comprises administering to the subject an effective amount of BVD-523 or a pharmaceutically acceptable salt thereof.

[0095] Suitable and preferred subjects are as disclosed herein. In this embodiment, the methods may be used to treat the cancers disclosed above, including those cancers with the mutational backgrounds, resistance profiles, and MAPK activity identified above. Methods of identifying such mutations are also as set forth above.

[0096] In a further aspect of this embodiment, the method further comprises administering to the subject at least one additional therapeutic agent, preferably an inhibitor of the PI3K/Akt pathway, as disclosed herein.

[0097] Another embodiment of the present invention is a method for identifying a subject having cancer who would benefit from therapy with an ERK inhibitor. The method comprises:

(a) obtaining a biological sample from the subject; and

(b) screening the sample to determine whether the subject has one or more of the following markers:

(i) a switch between RAF isoforms,

(ii) upregulation of RTK or NRAS signaling,

(iii) reactivation of mitogen activated protein kinase (MAPK) signaling,

(iv) the presence of a MEK activating mutation,

(v) amplification of mutant BRAF,

(vi) STAT3 upregulation,

(vii) mutations in the allosteric pocket of MEK that directly block binding of inhibitors to MEK or lead to constitutive MEK activity,

wherein the presence of one or more of the markers confirms that the subject's cancer is refractory or resistant to BRAF and/or MEK inhibitor therapy and that the subject would benefit from therapy with an ERK inhibitor, which is BVD-523 or a pharmaceutically acceptable salt thereof.

[0098] Suitable and preferred subjects are as disclosed herein. In this embodiment, the methods may be used to identify a subject having cancers

disclosed above, including those cancers with the mutational backgrounds, resistance profiles, and MAPK activity identified above. Methods of identifying such mutations are also as set forth above.

[0099] In one aspect of this embodiment, the method further comprises administering BVD-523 or a pharmaceutically acceptable salt thereof to a subject having one or more of the markers. Preferably, the method additionally comprises administering to the subject having one or more of the markers at least one additional therapeutic agent, preferably an inhibitor of the PI3K/Akt pathway, as disclosed herein.

[0100] An additional embodiment of the present invention is a pharmaceutical composition for treating or ameliorating the effects of a cancer in a subject, which cancer is refractory or resistant to non-ERK MAPK pathway therapy. The composition comprises a pharmaceutically acceptable carrier or diluent and an effective amount of BVD-523 or a pharmaceutically acceptable salt thereof.

[0101] Suitable and preferred subjects and types of non-ERK MAPK pathway inhibitor therapy are as disclosed herein. In this embodiment, the pharmaceutical composition may be used to treat the cancers disclosed above, including those cancers with the mutational backgrounds, resistance profiles, and MAPK activity identified above. Methods of identifying such mutations are also as set forth above.

[0102] In one aspect of this embodiment, the pharmaceutical composition further comprises at least one additional therapeutic agent, preferably an inhibitor of the PI3K/Akt pathway, as disclosed herein.

[0103] Another embodiment of the present invention is a kit for treating or ameliorating the effects of a cancer in a subject, which cancer is refractory or resistant to non-ERK MAPK pathway therapy. This kit comprises any pharmaceutical composition according to the present invention packaged together with instructions for its use.

[0104] The kits may also include suitable storage containers, *e.g.*, ampules, vials, tubes, etc., for each pharmaceutical composition and other reagents, *e.g.*, buffers, balanced salt solutions, etc., for use in administering the pharmaceutical compositions to subjects. The pharmaceutical compositions and other reagents may be present in the kits in any convenient form, such as, *e.g.*, in a solution or in a powder form. The kits may further include a packaging container, optionally having one or more partitions for housing the pharmaceutical composition and other optional reagents.

[0105] Suitable and preferred subjects and types of non-ERK MAPK pathway inhibitor therapy are as disclosed herein. In this embodiment, the kit may be used to treat the cancers disclosed above, including those cancers with the mutational backgrounds, resistance profiles, and MAPK activity identified herein. Methods of identifying such mutations are as set forth above.

[0106] In one aspect of this embodiment, the kit further comprises at least one additional therapeutic agent, preferably an inhibitor of the PI3K/Akt pathway, as disclosed herein.

[0107] Another embodiment of the present invention is a method for inhibiting phosphorylation of RSK in a cancer cell that is refractory or resistant to a non-ERK MAPK pathway inhibitor. The method comprises contacting the

cancer cell with an effective amount of BVD-523 or a pharmaceutically acceptable salt thereof for a period of time sufficient for phosphorylation of RSK in the cancer cell to be inhibited. In this embodiment, "contacting" means bringing BVD-523 or a pharmaceutically acceptable salt thereof and optionally one or more additional therapeutic agents into close proximity to the cancer cells. This may be accomplished using conventional techniques of drug delivery to mammals, or in the *in vitro* situation by, *e.g.*, providing BVD-523 or a pharmaceutically acceptable salt thereof and optionally other therapeutic agents to a culture media in which the cancer cells are located. In the *ex vivo* situation, contacting may be carried out by, *e.g.*, providing BVD-523 or a pharmaceutically acceptable salt thereof and optionally other therapeutic agents to a cancerous tissue.

[0108] Suitable and preferred types of non-ERK MAPK pathway inhibitors are as disclosed herein. In this embodiment, effecting cancer cell death may be accomplished in cancer cells having various mutational backgrounds, resistance profiles, and MAPK activity as disclosed above. Methods of identifying such mutations are also as set forth above.

[0109] The methods of this embodiment, which may be carried out in vitro, ex vivo, or in vivo, may be used to effect cancer cell death, by e.g., killing cancer cells, in cells of the types of cancer disclosed herein.

[0110] In one aspect of this embodiment, greater than 50% of RSK phosphorylation is inhibited. In another aspect of this embodiment, greater than 75% of RSK phosphorylation is inhibited. In an additional aspect of this embodiment, greater than 90% of RSK phosphorylation is inhibited. In a further aspect of this embodiment, greater than 95% of RSK phosphorylation

is inhibited. In another aspect of this embodiment, greater than 99% of RSK phosphorylation is inhibited. In an additional aspect of this embodiment, 100% of RSK phosphorylation is inhibited.

[0111] In a further aspect of this embodiment, the cancer cell is a mammalian cancer cell. Preferably, the mammalian cancer cell is obtained from a mammal selected from the group consisting of humans, primates, farm animals, and domestic animals. More preferably, the mammalian cancer cell is a human cancer cell.

[0112] In a further aspect of this embodiment, the contacting step comprises administering BVD-523 or a pharmaceutically acceptable salt to a subject from whom the cancer cell was obtained.

[0113] In the present invention, an "effective amount" or a "therapeutically effective amount" of a compound or composition disclosed herein is an amount of such compound or composition that is sufficient to effect beneficial or desired results as described herein when administered to a subject. Effective dosage forms, modes of administration, and dosage amounts may be determined empirically, and making such determinations is within the skill of the art. It is understood by those skilled in the art that the dosage amount will vary with the route of administration, the rate of excretion, the duration of the treatment, the identity of any other drugs being administered, the age, size, and species of mammal, *e.g.*, human patient, and like factors well known in the arts of medicine and veterinary medicine. In general, a suitable dose of a compound or composition according to the invention will be that amount of the composition, which is the lowest dose effective to produce the desired effect. The effective dose of a compound or

composition of the present invention may be administered as two, three, four, five, six or more sub-doses, administered separately at appropriate intervals throughout the day.

[0114] A suitable, non-limiting example of a dosage of a BVD-523 and other anti-cancer agents disclosed herein is from about 1 mg/kg to about 2400 mg/kg per day, such as from about 1 mg/kg to about 1200 mg/kg per day, 75 mg/kg per day to about 300 mg/kg per day, including from about 1 mg/kg to about 100 mg/kg per day. Other representative dosages of such agents include about 1 mg/kg, 5 mg/kg, 10 mg/kg, 15 mg/kg, 20 mg/kg, 25 mg/kg, 30 mg/kg, 35 mg/kg, 40 mg/kg, 45 mg/kg, 50 mg/kg, 60 mg/kg, 70 mg/kg, 75 mg/kg, 80 mg/kg, 90 mg/kg, 100 mg/kg, 125 mg/kg, 150 mg/kg, 175 mg/kg, 200 mg/kg, 250 mg/kg, 300 mg/kg, 400 mg/kg, 500 mg/kg, 600 mg/kg, 700 mg/kg, 800 mg/kg, 900 mg/kg, 1000 mg/kg, 1100 mg/kg, 1200 mg/kg, 1300 mg/kg, 1400 mg/kg, 1500 mg/kg, 1600 mg/kg, 1700 mg/kg, 1800 mg/kg, 1900 mg/kg, 2000 mg/kg, 2100 mg/kg, 2200 mg/kg, and 2300 mg/kg per day. The effective dose of BVD-523 and other anti-cancer agents disclosed herein, may be administered as two, three, four, five, six or more sub-doses, administered separately at appropriate intervals throughout the day.

[0115] The BVD-523, other inhibitors, and various other anti-cancer agents disclosed herein, or a pharmaceutical composition of the present invention may be administered in any desired and effective manner: for oral ingestion, or as an ointment or drop for local administration to the eyes, or for parenteral or other administration in any appropriate manner such as intraperitoneal, subcutaneous, topical, intradermal, inhalation, intrapulmonary, rectal, vaginal, sublingual, intramuscular, intravenous, intraarterial, intrathecal,

or intralymphatic. Further, BVD-523, other inhibitors, and various other anti-cancer agents disclosed herein, or a pharmaceutical composition of the present invention may be administered in conjunction with other treatments. BVD-523, other inhibitors, and various other anti-cancer agents disclosed herein, or a pharmaceutical composition of the present invention may be encapsulated or otherwise protected against gastric or other secretions, if desired.

[0116] The pharmaceutical compositions of the invention comprise one or more active ingredients in admixture with one or more pharmaceutically-acceptable diluents or carriers and, optionally, one or more other compounds, drugs, ingredients and/or materials. Regardless of the route of administration selected, the agents/compounds of the present invention are formulated into pharmaceutically-acceptable dosage forms by conventional methods known to those of skill in the art. See, *e.g.*, Remington, The Science and Practice of Pharmacy (21st Edition, Lippincott Williams and Wilkins, Philadelphia, PA.).

[0117] Pharmaceutically acceptable diluents or carriers are well known in the art (see, *e.g.*, Remington, The Science and Practice of Pharmacy (21st Edition, Lippincott Williams and Wilkins, Philadelphia, PA.) and The National Formulary (American Pharmaceutical Association, Washington, D.C.)) and include sugars (*e.g.*, lactose, sucrose, mannitol, and sorbitol), starches, cellulose preparations, calcium phosphates (*e.g.*, dicalcium phosphate, tricalcium phosphate and calcium hydrogen phosphate), sodium citrate, water, aqueous solutions (*e.g.*, saline, sodium chloride injection, Ringer's injection, dextrose injection, dextrose and sodium chloride injection, lactated Ringer's injection), alcohols (*e.g.*, ethyl alcohol, propyl alcohol, and benzyl alcohol),

polyols (*e.g.*, glycerol, propylene glycol, and polyethylene glycol), organic esters (*e.g.*, ethyl oleate and tryglycerides), biodegradable polymers (*e.g.*, polylactide-polyglycolide, poly(orthoesters), and poly(anhydrides)), elastomeric matrices, liposomes, microspheres, oils (*e.g.*, corn, germ, olive, castor, sesame, cottonseed, and groundnut), cocoa butter, waxes (*e.g.*, suppository waxes), paraffins, silicones, talc, silicylate, etc. Each pharmaceutically acceptable diluent or carrier used in a pharmaceutical composition of the invention must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not injurious to the subject. Diluents or carriers suitable for a selected dosage form and intended route of administration are well known in the art, and acceptable diluents or carriers for a chosen dosage form and method of administration can be determined using ordinary skill in the art.

[0118] The pharmaceutical compositions of the invention may, optionally, contain additional ingredients and/or materials commonly used in pharmaceutical compositions. These ingredients and materials are well known in the art and include (1) fillers or extenders, such as starches, lactose, sucrose, glucose, mannitol, and silicic acid; (2) binders, such as carboxymethylcellulose, alginates, gelatin, polyvinyl pyrrolidone, hydroxypropylmethyl cellulose, sucrose and acacia; (3) humectants, such as glycerol; (4) disintegrating agents, such as agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates, sodium starch glycolate, cross-linked sodium carboxymethyl cellulose and sodium carbonate; (5) solution retarding agents, such as paraffin; (6) absorption accelerators, such as quaternary ammonium compounds; (7) wetting agents,

such as cetyl alcohol and glycerol monostearate; (8) absorbents, such as kaolin and bentonite clay; (9) lubricants, such as talc, calcium stearate, magnesium stearate, solid polyethylene glycols, and sodium lauryl sulfate; (10) suspending agents, such as ethoxylated isostearyl alcohols, polyoxyethylene sorbitol and sorbitan esters, microcrystalline cellulose, aluminum metahydroxide, bentonite, agar-agar and tragacanth; (11) buffering agents; (12) excipients, such as lactose, milk sugars, polyethylene glycols, animal and vegetable fats, oils, waxes, paraffins, cocoa butter, starches, tragacanth, cellulose derivatives, polyethylene glycol, silicones, bentonites, silicic acid, talc, salicylate, zinc oxide, aluminum hydroxide, calcium silicates, and polyamide powder; (13) inert diluents, such as water or other solvents; (14) preservatives; (15) surface-active agents; (16) dispersing agents; (17) control-release or absorption-delaying agents, such as hydroxypropylmethyl cellulose, other polymer matrices, biodegradable polymers, liposomes, microspheres, aluminum monostearate, gelatin, and waxes; (18) opacifying agents; (19) adjuvants; (20) wetting agents; (21) emulsifying and suspending agents; (22), solubilizing agents and emulsifiers, such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, oils (in particular, cottonseed, groundnut, corn, germ, olive, castor and sesame oils), glycerol, tetrahydrofuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan; (23) propellants, such as chlorofluorohydrocarbons and volatile unsubstituted hydrocarbons, such as butane and propane; (24) antioxidants; (25) agents which render the formulation isotonic with the blood of the intended recipient, such as sugars and sodium chloride; (26) thickening agents; (27) coating

materials, such as lecithin; and (28) sweetening, flavoring, coloring, perfuming and preservative agents. Each such ingredient or material must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not injurious to the subject. Ingredients and materials suitable for a selected dosage form and intended route of administration are well known in the art, and acceptable ingredients and materials for a chosen dosage form and method of administration may be determined using ordinary skill in the art.

[0119] The pharmaceutical compositions of the present invention suitable for oral administration may be in the form of capsules, cachets, pills, tablets, powders, granules, a solution or a suspension in an aqueous or non-aqueous liquid, an oil-in-water or water-in-oil liquid emulsion, an elixir or syrup, a pastille, a bolus, an electuary or a paste. These formulations may be prepared by methods known in the art, *e.g.*, by means of conventional pan-coating, mixing, granulation or lyophilization processes.

[0120] Solid dosage forms for oral administration (capsules, tablets, pills, dragees, powders, granules and the like) may be prepared, *e.g.*, by mixing the active ingredient(s) with one or more pharmaceutically-acceptable diluents or carriers and, optionally, one or more fillers, extenders, binders, humectants, disintegrating agents, solution retarding agents, absorption accelerators, wetting agents, absorbents, lubricants, and/or coloring agents. Solid compositions of a similar type may be employed as fillers in soft and hard-filled gelatin capsules using a suitable excipient. A tablet may be made by compression or molding, optionally with one or more accessory ingredients. Compressed tablets may be prepared using a suitable binder,

lubricant, inert diluent, preservative, disintegrant, surface-active or dispersing agent. Molded tablets may be made by molding in a suitable machine. The tablets, and other solid dosage forms, such as dragees, capsules, pills and granules, may optionally be scored or prepared with coatings and shells, such as enteric coatings and other coatings well known in the pharmaceutical-formulating art. They may also be formulated so as to provide slow or controlled release of the active ingredient therein. They may be sterilized by, for example, filtration through a bacteria-retaining filter. These compositions may also optionally contain opacifying agents and may be of a composition such that they release the active ingredient only, or preferentially, in a certain portion of the gastrointestinal tract, optionally, in a delayed manner. The active ingredient can also be in microencapsulated form.

[0121] Liquid dosage forms for oral administration include pharmaceutically-acceptable emulsions, microemulsions, solutions, suspensions, syrups and elixirs. The liquid dosage forms may contain suitable inert diluents commonly used in the art. Besides inert diluents, the oral compositions may also include adjuvants, such as wetting agents, emulsifying and suspending agents, sweetening, flavoring, coloring, perfuming and preservative agents. Suspensions may contain suspending agents.

[0122] The pharmaceutical compositions of the present invention for rectal or vaginal administration may be presented as a suppository, which may be prepared by mixing one or more active ingredient(s) with one or more suitable nonirritating diluents or carriers which are solid at room temperature, but liquid at body temperature and, therefore, will melt in the rectum or vaginal cavity and release the active compound. The pharmaceutical compositions of

the present invention which are suitable for vaginal administration also include pessaries, tampons, creams, gels, pastes, foams or spray formulations containing such pharmaceutically-acceptable diluents or carriers as are known in the art to be appropriate.

[0123] Dosage forms for the topical or transdermal administration include powders, sprays, ointments, pastes, creams, lotions, gels, solutions, patches, drops and inhalants. The active agent(s)/compound(s) may be mixed under sterile conditions with a suitable pharmaceutically-acceptable diluent or carrier. The ointments, pastes, creams and gels may contain excipients. Powders and sprays may contain excipients and propellants.

[0124] The pharmaceutical compositions of the present invention suitable for parenteral administrations may comprise one or more agent(s)/compound(s) in combination with one or more pharmaceutically-acceptable sterile isotonic aqueous or non-aqueous solutions, dispersions, suspensions or emulsions, or sterile powders which may be reconstituted into sterile injectable solutions or dispersions just prior to use, which may contain suitable antioxidants, buffers, solutes which render the formulation isotonic with the blood of the intended recipient, or suspending or thickening agents. Proper fluidity can be maintained, for example, by the use of coating materials, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants. These pharmaceutical compositions may also contain suitable adjuvants, such as wetting agents, emulsifying agents and dispersing agents. It may also be desirable to include isotonic agents. In addition, prolonged absorption of the injectable

pharmaceutical form may be brought about by the inclusion of agents which delay absorption.

[0125] In some cases, in order to prolong the effect of a drug (*e.g.*, pharmaceutical formulation), it is desirable to slow its absorption from subcutaneous or intramuscular injection. This may be accomplished by the use of a liquid suspension of crystalline or amorphous material having poor water solubility.

[0126] The rate of absorption of the active agent/drug then depends upon its rate of dissolution which, in turn, may depend upon crystal size and crystalline form. Alternatively, delayed absorption of a parenterally-administered agent/drug may be accomplished by dissolving or suspending the active agent/drug in an oil vehicle. Injectable depot forms may be made by forming microencapsule matrices of the active ingredient in biodegradable polymers. Depending on the ratio of the active ingredient to polymer, and the nature of the particular polymer employed, the rate of active ingredient release can be controlled. Depot injectable formulations are also prepared by entrapping the drug in liposomes or microemulsions which are compatible with body tissue. The injectable materials can be sterilized for example, by filtration through a bacterial-retaining filter.

[0127] The formulations may be presented in unit-dose or multi-dose sealed containers, for example, ampules and vials, and may be stored in a lyophilized condition requiring only the addition of the sterile liquid diluent or carrier, for example water for injection, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets of the type described above.

[0128] The present invention provides treatment of cancer which is refractory or resistant to non-ERK MAPK pathway inhibitor therapy and discloses combinations shown to enhance the effects of ERK inhibitors. Herein, applicants have also shown that the combination of different ERK inhibitors is likewise synergistic. Therefore, it is contemplated that the effects of the combinations described herein can be further improved by the use of one or more additional ERK inhibitors. Accordingly, some embodiments of the present invention include one or more additional ERK inhibitors.

[0129] The following examples are provided to further illustrate the methods of the present invention. These examples are illustrative only and are not intended to limit the scope of the invention in any way.

EXAMPLES

Example 1

MATERIALS AND METHODS

[0130] Cancer cell lines were maintained in cell culture under standard media and serum conditions. For dose escalation studies, A375 cells were split, grown to about 40-60% confluence, and then treated with the initial dose of the specified drug. Table 4 shows a summary of drug treatments that were escalated.

Table 4 - Summary of Treatments Being Escalated

Treatment	Inhibitor
1	Trametinib (MEKi)
2	Dabrafenib (BRAFi)
3	BVD-523 (ERKi)
4	Dabrafenib (BRAFi) + Trametinib (MEKi)
5	Dabrafenib (BRAFi) + BVD-523 (ERKi)

6	Trametinib (MEKi) + BVD-523 (ERKi)

[0131] Single agent dose escalations were performed based on Little *et al.*, 2011 and are outlined in FIG. 20. Cells were then allowed to grow until 70-90% confluence and split. Split ratios were kept as "normal" as possible and reasonably consistent between treatments (*e.g.* a minimum of 50% of the normal split ratio of the parentals). Medium was refreshed every 3-4 days. When cells again reached about 40-60% confluence, the dose was escalated. In the event that the 40-60% window was missed, the cells were split again and dosed once they reached 40-60% confluence. Again, medium was refreshed every 3-4 days. The process was repeated as required (FIG. 20).

[0132] For single agent treatments, starting concentrations and dose increases were conducted by starting with the approximate IC_{50}, escalating in small increments or, gently, for the initial 4-5 doses, doubling the dose, increasing by the same increment for the next 4 doses, then moving to 1.5-fold increases in concentration for subsequent doses.

[0133] For combination treatments, starting concentrations and dose increases were conducted by starting with half of the approximate IC_{50} of each compound (combination assay suggests this will result in about 40-70% inhibition range), escalating as per single agents (i.e. doing an initial doubling and then increasing by the same increment for the next 4 doses, then moving to 1.5-fold increases in concentration). Table 5 shows the projected dose increases using these schemes.

Table 5 - Projected Dose Increases – Month 1

				Dab/Tram		Dab/523		Tram/523	
Dose	**Tram (nM)**	**Dab (nM)**	**BVD-523 (μM)**	**Dab (nM)**	**Tram (nM)**	**Dab (nM)**	**523 (μM)**	**Tram (nM)**	**523 (μM)**
1	1	5	0.16	2.5	0.5	2.5	0.08	0.5	0.08
2	2	10	0.32	5	1	5	0.16	1	0.16
3	3	15	0.48	7.5	1.5	7.5	0.24	1.5	0.24
4	4	20	0.64	10	2	10	0.32	2	0.32
5	5	25	0.80	12.5	2.5	12.5	0.40	2.5	0.40
6	8	38	1.2	19	4	19	0.6	4	0.6
7	11	56	1.8	28	6	28	0.9	6	0.9
8	17	84	2.7	42	8	42	1.4	8	1.4
9	25	127	4.1	63	13	63	2.0	13	2.0
10	38	190	6.1	95	19	95	3.0	19	3.0
11	57	285	9.1	142	28	142	4.6	28	4.6
12	85	427	13.7	214	43	214	6.8	43	6.8
13	128	641	20.5	320	64	320	10.3	64	10.3
14	192	961	30.8	481	96	481	15.4	96	15.4
15	288	1442	46.1	721	144	721	23.1	144	23.1
16	432	2162	69.2	1081	216	1081	34.6	216	34.6
17	649	3244	103.8	1622	324	1622	51.9	324	51.9
18	973	4865	155.7	2433	487	2433	77.8	487	77.8
19	1460	7298	233.5	3649	730	3649	116.8	730	116.8
20	2189	10947	350.3	5474	1095	5474	175.2	1095	175.2

[0134] Clonal resistant cell populations were derived from resistant cell pools by limiting dilution.

[0135] Proliferation assays were used to track changes in sensitivity to the escalated agent(s) at appropriate time intervals (*e.g.* each month, although the timing is dependent on adequate cell numbers being available). For proliferation assays, cells were seeded in 96-well plates at 3000 cells per well in drug-free DMEM medium containing 10% FBS and allowed to adhere overnight prior to addition of compound or vehicle control. Compounds were prepared from DMSO stocks to give a final concentration range as shown in

FIGS. 2A-H. The final DMSO concentration was constant at 0.1%. Test compounds were incubated with the cells for 96 hours at 37°C and 5% CO_2 in a humidified atmosphere. Alamar Blue 10% (v/v) was then added and incubated for 4 hours and fluorescent product was detected using a BMG FLUOstar plate reader. The average media only background value was deducted and the data analyzed using a 4-parameter logistic equation in GraphPad Prism. Paclitaxel was used as a positive control.

[0136] Proliferation assays for month 1 were initiated at day 28 using cells growing in the concentrations of each agent indicated in Table 6.

Table 6 - Initial Concentrations of Drugs Used in Proliferation Assays – Month 1

Line	Dab	Tram	BVD-523
Parental	-	-	-
Tram	-	2 nM	-
Dab	15 nM	-	-
BVD-523	-	-	0.48 μM
Tram + Dab	5 nM	1 nM	-
Dab + BVD-523	7.5 nM	-	0.24 μM
Tram + BVD-523	-	1 nM	0.16 μM

[0137] Proliferation assays for month 2 were initiated at day 56 using cells growing in the concentrations of each agent indicated in Table 7.

Table 7 - Initial Concentrations of Drugs Used in Proliferation Assays - Month 2

Line	Dab	Tram	BVD-523
Parental	-	-	-
Tram	-	8 nM	-

Dab	127 nM	-	-
BVD-523	-	-	0.8 μM
Tram + Dab	10 nM	2 nM	-
Dab + BVD-523	12.5 nM	-	0.4 μM
Tram + BVD-523	-	2 nM	0.32 μM

[0138] At the end of the 3 month escalation period, cultures were maintained at the top concentration for 2 weeks prior to the final round of proliferation assays and potential single cell cloning. As the proliferation assays/single cell cloning required actively proliferating cells, for treatments where cells were proliferating very slowly at the top concentration or that were only recently escalated, a backup culture was also maintained at a lower concentration (Table 8). For the BVD-523 treatment, where cells appeared to have almost completely stopped growing and looked particularly fragile at the top concentration (1.8μM), cultures were maintained at a lower concentration for the 2 week period.

Table 8 - Details of Treatments Being Cultured at a Fixed Concentration for 2 Weeks

Treatment	Inhibitor	Culture 1	Backup Culture
1	Tram	160 nM	80 nM
2	Dab	3.2 μM	-
3	BVD-523	1.2 μM	0.8 μM
4	Dab + Tram	D: 160 nM T: 30 nM	D: 80 nM T: 16 nM
5	Dab + BVD-523	D: 42 nM 523: 1.4 μM	D: 28 nM 523: 0.9 μM
6	Tram + BVD-523	T: 4 nM 523: 0.6 μM	T: 2.5 nM 523: 0.4 μM

[0139] Proliferation assays for month 3 used cells growing in the concentrations of each agent indicated in Table 9.

Table 9 - Initial Concentrations of Drugs Used in Proliferation Assays - Month 3

Line	Dab	Tram	BVD-523
Parental	-	-	-
Tram	-	160 nM	-
Dab	3.2 μM	-	-
BVD-523	-	-	1.2 μM
Tram + Dab	80 nM	16 nM	-
Dab + BVD-523	28 nM	-	0.9 μM
Tram + BVD-523	-	2.5 nM	0.4 μM

[0140] For combination studies, A375 cells (ATCC) were seeded into triplicate 96-well plates at a cell density of 3000 cells/well in DMEM plus 10% FBS and allowed to adhere overnight prior to addition of test compound or vehicle control. Combinations were tested using a 10x8 dose matrix with a final DMSO concentration of 0.2%. A 96 hour assay incubation period followed, with subsequent addition of Alamar Blue 10% (v/v) and 4 hours incubation prior to reading on a fluorescent plate reader. After reading Alamar Blue, the medium/Alamar Blue mix was flicked off and 100μl of CellTiter-Glo/PBS (1:1) added and the plates processed as per the manufacturers instructions (Promega). Media only background values were subtracted before the data was analysed. The Bliss additivity model was then applied.

[0141] In brief, predicted fractional inhibition values for combined inhibition were calculated using the equation $C_{bliss} = A + B - (A \times B)$ where A and B are the fractional inhibitions obtained by drug A alone or drug B alone

at specific concentrations. C_{bliss} is the fractional inhibition that would be expected if the combination of the two drugs were exactly additive. C_{bliss} values are subtracted from the experimentally observed fractional inhibition values to give an 'excess over Bliss' value. Excess over Bliss values greater than 0 indicate synergy, whereas values less than 0 indicate antagonism. Excess over Bliss values are plotted as heat maps ± SD.

[0142] The single and combination data are also presented as dose-response curves generated in GraphPad Prism (plotted using % viability relative to DMSO only treated controls).

[0143] For focused combination studies, the Alamar Blue viability assays were performed as described above for combination studies. Additionally, Caspase-Glo 3/7 assays were performed. In brief, HCT116 cells were seeded in triplicate in white 96-well plates at a cell density of 5000 cells/well in McCoy's 5A plus 10% FBS. A375 cells were seeded at a density of 5000 cells/well in DMEM plus 10% FBS. Cells were allowed to adhere overnight prior to addition of test compound or vehicle control. The final concentration of DMSO was 0.2%, and 800nM staurosporine was included as a positive control. 24 and 48 hour assay incubation periods were used. Then, Caspase-Glo® 3/7 50% (v/v) was added, plates were mixed for 5 minutes on an orbital shaker and incubated for 1 hour at room temperature prior to reading on a luminescent plate reader. Media only background values were subtracted before the data was analysed.

Example 2

Dose Escalation and Proliferation Assays – Month 1

Dose Escalation Progress – Month 1

[0144] A375 cells were dose escalated using BVD-523, dabrafenib, and trametinib either as single agents or in combination. Doses were increased in small increments during the first month. Other than a marked reduction in growth rate, cells generally tolerated the escalations well and the doses were planned to be more aggressively escalated using larger increments in month 2. FIGS. 1A-C show month 1 progress for the dose escalation studies.

Proliferation Assay Results – Month 1

[0145] Proliferation assays were performed to assess the response of the escalated cells lines vs. parental cell line, to BVD-523, dabrafenib, and trametinib treatments.

[0146] FIGS. 2A-H show normalized and raw proliferation assay results from month 1 of the studies. Note that differences in max signals in DMSO controls between different treatments (FIGS. 2D-F, 2H) suggest differential growth rates between treatments. These differences may influence the responses of lines to inhibitors in the proliferation assays.

[0147] Table 10 shows IC_{50} data for month 1 of the studies.

Table 10 - IC_{50} Data - Month 1

Compound	Cell Line, Relative IC_{50} (nM)						
	Par*	Tram	Dab	BVD-523	Dab/Tram	Dab/523	Tram/523
Dabrafenib	6	29	about 161	8	58	68	11
Trametinib	0.5	2.2	2.5	0.7	3.9	3.1	2.5
BVD-523	189	335	350	268	300	412	263
Paclitaxel	2.2	3.0	3.3	3.4	3.5	3.4	3.4

*Par = Parental cell line

[0148] There were early hints that cells grown in the presence of escalating doses of dabrafenib or trametinib, either as single agents or in combinations, were exhibiting decreased responses to these two agents in proliferation assays.

[0149] In the early stages of month 2, the growth rate of cells in the dabrafenib only treatment notably increased relative to the early stages of month 1. This enabled an increased rate of progression and suggested that resistance was becoming apparent.

Example 3

Dose Escalation and Proliferation Assays – Month 2

Dose Escalation Progress – Month 2

[0150] The second month of studies saw most treatments move into a phase where doses were increased in greater increments (1.5-fold) compared to the initial gentle escalation phase. The single agent escalation of dabrafenib and trametinib was quickest, with cells growing in concentrations equivalent to 100x parental cell IC_{50} (FIGS. 3A,B). The single agent escalation of BVD-523 progressed more slowly compared to dabrafenib and

trametinib (FIG. 3C). See FIG. 3D for a comparison of the single agent escalations. BVD-523 escalated cells had a more "fragile" appearance and there was a greater number of floating cells compared to the dabrafenib and trametinib escalated populations.

[0151] The combined agent escalations progressed more slowly than the single agent treatments. The BVD-523/trametinib combination was particularly effective in preventing cells from progressing.

Proliferation Assay Results – Month 2

[0152] Proliferation assays on single agent escalated dabrafenib and trametinib cell populations revealed modest shifts in the dose response curves, suggesting that an additional period of escalation would be beneficial to further enrich for resistant cells. Interestingly, in the proliferations assay, there was evidence to suggest that cells exposed to BVD-523 grew less well upon inhibitor withdrawal, perhaps indicating a level of addiction.

[0153] FIGS. 4A-H show normalized and raw proliferation assay results from month 2 of the studies. Note that differences in max signals in DMSO controls between different treatments (FIGS. 4D-F, 4H) suggest differential growth rates between treatments. These differences may influence the responses of lines to inhibitors in the proliferation assays.

[0154] FIGS. 5A-H show normalized and raw proliferation assay results from month 2 of the studies with a focus on parental and BVD-523 line data only.

[0155] Table 11 shows IC_{50} data for month 2 of the studies. Relative IC_{50}s were determined from 4-parameter curve fits in Prism.

Table 11 - IC_{50} Data - Month 2

Compound	Cell Line, Relative IC_{50} (nM)						
	Par*	Tra	Dab	BVD-523	Dab/Tram	Dab/523	Tram/523
Dabrafenib	4.1	6.2	11.5	697	256	218	68
Trametinib	0.4	0.7	1.1	24.3	12.6	6.2	4.6
BVD-523	187	252	284	1706	561	678	435
Paclitaxel	3.7	8.9	1.9	6.5	4.7	4.2	8.9

*Par = Parental cell line

Example 4

Dose Escalation and Proliferation Assays – Month 3

Dose Escalation Progress – Month 3

[0156] FIGS. 6A-C show single and combination agent escalation for month 3 of the studies. FIG. 6D shows a comparison of single agent escalations.

Proliferation Assay Results – Month 3

[0157] FIG. 7 shows an assessment of growth during the proliferation assay in DMSO control wells. FIGS. 8A-D show results from month 3 of the studies. FIGS. 9A-D show results from month 3 of the studies with a focus on single treatment cell lines.

[0158] Table 12 shows IC_{50} data for month 3 of the studies. Relative IC_{50}s were determined from 4-parameter curve fits in Prism. IC_{50} values were not determined for the cell line escalated with trametinib due to a lack of growth during the assay (ND: not done).

Table 12 - IC_{50} Data - Month 3

Compound	Cell Line, Relative IC_{50} (nM)						
	Par*	Tram	Dab	BVD-523	Dab/Tram	Dab/523	Tram/523
Dabrafenib	2.1	ND	2.5	18.4	17.9	337	73
Trametinib	0.2	ND	0.4	1.7	2.7	90	11.2
BVD-523	129	ND	198	433	323	1151	296
Paclitaxel	1.9	ND	1.9	6.5	4.7	4.2	8.9

*Par = Parental cell line

[0159] Figure 19 shows single and combination agent escalation for month 3 of the studies. Cell line variants were obtained that could grow in the presence of dabrafenib or trametinib at concentrations greater than 100 times the IC_{50} of these agents in parental A375 cell. In comparison, cell lines resistant to BVD-523 could only be maintained in less than 10X of parental IC_{50} concentration. Sensitivity testing suggested dabrafenib and trametinib-resistant cell lines remained relatively sensitive to BVD-523; the increased IC_{50} "shift" for BVD-523 in resistant cell lines was more modest than those corresponding IC_{50} increases following dabrafenib or trametinib treatment. Likewise, compared to dabrafenib or trametinib treatment, more complete inhibition of cell growth was observed when resistant cell lines were treated with BVD-523 at concentrations 10-fold above its IC_{50} in the parental A375 line. In total, patterns of resistance and cross-sensitivity suggest BVD-523 may remain effective in settings of acquired resistance.

Example 5

Combination Study Results

[0160] As expected, A375 cells, which carry a BRAF (V600E) mutation, were sensitive to dabrafenib. Single agent IC_{50} values calculated using

Alamar Blue (FIGS. 10, 12, 14) were generally slightly lower for Dabrafenib and BVD-523 compared to those derived using CellTiter-Glo (FIGS. 11, 13, 15). Published IC_{50} values for Dabrafenib and Trametinib in a 72 hour CellTiter-Glo assay were 28 ± 16nM and 5 ± 3nM respectively (Greger *et al.*, 2012; King *et al.*, 2013) – the single agent results reported here are consistent with these values. There was some evidence for a window of synergy in all treatments. Variation between triplicates was low, however, there was some evidence of edge effects that likely explains the apparent enhanced growth observed in some treatments versus the no drug control (*e.g.* particularly apparent in the Trametinib/BVD-523 combination). This makes the interpretation of the Bliss analysis more challenging as in some treatments it may have resulted in the artefactual enhancement in the level of synergy.

[0161] The combination assays were repeated for A375 cells. Single agent BVD-523, Trametinib and Dabrafenib potencies were consistent with those reported in the previous studies disclosed herein.

[0162] In sum, taken together the data show that MEK and BRAF resistant cells could be overcome by treatment with the ERK inhibitor, BVD-523.

Example 6

BVD-523 altered markers of MAPK kinase activity and effector function

[0163] For Western blot studies, HCT116 cells (5 x 10^6) were seeded into 10 cm dishes in McCoy's 5A plus 10% FBS. A375 cells (2.5 x 10^6) were seeded into 10 cm dishes in DMEM plus 10% FBS. Cells were allowed to adhere overnight prior to addition of the indicated amount of test compound (BVD-523) or vehicle control. Cells were treated for either 4 or 24 hours

before isolation of whole-cell protein lysates, as specified below. Cells were harvested by trypsinisation, pelleted and snap frozen. Lysates were prepared with RIPA (Radio-Immunoprecipitation Assay) buffer, clarified by centrifugation and quantitated by bicinchoninic acid assay (BCA) assay. 20-50 µg of protein was resolved by SDS-PAGE electrophoresis, blotted onto PVDF membrane and probed using the antibodies detailed in Table 13 (for the 4-hour treatment) and Table 14 (for the 24-hour treatment) below.

Table 13 – Antibody Details

Antigen	Size (kDa)	Supplier	Cat No	Dilution	Incubation / Block Conditions	Secondary
pRSK1/2 pS380	90	Cell Signaling	9335	1:1000	o/n 4˚C 5% BSA	anti-rabbit
pRSK1/2 pS380	90	Cell Signaling	11989	1:2000	o/n 4˚C 5% BSA	anti-rabbit
pRSK-T359/S363	90	Millipore	04-419	1:40000	o/n 4˚C 5% BSA	anti-rabbit
Total RSK	90	Cell Signaling	9333	1:1000	o/n 4˚C 5% BSA	anti-rabbit
pErk 1/2	42/44	Cell Signaling	9106S	1:500	o/n 4˚C 5% milk	anti-mouse
Total ERK	42/44	Cell Signaling	9102	1:2000	o/n 4˚C 5% milk	anti-rabbit
pMEK1/2	45	Cell Signaling	9154	1:1000	o/n 4˚C 5% BSA	anti-rabbit
Total MEK	45	Cell Signaling	9126	1:1000	o/n 4˚C 5% BSA	anti-rabbit
pS6-pS235	32	Cell Signaling	2211S	1:3000	o/n 4˚C 5% milk	anti-rabbit
Total S6	32	Cell Signaling	2217	1:2000	o/n 4˚C 5% milk	anti-rabbit

Antigen	Size (kDa)	Supplier	Cat No	Dilution	Incubation / Block Conditions	Secondary
DUSP6	48	Cell Signaling	3058S	1:1000	o/n 4˚C 5% BSA	anti-rabbit
Total CRAF	73	BD Bio-sciences	610152	1:2000	o/n 4˚C 5% milk	anti-mouse
pCRAF-Ser338	73	Cell Signaling	9427	1:1000	o/n 4˚C 5% BSA	anti-rabbit
pRB (Ser780)	105	Cell Signaling	9307	1:2000	o/n 4˚C 5% BSA	anti-rabbit
β-Actin	42	Sigma	A5441	1:500,000	o/n 4˚C 5% milk	anti-mouse

Table 14 – Antibody details

Antigen	Size (kDa)	Supplier	Cat No	Dilution	Incubation / Block Conditions	Secondary
pRB (Ser780)	105	Cell Signaling	9307	1:2000	o/n 4˚C 5% BSA	anti-rabbit
CCND1	34	Abcam	ab6152	1:500	o/n 4˚C 5% milk	anti-mouse
Bim-EL	23	Millipore	AB17003	1:1000	o/n 4˚C 5% BSA	anti-rabbit
Bim-EL	23	Cell Signaling	2933	1:1000	o/n 4˚C 5% BSA	anti-rabbit
BCL-xL	30	Cell Signaling	2762	1:2000	o/n 4˚C 5% BSA	anti-rabbit
PARP	116/89	Cell Signaling	9542	1:1000	o/n 4˚C 5% milk	anti-rabbit
Cleaved Caspase 3	17,19	Cell Signaling	9664X	1:1000	o/n 4˚C 5% milk	anti-rabbit
DUSP6	48	Cell Signaling	3058S	1:1000	o/n 4°C 5% BSA	anti-rabbit
pRSK1/2	90	Cell	9335	1:1000	o/n 4°C 5%	anti-rabbit

Antigen	Size (kDa)	Supplier	Cat No	Dilution	Incubation / Block Conditions	Secondary
pS380		Signaling			BSA	
pRSK1/2 pS380	90	Cell Signaling	11989	1:2000	o/n 4°C 5% BSA	anti-rabbit
pRSK-T359/S363	90	Millipore	04-419	1:40000	o/n 4°C 5% BSA	anti-rabbit
Total RSK	90	Cell Signaling	9333	1:1000	o/n 4°C 5% BSA	anti-rabbit
pErk 1/2	42/44	Cell Signaling	9106S	1:500	o/n 4°C 5% milk	anti-mouse
Total ERK	42/44	Cell Signaling	9102	1:2000	o/n 4°C 5% milk	anti-rabbit
B-Actin	42	Sigma	A5441	1:500,000	o/n 4°C 5% milk	anti-mouse

[0164] Figure 16-18 shows Western blot analyses of cells treated with BVD-523 at various concentrations for the following: 1) MAPK signaling components in A375 cells after 4 hours; 2) cell cycle and apoptosis signaling in A375 24 hours treatment with various amounts of BVD-523; and 3) MAPK signaling in HCT-116 cells treated for 4 hours. The results show that acute and prolonged treatment with BVD-523 in RAF and RAS mutant cancer cells in-vitro affects both substrate phosphorylation and effector targets of ERK kinases. The concentrations of BVD-523 required to induce these changes is typically in the low micromolar range.

[0165] Changes in several specific activity markers are noteworthy. First, the abundance of slowly migrating isoforms of ERK kinase increase following BVD-523 treatment; modest changes can be observed acutely, and

increase following prolonged treatment. While this could indicate an increase in enzymatically active, phosphorylated forms of ERK, it remains noteworthy that multiple proteins subject to both direct and indirect regulation by ERK remain "off" following BVD-523 treatment. First, RSK1/2 proteins exhibit reduced phosphorylation at residues that are strictly dependent on ERK for protein modification (T359/S363). Second, BVD-523 treatment induces complex changes in the MAPK feedback phosphatase, DUSP6: slowly migrating protein isoforms are reduced following acute treatment, while total protein levels are greatly reduced following prolonged BVD-523 treatment. Both of these findings are consistent with reduced activity of ERK kinases, which control DUSP6 function through both post-translational and transcriptional mechanisms. Overall, despite increases in cellular forms of ERK that are typically thought to be active, it appears likely that cellular ERK enzyme activity is fully inhibited following either acute or prolonged treatment with BVD-523.

[0166] Consistent with these observations, effector genes that require MAPK pathway signaling are altered following treatment with BVD-523. The G1/S cell-cycle apparatus is regulated at both post-translational and transcriptional levels by MAPK signaling, and cyclin-D1 protein levels are greatly reduced following prolonged BVD-523 treatment. Similarly, gene expression and protein abundance of apoptosis effectors often require intact MAPK signaling, and total levels of Bim-EL increase following prolonged BVD-523 treatment. As noted above, however, PARP protein cleavage and increased apoptosis were not noted in the A375 cell background; this suggests that additional factors may influence whether changes in BVD-

523/ERK-dependent effector signaling are translated into definitive events such as cell death and cell cycle arrest.

[0167] Consistent with the cellular activity of BVD-523, marker analysis suggests that ERK inhibition alters a variety of molecular signaling events in cancer cells, making them susceptible to both decreased cell proliferation and survival.

[0168] In sum, FIGS. 16-18 show that BVD-523 inhibits the MAPK signaling pathway and may be more favorable compared to RAF or MEK inhibition in this setting.

[0169] Finally, properties of BVD-523 may make this a preferred agent for use as an ERK inhibitor, compared to other agents with a similar activity. It is known that kinase inhibitor drugs display unique and specific interactions with their enzyme targets, and that drug efficacy is strongly influenced by both the mode of direct inhibition, as well as susceptibility to adaptive changes that occur following treatment. For example, inhibitors of ABL, KIT, EGFR and ALK kinases are effective only when their cognate target is found in active or inactive configurations. Likewise, certain of these inhibitors are uniquely sensitive to either secondary genetic mutation, or post-translational adaptive changes, of the protein target. Finally, RAF inhibitors show differential potency to RAF kinases present in certain protein complexes and/or subcellular localizations. In summary, as ERK kinases are similarly known to exist in diverse, variable, and complex biochemical states, it appears likely that BVD-523 may interact with and inhibit these targets in a fashion that is distinct and highly preferable to other agents.

Example 7

Effects of BVD-523 and Benchmark ERK BRAF and MEK Inhibitors on Viability and MAPK Signalling

Single Agent Proliferation Assay

[0170] Cells were seeded in 96-well plates at the densities indicated in Table 15 in McCoy's 5A containing 10% FBS and allowed to adhere overnight prior to addition of compound or vehicle control. Compounds were prepared from DMSO stocks to give the desired final concentrations. The final DMSO concentration was constant at 0.1%. Test compounds were incubated with the cells for 96h at 37°C, 5% CO_2 in a humidified atmosphere. CellTiter-Glo® reagent (Promega, Madison, WI) was added according to manufacturer's instructions and luminescence detected using the BMG FLUOstar plate reader (BMG Labtech, Ortenberg, Germany). The average media only background value was deducted and the data analysed using a 4-parameter logistic equation in GraphPad Prism (GraphPad Software, La Jolla, CA).

Combination Proliferation Assay

[0171] Cells were seeded into triplicate 96-well plates at the densities indicated in Table 15 in McCoy's 5A containing 10% FBS and allowed to adhere overnight prior to addition of test compound or vehicle control. Combinations were tested using a 10x8 dose matrix. The final DMSO concentration was constant at 0.2%.

[0172] Test compounds were incubated with the cells for 96h at 37°C, 5% CO_2 in a humidified atmosphere. Cells were stained with Hoechst stain and fluorescence detected as described above. The average media only background value was deducted and the data analysed.

[0173] Combination interactions across the dose matrix were determined by the Loewe Additivity and Bliss independence models using Chalice™ Combination Analysis Software (Horizon Discovery Group, Cambridge, MA) as outlined in the user manual (available at chalice.horizondiscovery.com/chalice-portal/documentation/analyzer/home.jsp). Synergy is determined by comparing the experimentally observed level of inhibition at each combination point with the value expected for additivity, which is derived from the single-agent responses along the edges of the matrix. Potential synergistic interactions were identified by displaying the calculated excess inhibition over that predicted as being additive across the dose matrix as a heat map, and by reporting a quantitative 'Synergy Score' based on the Loewe model. The single agent data derived from the combination assay plates were presented as dose-response curves generated in Chalice™.

Table 15 - Cell Line Seeding Density

Cell Line	Seeding density (cells/well)		
	96-well Proliferation	6-Well Western	10cm dish Westerns
RKO Parental	1000	$1x10^6$	$2.9x10^6$
RKO MEK1 (Q56P/+) Clone 1	1250	Not tested	Not tested
RKO MEK1 (Q56P/+) Clone 2	1000	$7.5x10^5$	$2x10^6$

Western Blotting

[0174] Cells were seeded into 6-well plates (Experiment 1) or 10cm dishes (Experiment 2) at the densities indicated in Table 15 in McCoy's 5A containing 10% FBS and allowed to adhere overnight prior to addition of compound or vehicle control. Test compounds were added and incubated with

the cells for 4 or 24h at 37°C, 5% CO_2 in a humidified atmosphere. Cells were harvested by trypsinisation, pelleted by centrifugation and snap frozen on dry ice.

[0175] Lysates were prepared using RIPA buffer (50mM Tris-hydrochloride, pH 8.0; 150mM sodium chloride; 1.0% Igepal CA-630 (NP-40); 0.5% sodium deoxycholate; 0.1% sodium dodecyl sulphate; 1xcomplete EDTA-free protease inhibitor cocktail (Roche, Nutley, NJ; cat 05 892 791 001); 1xphosSTOP phosphatase inhibitor cocktail (Roche Nutley, NJ; cat. 04 906 837 001)) and clarified by centrifugation at 11,000 rpm for 10min in a bench-top centrifuge.

[0176] Total protein in the lysates was quantitated by BCA assay according to the manufacturer's instructions (Pierce™ BCA Protein Assay Kit; Thermo Scientific, Waltham, MA; cat. 23225), boiled in sample buffer (NuPAGE LDS Sample Buffer; (Invitrogen, Carlsbad, CA; cat. NP0007)) and stored at -80°C.

[0177] Equal amounts of protein (40µg) were resolved on NuPAGE 4-12% Bis-Tris gels (Invitrogen, Carlsbad, CA; cat. WG1402BOX) and blotted onto PVDF membranes using iBlot gel transfer stacks (Invitrogen, Carlsbad, CA; cat. IB4010-01) on an iBlot gel transfer device (Invitrogen Carlsbad, CA) according to the manufacturer's instructions.

[0178] Blots were probed using the antibodies and block conditions detailed in Table 16. Western blots were developed using Pierce™ ECL2 Western blotting substrate (Thermo Scientific, Waltham, MA; cat. 80196) and imaged using a FluorChem M Western blot imager (ProteinSimple, San Jose, CA).

Table 16 – Antibodies and Western Blotting Conditions

Antigen	Size (kDa)	Supplier	Cat No	Dilution	Incubation/block Conditions	Secondary
pRSK-T359/S363	90	Millipore	04-419	1:20000	o/n 4°C 5% BSA	anti-rabbit
Total RSK	90	Cell Signaling	9333	1:1000	o/n 4°C 5% BSA	anti-rabbit
pErk 1/2	42/44	Cell Signaling	9106S	1:500	o/n 4°C 5% milk	anti-mouse
Total ERK	42/44	Cell Signaling	9102	1:2000	o/n 4°C 5% milk	anti-rabbit
pMEK1/2	45	Cell Signaling	9154	1:1000	o/n 4°C 5% BSA	anti-rabbit
Total MEK	45	Cell Signaling	9126	1:1000	o/n 4°C 5% BSA	anti-rabbit
DUSP6	48	Cell Signaling	3058S	1:1000	o/n 4°C 5% BSA	anti-rabbit
pRB (Ser780)	105	Cell Signaling	9307	1:2000	o/n 4°C 5% BSA	anti-rabbit
CCND1	34	Abcam	ab6152	1:500	o/n 4°C 5% milk	anti-mouse
B-Actin	42	Sigma	A5441	1 100,000	o/n 4°C 5% milk	anti-mouse
Anti-rabbit HRP-conjugated secondary	-	Cell Signaling	7074S	1:2000	1h room temp; Block matched to primary Antibody	-
Anti-mouse HRP-conjugated secondary	-	Cell Signaling	7076	1:5000	1h room temp; Block matched to primary Antibody	-

[0179] The MEK1 (Q56P) mutation exemplifies a class of clinically relevant MEK1/2 activating mutations known to up-regulate the MAPK pathway and drive acquired resistance to BRAF or MEK inhibitors.

[0180] This study used a pair of RKO BRAF(V600E) cell lines that are isogenic for the presence or absence of a MEK1 (Q56P) activating mutation, to assess the effect that activating MEK mutations have in response to the novel ERK inhibitor BVD-523 versus other benchmark MAPK inhibitors.

[0181] Effects of on cell viability were assessed by quantitating cellular ATP levels using CellTiter-Glo® after 96h. Single agent assays demonstrated that the double mutant BRAF(V600E)::MEK1(Q56P) cells displayed a

markedly reduced sensitivity to inhibition with benchmark clinical BRAF (exemplified by Dabrafenib) or MEK (exemplified by Trametinib) inhibitors relative to the parental BRAF(V600E) cells, which demonstrates the suitability of this isogenic model for recapitulating the acquired resistance known to be associated with this class of mutation in the clinic (Table 17).

Table 17 – Single Agent IC_{50} Values

Compound	RKO Parental	RKO MEK1 Q56P/+ Cl.1	RKO MEK1 Q56P/+ Cl.2
BVD-523	0.20	0.17	0.18
SCH772984	0.04	0.14	0.12
Dabrafenib	n.d.	n.d.	n.d.
Trametinib	0.006	0.093	0.080
Paclitaxel	0.002	0.002	0.002

n.d. – not determined, only a partial dose response achieved

[0182] In contrast, response to BVD-523 was identical in both the parental and double mutant cells, indicating that BVD-523 is not susceptible to this mechanism of acquired resistance.

[0183] These results were identical in two independently derived double mutant BRAF(V600E)::MEK1(Q56P) cell line clones confirming that these differences in response versus the parental cells were specifically related to the presence of the MEK1 mutation rather than an unrelated clonal artifact (FIG. 22). Similar results were also observed with a second mechanistically distinct benchmark ERK inhibitor (SCH772984), which supports the notion that these observations are specifically related to inhibition of ERK and not due to an off-target effect.

[0184] The effect of combining BVD-523 with a BRAF inhibitor (exemplified by Dabrafenib) was also assessed in these cell lines across a matrix of concentrations using the Loewe Addivity or Bliss Independence

models with Horizon's Chalice™ combination analysis software (FIG. 23 – FIG. 24). The presence of potentially synergistic interactions was then assessed by displaying the calculated excess inhibition over that predicted as being additive across the dose matrix as a heat map, and by calculating a 'Volume Score' that shows whether the overall response to a combination is synergistic (positive values), antagonistic (negative values) or additive (~ 0).

[0185] The results suggest that the BVD-523::Dabrafenib combination was mainly additive in the parental and mutant cell line. In contrast, the combination of a MEK inhibitor (trametinib) plus Dabrafenib, while being mostly additive in the parental cell line, showed strong synergy in the double mutant BRAF(V600E)::MEK1(Q56P) cell line (FIG. 25). Loewe Volumes, Bliss Volumes and Synergy scores for the combinations tested are shown in Tables 18 - 20, respectively and are shown graphed in FIG. 26.

Table 18 – Loewe Volumes

	RKO Parental	RKO MEK1 (Q56P) - Clone 1	RKO MEK1 (Q56P) - Clone 2
BVD-523 x Dabrafenib	3.54	2.88	2.35
Dabrafenib x SCH772984	5.2	6.79	6.14
Dabrafenib x Trametinib	5.68	17.6	15.4

Table 19 – Bliss Volumes

	RKO Parental	RKO MEK1 (Q56P) - Clone 1	RKO MEK1 (Q56P) - Clone 2
BVD-523 x Dabrafenib	-0.894	0.527	1.42
Dabrafenib x SCH772984	0.209	4.3	5.07
Dabrafenib x Trametinib	0.353	10.8	9.87

Table 20 – Synergy Scores

	RKO Parental	RKO MEK1 (Q56P) - Clone 1	RKO MEK1 (Q56P) - Clone 2
BVD-523 x Dabrafenib	3.18	2.31	1.77
Dabrafenib x SCH772984	4.56	5.57	4.36
Dabrafenib x Trametinib	5.58	11	9.84

[0186] Effects on MAPK pathway signally was assessed by Western blotting. The levels of basal ERK phosphorylation (DMSO samples) was markedly up-regulated in the MEK1(Q56P)-expressing line relative to parental further confirming that this isogenic model faithfully recapitulates the expected phenotype for the expression of MEK activating acquired resistance mutations.

[0187] In the parental BRAF(V600E) RKO cells, a reduced level of RSK1/2 phosphorylation is observed following acute treatment with RAF, MEK and ERK kinase inhibitors at pharmacologically active concentrations. In contrast, isogenic, double mutant BRAFV600E::MEK1Q56P cells do not exhibit reduced RSK phosphorylation following BRAF or MEK inhibitor treatment, while BVD-523 remains effective at similar concentrations (FIG. 27). The dotted lines indicate that the trametinib-treated samples (plus

matched DMSO control) and blots are derived from a separate experiment to the BRAFi and BVD-523 treated samples.

[0188] Changes in effector gene signaling consistent with cell growth inhibition patterns are observed following prolonged inhibitor treatment. In parental RKO lines, a reduced level of phosphorylated pRB is observed following prolonged MEK and ERK inhibitor treatment. At the level of pRB modulation, MEK1 mutant lines appear insensitive to low concentration MEK inhibitor treatment, while higher concentrations remain effective. Critically, BVD-523 potency against pRB activity does not appear to be strongly affected by MEK mutation. Surprisingly, RAF inhibitor treatment does not affect pRB status, despite potent inhibition of upstream signaling, in both parental and MEK mutant backgrounds.

[0189] In summary, these results show that BVD-523 is not susceptible to acquired resistance driven by MEK activating mutations such as MEK1 (Q56P). In addition they suggest that in combination the interactions between BVD-523 and BRAFi (exemplified by Dabrafenib) are additive irrespective of the presence of a MEK activating mutation.

Example 8

Combination Interactions Between ERK inhibitors

[0190] RAF mutant melanoma cell line A375 cells were cultured in DMEM with 10% FBS and seeded into triplicate 96-well plates at an initial density of 2000 cells per well. Combination interactions between ERK inhibitors BVD-523 and SCH772984 were analized after 72 hours as described above in Example 4. Viability was determined using CellTiter-Glo® reagent (Promega, Madison, WI) according to manufacturer's instructions and

luminescence was detected using the BMG FLUOstar plate reader (BMG Labtech, Ortenberg, Germany).

[0191] Visualization of the Loewe and Bliss 'excess inhibition' heat maps suggested that the combination of BVD-523 and SCH772984 was mainly additive with windows of potential synergy in mid-range doses (FIG. 28).

[0192] In summary, these results suggest that interactions between BVD-523 and SCH772984 are at least additive, and in some cases synergistic.

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[0193] All documents cited in this application are hereby incorporated by reference as if recited in full herein.

[0194] Although illustrative embodiments of the present invention have been described herein, it should be understood that the invention is not limited to those described, and that various other changes or modifications may be made by one skilled in the art without departing from the scope or spirit of the invention.

WHAT IS CLAIMED IS:

1. A method for treating or ameliorating the effects of a cancer in a subject, which cancer is refractory or resistant to non-ERK MAPK pathway inhibitor therapy, the method comprising administering to the subject an effective amount of BVD-523 or a pharmaceutically acceptable salt thereof.

2. The method according to claim 1, wherein the non-ERK MAPK pathway inhibitor therapy is selected from the group consisting of a RAS inhibitor, a RAF inhibitor, a MEK inhibitor, and combinations thereof.

3. The method according to claim 1, wherein the non-ERK MAPK pathway inhibitor therapy is selected from the group consisting of a BRAF inhibitor, a MEK inhibitor, and combinations thereof.

4. The method according to claim 1, wherein substantially all phosphorylation of RSK is inhibited after administration of BVD-523 or a pharmaceutically acceptable salt thereof.

5. The method according to claim 1, wherein the subject is a mammal.

6. The method according to claim 5, wherein the mammal is selected from the group consisting of humans, primates, farm animals, and domestic animals.

7. The method according to claim 5, wherein the mammal is a human.

8. The method according to claim 1, wherein the cancer has MAPK activity.

9. The method according to claim 8, wherein the cancer is a solid tumor cancer or a hematologic cancer.

10. The method according to claim 8, wherein the cancer is selected from the group consisting of a cancer of the large intestine, breast cancer, pancreatic cancer, skin cancer, and endometrial cancers.

11. The method according to claim 8, wherein the cancer is melanoma.

12. The method according to claim 1 further comprising administering to the subject at least one additional therapeutic agent selected from the group consisting of an antibody or fragment thereof, a cytotoxic agent, a toxin, a radionuclide, an immunomodulator, a photoactive therapeutic agent, a radiosensitizing agent, a hormone, an anti-angiogenesis agent, and combinations thereof.

13. The method according to claim 12, wherein the additional therapeutic agent is an inhibitor of the PI3K/Akt pathway.

14. The method according to claim 13, wherein the inhibitor of the PI3K/Akt pathway is selected from the group consisting of A-674563 (CAS # 552325-73-2), AGL 2263, AMG-319 (Amgen, Thousand Oaks, CA), AS-041164 (5-benzo[1,3]dioxol-5-ylmethylene-thiazolidine-2,4-dione), AS-604850 (5-(2,2-Difluoro-benzo[1,3]dioxol-5-ylmethylene)-thiazolidine-2,4-dione), AS-605240 (5-quinoxilin-6-methylene-1,3-thiazolidine-2,4-dione), AT7867 (CAS # 857531-00-1), benzimidazole series, Genentech (Roche Holdings Inc., South San Francisco, CA), BML-257 (CAS # 32387-96-5), CAL-120 (Gilead Sciences, Foster City, CA), CAL-129 (Gilead Sciences), CAL-130 (Gilead

Sciences), CAL-253 (Gilead Sciences), CAL-263 (Gilead Sciences), CAS # 612847-09-3, CAS # 681281-88-9, CAS # 75747-14-7, CAS # 925681-41-0, CAS # 98510-80-6, CCT128930 (CAS # 885499-61-6), CH5132799 (CAS # 1007207-67-1), CHR-4432 (Chroma Therapeutics, Ltd., Abingdon, UK), FPA 124 (CAS # 902779-59-3), GS-1101 (CAL-101) (Gilead Sciences), GSK 690693 (CAS # 937174-76-0), H-89 (CAS # 127243-85-0), Honokiol, IC87114 (Gilead Science), IPI-145 (Intellikine Inc.), KAR-4139 (Karus Therapeutics, Chilworth, UK), KAR-4141 (Karus Therapeutics), KIN-1 (Karus Therapeutics), KT 5720 (CAS # 108068-98-0), Miltefosine, MK-2206 dihydrochloride (CAS # 1032350-13-2), ML-9 (CAS # 105637-50-1), Naltrindole Hydrochloride, OXY-111A (NormOxys Inc., Brighton, MA), perifosine, PHT-427 (CAS # 1191951-57-1), PI3 kinase delta inhibitor, Merck KGaA (Merck & Co., Whitehouse Station, NJ), PI3 kinase delta inhibitors, Genentech (Roche Holdings Inc.), PI3 kinase delta inhibitors, Incozen (Incozen Therapeutics, Pvt. Ltd., Hydrabad, India), PI3 kinase delta inhibitors-2, Incozen (Incozen Therapeutics), PI3 kinase inhibitor, Roche-4 (Roche Holdings Inc.), PI3 kinase inhibitors, Roche (Roche Holdings Inc.), PI3 kinase inhibitors, Roche-5 (Roche Holdings Inc.), PI3-alpha/delta inhibitors, Pathway Therapeutics (Pathway Therapeutics Ltd., South San Francisco, CA), PI3-delta inhibitors, Cellzome (Cellzome AG, Heidelberg, Germany), PI3-delta inhibitors, Intellikine (Intellikine Inc., La Jolla, CA), PI3-delta inhibitors, Pathway Therapeutics-1 (Pathway Therapeutics Ltd.), PI3-delta inhibitors, Pathway Therapeutics-2 (Pathway Therapeutics Ltd.), PI3-delta/gamma inhibitors, Cellzome (Cellzome AG), PI3-delta/gamma inhibitors, Cellzome (Cellzome AG), PI3-delta/gamma inhibitors, Intellikine (Intellikine Inc.), PI3-delta/gamma inhibitors, Intellikine (Intellikine Inc.), PI3-

delta/gamma inhibitors, Pathway Therapeutics (Pathway Therapeutics Ltd.), PI3-delta/gamma inhibitors, Pathway Therapeutics (Pathway Therapeutics Ltd.), PI3-gamma inhibitor Evotec (Evotec), PI3-gamma inhibitor, Cellzome (Cellzome AG), PI3-gamma inhibitors, Pathway Therapeutics (Pathway Therapeutics Ltd.), PI3K delta/gamma inhibitors, Intellikine-1 (Intellikine Inc.), PI3K delta/gamma inhibitors, Intellikine-1 (Intellikine Inc.), pictilisib (Roche Holdings Inc.), PIK-90 (CAS # 677338-12-4), SC-103980 (Pfizer, New York, NY), SF-1126 (Semafore Pharmaceuticals, Indianapolis, IN), SH-5, SH-6, Tetrahydro Curcumin, TG100-115 (Targegen Inc., San Diego, CA), Triciribine, X-339 (Xcovery, West Palm Beach, FL), XL-499 (Evotech, Hamburg, Germany), pharmaceutically acceptable salts thereof, and combinations thereof.

15. A method for treating or ameliorating the effects of a cancer in a subject comprising:

(a) identifying a subject with cancer that has become refractory or resistant to BRAF inhibitor therapy, MEK inhibitor therapy, or BRAF and MEK inhibitor therapy; and

(b) administering to the subject with said refractory or resistant cancer an effective amount of an ERK inhibitor, which is BVD-523 or a pharmaceutically acceptable salt thereof.

16. The method according to claim 15, wherein the subject is a mammal.

17. The method according to claim 16, wherein the mammal is selected from the group consisting of humans, primates, farm animals, and domestic animals.

18. The method according to claim 16, wherein the mammal is a human.

19. The method according to claim 15, wherein the cancer has MAPK activity.

20. The method according to claim 19, wherein the cancer is a solid tumor cancer or a hematologic cancer.

21. The method according to claim 19, wherein the cancer is selected from the group consisting of a cancer of the large intestine, breast cancer, pancreatic cancer, skin cancer, and endometrial cancers.

22. The method according to claim 19, wherein the cancer is melanoma.

23. The method according to claim 15, wherein identifying a subject with cancer that is refractory or resistant to BRAF and/or MEK inhibitor therapy comprises:

(a) obtaining a biological sample from the subject; and

(b) screening the sample to determine whether the subject has become resistant to an inhibitor therapy selected from the group consisting of BRAF inhibitor therapy, MEK inhibitor therapy, and combinations thereof.

24. The method according to claim 23, wherein the screening for a cancer that is refractory or resistant to BRAF inhibitor therapy comprises identifying

(i) a switch between RAF isoforms, (ii) upregulation of RTK or NRAS signaling, (iii) reactivation of mitogen activated protein kinase (MAPK) signaling, (iv) the presence of a MEK activating mutation, and combinations thereof.

25. The method according to claim 23, wherein the screening for a cancer that is refractory or resistant to MEK inhibitor therapy comprises identifying (i) amplification of mutant BRAF, (ii) STAT3 upregulation, (iii) mutations in the allosteric pocket of MEK that directly block binding of inhibitors to MEK or lead to constitutive MEK activity, and combinations thereof.

26. The method according to claim 15 further comprising administering at least one additional therapeutic agent selected from the group consisting of an antibody or fragment thereof, a cytotoxic agent, a toxin, a radionuclide, an immunomodulator, a photoactive therapeutic agent, a radiosensitizing agent, a hormone, an anti-angiogenesis agent, and combinations thereof.

27. The method according to claim 26, wherein the additional therapeutic agent is an inhibitor of the PI3K/Akt pathway.

28. The method according to claim 27, wherein the inhibitor of the PI3K/Akt pathway is selected from the group consisting of A-674563 (CAS # 552325-73-2), AGL 2263, AMG-319 (Amgen, Thousand Oaks, CA), AS-041164 (5-benzo[1,3]dioxol-5-ylmethylene-thiazolidine-2,4-dione), AS-604850 (5-(2,2-Difluoro-benzo[1,3]dioxol-5-ylmethylene)-thiazolidine-2,4-dione), AS-605240 (5-quinoxilin-6-methylene-1,3-thiazolidine-2,4-dione), AT7867 (CAS # 857531-00-1), benzimidazole series, Genentech (Roche Holdings Inc., South San Francisco, CA), BML-257 (CAS # 32387-96-5), CAL-120 (Gilead

Sciences, Foster City, CA), CAL-129 (Gilead Sciences), CAL-130 (Gilead Sciences), CAL-253 (Gilead Sciences), CAL-263 (Gilead Sciences), CAS # 612847-09-3, CAS # 681281-88-9, CAS # 75747-14-7, CAS # 925681-41-0, CAS # 98510-80-6, CCT128930 (CAS # 885499-61-6), CH5132799 (CAS # 1007207-67-1), CHR-4432 (Chroma Therapeutics, Ltd., Abingdon, UK), FPA 124 (CAS # 902779-59-3), GS-1101 (CAL-101) (Gilead Sciences), GSK 690693 (CAS # 937174-76-0), H-89 (CAS # 127243-85-0), Honokiol, IC87114 (Gilead Science), IPI-145 (Intellikine Inc.), KAR-4139 (Karus Therapeutics, Chilworth, UK), KAR-4141 (Karus Therapeutics), KIN-1 (Karus Therapeutics), KT 5720 (CAS # 108068-98-0), Miltefosine, MK-2206 dihydrochloride (CAS # 1032350-13-2), ML-9 (CAS # 105637-50-1), Naltrindole Hydrochloride, OXY-111A (NormOxys Inc., Brighton, MA), perifosine, PHT-427 (CAS # 1191951-57-1), PI3 kinase delta inhibitor, Merck KGaA (Merck & Co., Whitehouse Station, NJ), PI3 kinase delta inhibitors, Genentech (Roche Holdings Inc.), PI3 kinase delta inhibitors, Incozen (Incozen Therapeutics, Pvt. Ltd., Hydrabad, India), PI3 kinase delta inhibitors-2, Incozen (Incozen Therapeutics), PI3 kinase inhibitor, Roche-4 (Roche Holdings Inc.), PI3 kinase inhibitors, Roche (Roche Holdings Inc.), PI3 kinase inhibitors, Roche-5 (Roche Holdings Inc.), PI3-alpha/delta inhibitors, Pathway Therapeutics (Pathway Therapeutics Ltd., South San Francisco, CA), PI3-delta inhibitors, Cellzome (Cellzome AG, Heidelberg, Germany), PI3-delta inhibitors, Intellikine (Intellikine Inc., La Jolla, CA), PI3-delta inhibitors, Pathway Therapeutics-1 (Pathway Therapeutics Ltd.), PI3-delta inhibitors, Pathway Therapeutics-2 (Pathway Therapeutics Ltd.), PI3-delta/gamma inhibitors, Cellzome (Cellzome AG), PI3-delta/gamma inhibitors, Cellzome (Cellzome AG), PI3-delta/gamma inhibitors, Intellikine

(Intellikine Inc.), PI3-delta/gamma inhibitors, Intellikine (Intellikine Inc.), PI3-delta/gamma inhibitors, Pathway Therapeutics (Pathway Therapeutics Ltd.), PI3-delta/gamma inhibitors, Pathway Therapeutics (Pathway Therapeutics Ltd.), PI3-gamma inhibitor Evotec (Evotec), PI3-gamma inhibitor, Cellzome (Cellzome AG), PI3-gamma inhibitors, Pathway Therapeutics (Pathway Therapeutics Ltd.), PI3K delta/gamma inhibitors, Intellikine-1 (Intellikine Inc.), PI3K delta/gamma inhibitors, Intellikine-1 (Intellikine Inc.), pictilisib (Roche Holdings Inc.), PIK-90 (CAS # 677338-12-4), SC-103980 (Pfizer, New York, NY), SF-1126 (Semafore Pharmaceuticals, Indianapolis, IN), SH-5, SH-6, Tetrahydro Curcumin, TG100-115 (Targegen Inc., San Diego, CA), Triciribine, X-339 (Xcovery, West Palm Beach, FL), XL-499 (Evotech, Hamburg, Germany), pharmaceutically acceptable salts thereof, and combinations thereof.

29. A method for treating or ameliorating the effects of cancer in a subject, which cancer is refractory or resistant to BRAF inhibitor therapy, MEK inhibitor therapy, or both, the method comprising administering to the subject an effective amount of BVD-523 or a pharmaceutically acceptable salt thereof.

30. The method according to claim 29, wherein the subject is a mammal.

31. The method according to claim 30, wherein the mammal is selected from the group consisting of humans, primates, farm animals, and domestic animals.

32. The method according to claim 30, wherein the mammal is a human.

33. The method according to claim 29, wherein the cancer has MAPK activity.

34. The method according to claim 33, wherein the cancer is a solid tumor cancer or a hematologic cancer.

35. The method according to claim 33, wherein the cancer is selected from the group consisting of a cancer of the large intestine, breast cancer, pancreatic cancer, skin cancer, and endometrial cancers.

36. The method according to claim 33, wherein the cancer is melanoma.

37. The method according to claim 29, wherein the cancer is determined to be refractory or resistant to BRAF inhibitor therapy based on one or more of the following: (i) a switch between RAF isoforms, (ii) upregulation of RTK or NRAS signaling, (iii) reactivation of mitogen activated protein kinase (MAPK) signaling, (iv) the presence of a MEK activating mutation.

38. The method according to claim 29, wherein the cancer is determined to be refractory or resistant to MEK inhibitor therapy based on one or more of the following: (i) amplification of mutant BRAF, (ii) STAT3 upregulation, (iii) mutations in the allosteric pocket of MEK that directly block binding of inhibitors to MEK or lead to constitutive MEK activity.

39. The method according to claim 29 further comprising administering to the subject at least one additional therapeutic agent selected from the group consisting of an antibody or fragment thereof, a cytotoxic agent, a toxin, a radionuclide, an immunomodulator, a photoactive therapeutic agent, a

radiosensitizing agent, a hormone, an anti-angiogenesis agent, and combinations thereof.

40. The method according to claim 39, wherein the additional therapeutic agent is an inhibitor of the PI3K/Akt pathway.

41. The method according to claim 40, wherein the inhibitor of the PI3K/Akt pathway is selected from the group consisting of A-674563 (CAS # 552325-73-2), AGL 2263, AMG-319 (Amgen, Thousand Oaks, CA), AS-041164 (5-benzo[1,3]dioxol-5-ylmethylene-thiazolidine-2,4-dione), AS-604850 (5-(2,2-Difluoro-benzo[1,3]dioxol-5-ylmethylene)-thiazolidine-2,4-dione), AS-605240 (5-quinoxilin-6-methylene-1,3-thiazolidine-2,4-dione), AT7867 (CAS # 857531-00-1), benzimidazole series, Genentech (Roche Holdings Inc., South San Francisco, CA), BML-257 (CAS # 32387-96-5), CAL-120 (Gilead Sciences, Foster City, CA), CAL-129 (Gilead Sciences), CAL-130 (Gilead Sciences), CAL-253 (Gilead Sciences), CAL-263 (Gilead Sciences), CAS # 612847-09-3, CAS # 681281-88-9, CAS # 75747-14-7, CAS # 925681-41-0, CAS # 98510-80-6, CCT128930 (CAS # 885499-61-6), CH5132799 (CAS # 1007207-67-1), CHR-4432 (Chroma Therapeutics, Ltd., Abingdon, UK), FPA 124 (CAS # 902779-59-3), GS-1101 (CAL-101) (Gilead Sciences), GSK 690693 (CAS # 937174-76-0), H-89 (CAS # 127243-85-0), Honokiol, IC87114 (Gilead Science), IPI-145 (Intellikine Inc.), KAR-4139 (Karus Therapeutics, Chilworth, UK), KAR-4141 (Karus Therapeutics), KIN-1 (Karus Therapeutics), KT 5720 (CAS # 108068-98-0), Miltefosine, MK-2206 dihydrochloride (CAS # 1032350-13-2), ML-9 (CAS # 105637-50-1), Naltrindole Hydrochloride, OXY-111A (NormOxys Inc., Brighton, MA), perifosine, PHT-427 (CAS # 1191951-

57-1), PI3 kinase delta inhibitor, Merck KGaA (Merck & Co., Whitehouse Station, NJ), PI3 kinase delta inhibitors, Genentech (Roche Holdings Inc.), PI3 kinase delta inhibitors, Incozen (Incozen Therapeutics, Pvt. Ltd., Hydrabad, India), PI3 kinase delta inhibitors-2, Incozen (Incozen Therapeutics), PI3 kinase inhibitor, Roche-4 (Roche Holdings Inc.), PI3 kinase inhibitors, Roche (Roche Holdings Inc.), PI3 kinase inhibitors, Roche-5 (Roche Holdings Inc.), PI3-alpha/delta inhibitors, Pathway Therapeutics (Pathway Therapeutics Ltd., South San Francisco, CA), PI3-delta inhibitors, Cellzome (Cellzome AG, Heidelberg, Germany), PI3-delta inhibitors, Intellikine (Intellikine Inc., La Jolla, CA), PI3-delta inhibitors, Pathway Therapeutics-1 (Pathway Therapeutics Ltd.), PI3-delta inhibitors, Pathway Therapeutics-2 (Pathway Therapeutics Ltd.), PI3-delta/gamma inhibitors, Cellzome (Cellzome AG), PI3-delta/gamma inhibitors, Cellzome (Cellzome AG), PI3-delta/gamma inhibitors, Intellikine (Intellikine Inc.), PI3-delta/gamma inhibitors, Intellikine (Intellikine Inc.), PI3-delta/gamma inhibitors, Pathway Therapeutics (Pathway Therapeutics Ltd.), PI3-delta/gamma inhibitors, Pathway Therapeutics (Pathway Therapeutics Ltd.), PI3-gamma inhibitor Evotec (Evotec), PI3-gamma inhibitor, Cellzome (Cellzome AG), PI3-gamma inhibitors, Pathway Therapeutics (Pathway Therapeutics Ltd.), PI3K delta/gamma inhibitors, Intellikine-1 (Intellikine Inc.), PI3K delta/gamma inhibitors, Intellikine-1 (Intellikine Inc.), pictilisib (Roche Holdings Inc.), PIK-90 (CAS # 677338-12-4), SC-103980 (Pfizer, New York, NY), SF-1126 (Semafore Pharmaceuticals, Indianapolis, IN), SH-5, SH-6, Tetrahydro Curcumin, TG100-115 (Targegen Inc., San Diego, CA), Triciribine, X-339 (Xcovery, West Palm Beach, FL), XL-499 (Evotech, Hamburg,

Germany), pharmaceutically acceptable salts thereof, and combinations thereof.

42. A method for identifying a subject having cancer who would benefit from therapy with an ERK inhibitor, the method comprising:

(a) obtaining a biological sample from the subject; and

(b) screening the sample to determine whether the subject has one or more of the following markers:

(i) a switch between RAF isoforms,

(ii) upregulation of receptor tyrosine kinase (RTK) or NRAS signaling,

(iii) reactivation of mitogen activated protein kinase (MAPK) signaling,

(iv) the presence of a MEK activating mutation,

(v) amplification of mutant BRAF,

(vi) STAT3 upregulation,

(vii) mutations in the allosteric pocket of MEK that directly block binding of inhibitors to MEK or lead to constitutive MEK activity,

wherein the presence of one or more of the markers confirms that the subject's cancer is refractory or resistant to BRAF and/or MEK inhibitor

therapy and that the subject would benefit from therapy with an ERK inhibitor, which is BVD-523 or a pharmaceutically acceptable salt thereof.

43. The method according to claim 42, wherein the subject is a mammal.

44. The method according to claim 43, wherein the mammal is selected from the group consisting of humans, primates, farm animals, and domestic animals.

45. The method according to claim 43, wherein the mammal is a human.

46. The method according to claim 42, wherein the cancer has MAPK activity.

47. The method according to claim 46, wherein the cancer is a solid tumor cancer or a hematologic cancer.

48. The method according to claim 46, wherein the cancer is selected from the group consisting of a cancer of the large intestine, breast cancer, pancreatic cancer, skin cancer, and endometrial cancers.

49. The method according to claim 46, wherein the cancer is melanoma.

50. The method according to claim 42 further comprising administering BVD-523 or a pharmaceutically acceptable salt thereof to a subject having one or more of the markers.

51. The method according to claim 50 further comprising administering to the subject having one or more of the markers at least one additional therapeutic agent selected from the group consisting of an antibody or

fragment thereof, a cytotoxic agent, a toxin, a radionuclide, an immunomodulator, a photoactive therapeutic agent, a radiosensitizing agent, a hormone, an anti-angiogenesis agent, and combinations thereof.

52. The method according to claim 51, wherein the additional therapeutic agent is an inhibitor of the PI3K/Akt pathway.

53. The method according to claim 52, wherein the inhibitor of the PI3K/Akt pathway is selected from the group consisting of A-674563 (CAS # 552325-73-2), AGL 2263, AMG-319 (Amgen, Thousand Oaks, CA), AS-041164 (5-benzo[1,3]dioxol-5-ylmethylene-thiazolidine-2,4-dione), AS-604850 (5-(2,2-Difluoro-benzo[1,3]dioxol-5-ylmethylene)-thiazolidine-2,4-dione), AS-605240 (5-quinoxilin-6-methylene-1,3-thiazolidine-2,4-dione), AT7867 (CAS # 857531-00-1), benzimidazole series, Genentech (Roche Holdings Inc., South San Francisco, CA), BML-257 (CAS # 32387-96-5), CAL-120 (Gilead Sciences, Foster City, CA), CAL-129 (Gilead Sciences), CAL-130 (Gilead Sciences), CAL-253 (Gilead Sciences), CAL-263 (Gilead Sciences), CAS # 612847-09-3, CAS # 681281-88-9, CAS # 75747-14-7, CAS # 925681-41-0, CAS # 98510-80-6, CCT128930 (CAS # 885499-61-6), CH5132799 (CAS # 1007207-67-1), CHR-4432 (Chroma Therapeutics, Ltd., Abingdon, UK), FPA 124 (CAS # 902779-59-3), GS-1101 (CAL-101) (Gilead Sciences), GSK 690693 (CAS # 937174-76-0), H-89 (CAS # 127243-85-0), Honokiol, IC87114 (Gilead Science), IPI-145 (Intellikine Inc.), KAR-4139 (Karus Therapeutics, Chilworth, UK), KAR-4141 (Karus Therapeutics), KIN-1 (Karus Therapeutics), KT 5720 (CAS # 108068-98-0), Miltefosine, MK-2206 dihydrochloride (CAS # 1032350-13-2), ML-9 (CAS # 105637-50-1), Naltrindole Hydrochloride, OXY-

111A (NormOxys Inc., Brighton, MA), perifosine, PHT-427 (CAS # 1191951-57-1), PI3 kinase delta inhibitor, Merck KGaA (Merck & Co., Whitehouse Station, NJ), PI3 kinase delta inhibitors, Genentech (Roche Holdings Inc.), PI3 kinase delta inhibitors, Incozen (Incozen Therapeutics, Pvt. Ltd., Hydrabad, India), PI3 kinase delta inhibitors-2, Incozen (Incozen Therapeutics), PI3 kinase inhibitor, Roche-4 (Roche Holdings Inc.), PI3 kinase inhibitors, Roche (Roche Holdings Inc.), PI3 kinase inhibitors, Roche-5 (Roche Holdings Inc.), PI3-alpha/delta inhibitors, Pathway Therapeutics (Pathway Therapeutics Ltd., South San Francisco, CA), PI3-delta inhibitors, Cellzome (Cellzome AG, Heidelberg, Germany), PI3-delta inhibitors, Intellikine (Intellikine Inc., La Jolla, CA), PI3-delta inhibitors, Pathway Therapeutics-1 (Pathway Therapeutics Ltd.), PI3-delta inhibitors, Pathway Therapeutics-2 (Pathway Therapeutics Ltd.), PI3-delta/gamma inhibitors, Cellzome (Cellzome AG), PI3-delta/gamma inhibitors, Cellzome (Cellzome AG), PI3-delta/gamma inhibitors, Intellikine (Intellikine Inc.), PI3-delta/gamma inhibitors, Intellikine (Intellikine Inc.), PI3-delta/gamma inhibitors, Pathway Therapeutics (Pathway Therapeutics Ltd.), PI3-delta/gamma inhibitors, Pathway Therapeutics (Pathway Therapeutics Ltd.), PI3-gamma inhibitor Evotec (Evotec), PI3-gamma inhibitor, Cellzome (Cellzome AG), PI3-gamma inhibitors, Pathway Therapeutics (Pathway Therapeutics Ltd.), PI3K delta/gamma inhibitors, Intellikine-1 (Intellikine Inc.), PI3K delta/gamma inhibitors, Intellikine-1 (Intellikine Inc.), pictilisib (Roche Holdings Inc.), PIK-90 (CAS # 677338-12-4), SC-103980 (Pfizer, New York, NY), SF-1126 (Semafore Pharmaceuticals, Indianapolis, IN), SH-5, SH-6, Tetrahydro Curcumin, TG100-115 (Targegen Inc., San Diego, CA), Triciribine, X-339 (Xcovery, West Palm Beach, FL), XL-499 (Evotech, Hamburg,

Germany), pharmaceutically acceptable salts thereof, and combinations thereof.

54. A pharmaceutical composition for treating or ameliorating the effects of a cancer in a subject, which cancer is refractory or resistant to non-ERK MAPK pathway therapy, the composition comprising a pharmaceutically acceptable carrier or diluent and an effective amount of BVD-523 or a pharmaceutically acceptable salt thereof.

55. The pharmaceutical composition according to claim 54, wherein the non-ERK MAPK pathway inhibitor therapy is selected from the group consisting of a RAS inhibitor, a RAF inhibitor, a MEK inhibitor, and combinations thereof.

56. The pharmaceutical composition according to claim 54, wherein the non-ERK MAPK pathway inhibitor therapy is selected from the group consisting of a BRAF inhibitor, a MEK inhibitor, and combinations thereof.

57. The pharmaceutical composition according to claim 54, wherein the subject is a mammal.

58. The pharmaceutical composition according to claim 57, wherein the mammal is selected from the group consisting of humans, primates, farm animals, and domestic animals.

59. The pharmaceutical composition according to claim 57, wherein the mammal is a human.

60. The pharmaceutical composition according to claim 54, wherein the cancer has MAPK activity.

61. The pharmaceutical composition according to claim 60, wherein the cancer is a solid tumor cancer or a hematologic cancer.

62. The pharmaceutical composition according to claim 60, wherein the cancer is selected from the group consisting of a cancer of the large intestine, breast cancer, pancreatic cancer, skin cancer, and endometrial cancers.

63. The pharmaceutical composition according to claim 60, wherein the cancer is melanoma.

64. The pharmaceutical composition according to claim 54 further comprising at least one additional therapeutic agent selected from the group consisting of an antibody or fragment thereof, a cytotoxic agent, a toxin, a radionuclide, an immunomodulator, a photoactive therapeutic agent, a radiosensitizing agent, a hormone, an anti-angiogenesis agent, and combinations thereof.

65. The pharmaceutical composition according to claim 64, wherein the additional therapeutic agent is an inhibitor of the PI3K/Akt pathway.

66. The pharmaceutical composition according to claim 65, wherein the inhibitor of the PI3K/Akt pathway is selected from the group consisting of A-674563 (CAS # 552325-73-2), AGL 2263, AMG-319 (Amgen, Thousand Oaks, CA), AS-041164 (5-benzo[1,3]dioxol-5-ylmethylene-thiazolidine-2,4-dione), AS-604850 (5-(2,2-Difluoro-benzo[1,3]dioxol-5-ylmethylene)-thiazolidine-2,4-dione), AS-605240 (5-quinoxilin-6-methylene-1,3-thiazolidine-

2,4-dione), AT7867 (CAS # 857531-00-1), benzimidazole series, Genentech (Roche Holdings Inc., South San Francisco, CA), BML-257 (CAS # 32387-96-5), CAL-120 (Gilead Sciences, Foster City, CA), CAL-129 (Gilead Sciences), CAL-130 (Gilead Sciences), CAL-253 (Gilead Sciences), CAL-263 (Gilead Sciences), CAS # 612847-09-3, CAS # 681281-88-9, CAS # 75747-14-7, CAS # 925681-41-0, CAS # 98510-80-6, CCT128930 (CAS # 885499-61-6), CH5132799 (CAS # 1007207-67-1), CHR-4432 (Chroma Therapeutics, Ltd., Abingdon, UK), FPA 124 (CAS # 902779-59-3), GS-1101 (CAL-101) (Gilead Sciences), GSK 690693 (CAS # 937174-76-0), H-89 (CAS # 127243-85-0), Honokiol, IC87114 (Gilead Science), IPI-145 (Intellikine Inc.), KAR-4139 (Karus Therapeutics, Chilworth, UK), KAR-4141 (Karus Therapeutics), KIN-1 (Karus Therapeutics), KT 5720 (CAS # 108068-98-0), Miltefosine, MK-2206 dihydrochloride (CAS # 1032350-13-2), ML-9 (CAS # 105637-50-1), Naltrindole Hydrochloride, OXY-111A (NormOxys Inc., Brighton, MA), perifosine, PHT-427 (CAS # 1191951-57-1), PI3 kinase delta inhibitor, Merck KGaA (Merck & Co., Whitehouse Station, NJ), PI3 kinase delta inhibitors, Genentech (Roche Holdings Inc.), PI3 kinase delta inhibitors, Incozen (Incozen Therapeutics, Pvt. Ltd., Hydrabad, India), PI3 kinase delta inhibitors-2, Incozen (Incozen Therapeutics), PI3 kinase inhibitor, Roche-4 (Roche Holdings Inc.), PI3 kinase inhibitors, Roche (Roche Holdings Inc.), PI3 kinase inhibitors, Roche-5 (Roche Holdings Inc.), PI3-alpha/delta inhibitors, Pathway Therapeutics (Pathway Therapeutics Ltd., South San Francisco, CA), PI3-delta inhibitors, Cellzome (Cellzome AG, Heidelberg, Germany), PI3-delta inhibitors, Intellikine (Intellikine Inc., La Jolla, CA), PI3-delta inhibitors, Pathway Therapeutics-1 (Pathway Therapeutics Ltd.), PI3-delta inhibitors,

Pathway Therapeutics-2 (Pathway Therapeutics Ltd.), PI3-delta/gamma inhibitors, Cellzome (Cellzome AG), PI3-delta/gamma inhibitors, Cellzome (Cellzome AG), PI3-delta/gamma inhibitors, Intellikine (Intellikine Inc.), PI3-delta/gamma inhibitors, Intellikine (Intellikine Inc.), PI3-delta/gamma inhibitors, Pathway Therapeutics (Pathway Therapeutics Ltd.), PI3-delta/gamma inhibitors, Pathway Therapeutics (Pathway Therapeutics Ltd.), PI3-gamma inhibitor Evotec (Evotec), PI3-gamma inhibitor, Cellzome (Cellzome AG), PI3-gamma inhibitors, Pathway Therapeutics (Pathway Therapeutics Ltd.), PI3K delta/gamma inhibitors, Intellikine-1 (Intellikine Inc.), PI3K delta/gamma inhibitors, Intellikine-1 (Intellikine Inc.), pictilisib (Roche Holdings Inc.), PIK-90 (CAS # 677338-12-4), SC-103980 (Pfizer, New York, NY), SF-1126 (Semafore Pharmaceuticals, Indianapolis, IN), SH-5, SH-6, Tetrahydro Curcumin, TG100-115 (Targegen Inc., San Diego, CA), Triciribine, X-339 (Xcovery, West Palm Beach, FL), XL-499 (Evotech, Hamburg, Germany), pharmaceutically acceptable salts thereof, and combinations thereof.

67. A kit for treating or ameliorating the effects of a cancer in a subject, which cancer is refractory or resistant to non-ERK MAPK pathway therapy, the kit comprising a pharmaceutical composition according to any one of claims 54, 55, or 56 packaged together with instructions for its use.

68. A method for inhibiting phosphorylation of RSK in a cancer cell that is refractory or resistant to a non-ERK MAPK pathway inhibitor, the method comprising contacting the cancer cell with an effective amount of BVD-523 or a pharmaceutically acceptable salt thereof for a period of time sufficient for phosphorylation of RSK in the cancer cell to be inhibited.

69. The method according to claim 68, wherein greater than 50% of RSK phosphorylation is inhibited.

70. The method according to claim 68, wherein greater than 75% of RSK phosphorylation is inhibited.

71. The method according to claim 68, wherein greater than 90% of RSK phosphorylation is inhibited.

72. The method according to claim 68, wherein greater than 95% of RSK phosphorylation is inhibited.

73. The method according to claim 68, wherein greater than 99% of RSK phosphorylation is inhibited.

74. The method according to claim 68, wherein 100% of RSK phosphorylation is inhibited.

75. The method according to claim 68, which is carried out in vitro, ex vivo, or in vivo.

76. The method according to claim 68, wherein the contacting step comprises administering BVD-523 or a pharmaceutically acceptable salt to a subject from whom the cancer cell was obtained.

77. The method according to claim 68, wherein the non-ERK MAPK pathway inhibitor is selected from the group consisting of a RAS inhibitor, a RAF inhibitor, a MEK inhibitor, and combinations thereof.

78. The method according to claim 68, wherein the non-ERK MAPK pathway inhibitor is selected from the group consisting of BRAF inhibitors, MEK inhibitors, and combinations thereof.

79. The method according to claim 68, wherein the cancer is from a mammal.

80. The method according to claim 79, wherein the mammal is selected from the group consisting of humans, primates, farm animals, and domestic animals.

81. The method according to claim 79, wherein the mammal is a human.

82. The method according to claim 68, wherein the cancer has MAPK activity.

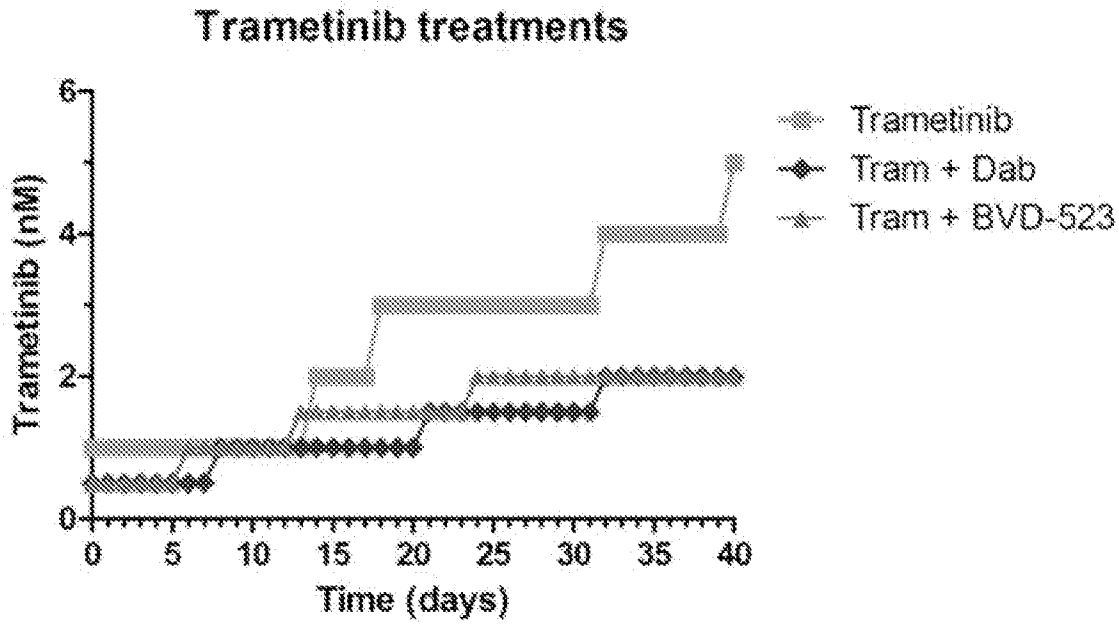
83. The method according to claim 82, wherein the cancer is a solid tumor cancer or a hematologic cancer.

84. The method according to claim 82, wherein the cancer is selected from the group consisting of a cancer of the large intestine, breast cancer, pancreatic cancer, skin cancer, and endometrial cancers.

85. The method according to claim 82, wherein the cancer is melanoma.

FIG. 1

A


Trametinib treatments
Trametinib (nM)
0
2
4
6
0 5 10 15 20 25 30 35 40
Time (days)
Trametinib
Tram + Dab
Tram + BVD-523

B

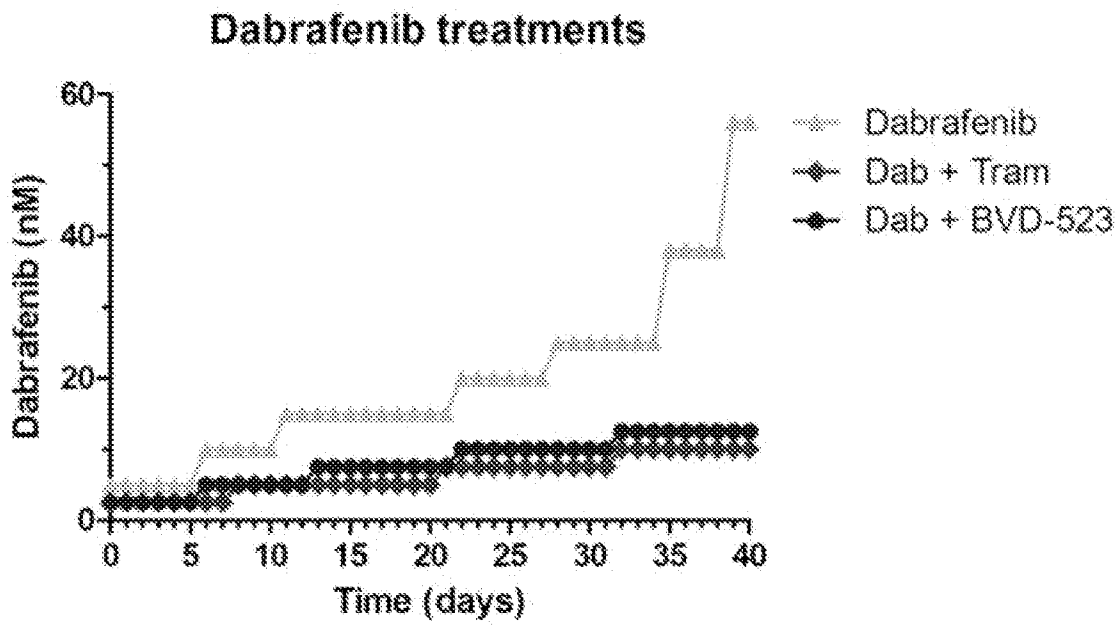

Dabrafenib treatments
Dabrafenib (nM)
0
20
40
60
0 5 10 15 20 25 30 35 40
Time (days)
Dabrafenib
Dab + Tram
Dab + BVD-523

FIG. 1, Con't

C

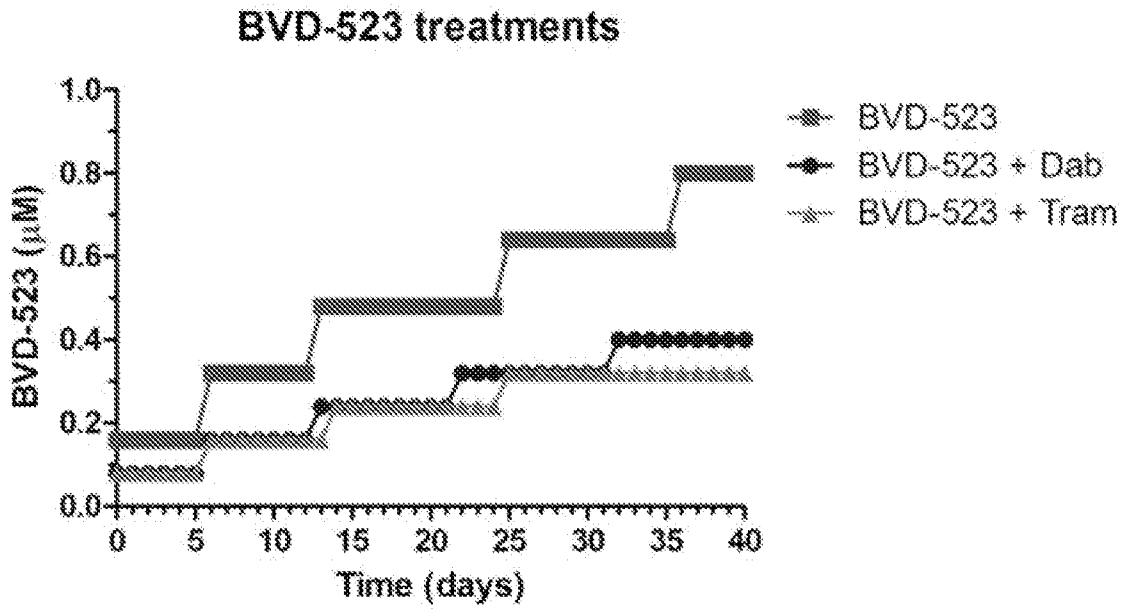

BVD-523 treatments
BVD-523 (μM)
1.0
0.8
0.6
0.4
0.2
0.0
0
5
10
15
20
25
30
35
40
Time (days)
BVD-523
BVD-523 + Dab
BVD-523 + Tram

FIG. 2

A

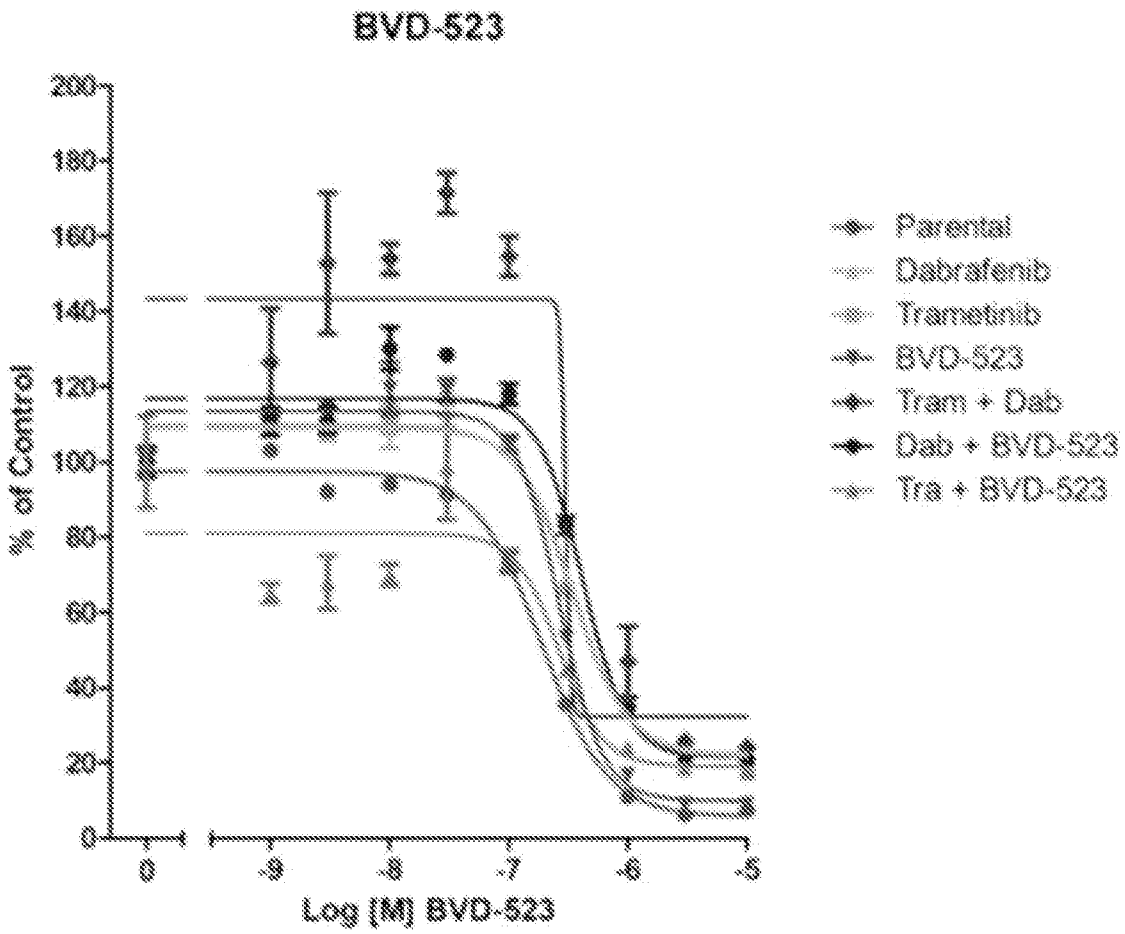

BVD-523
% of Control
200
180
160
140
120
100
80
60
40
20
0
0
-9
-8
-7
-6
-5
Log [M] BVD-523
Parental
Dabrafenib
Trametinib
BVD-523
Tram + Dab
Dab + BVD-523
Tra + BVD-523

FIG. 2, Con't

B

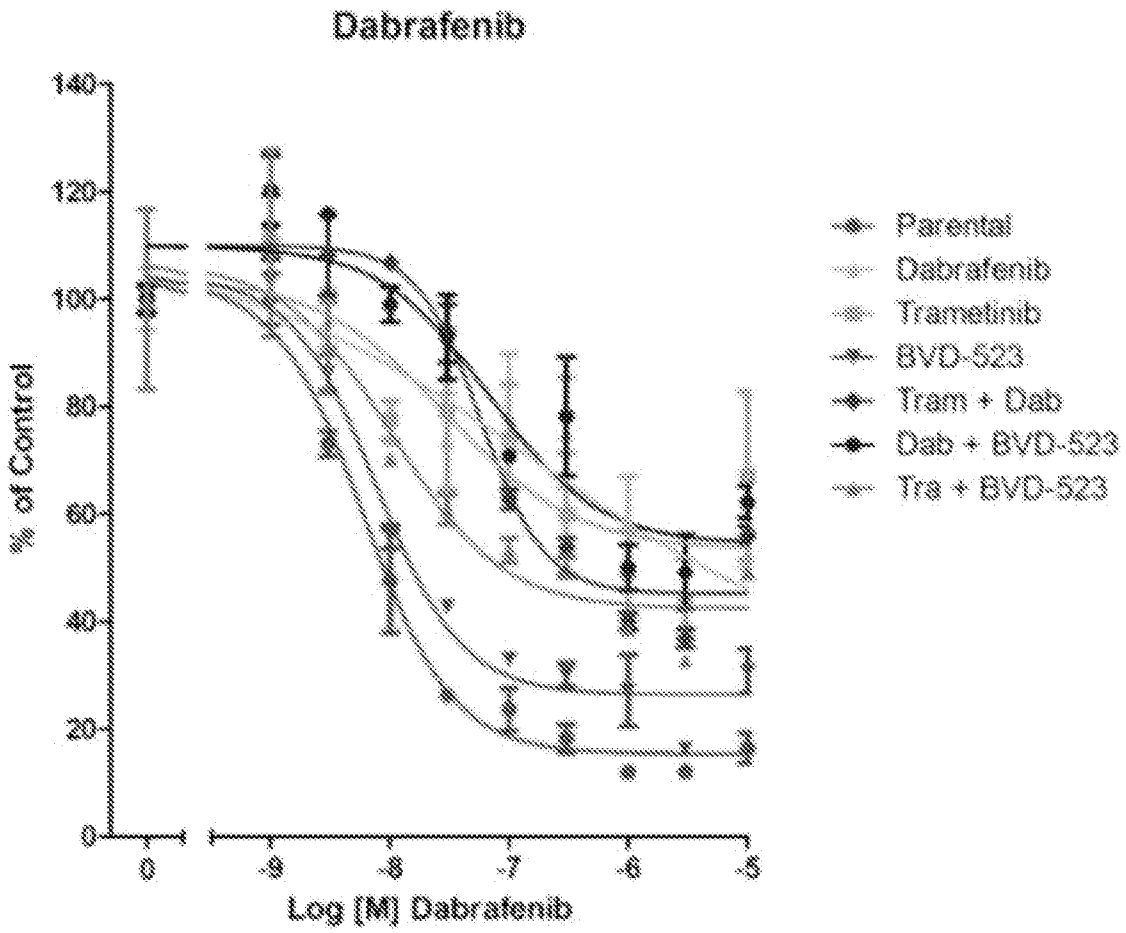

Dabrafenib
% of Control
140
120
100
80
60
40
20
0
0
-9
-8
-7
-6
-5
Log [M] Dabrafenib
Parental
Dabrafenib
Trametinib
BVD-523
Tram + Dab
Dab + BVD-523
Tra + BVD-523

FIG. 2, Con't

C

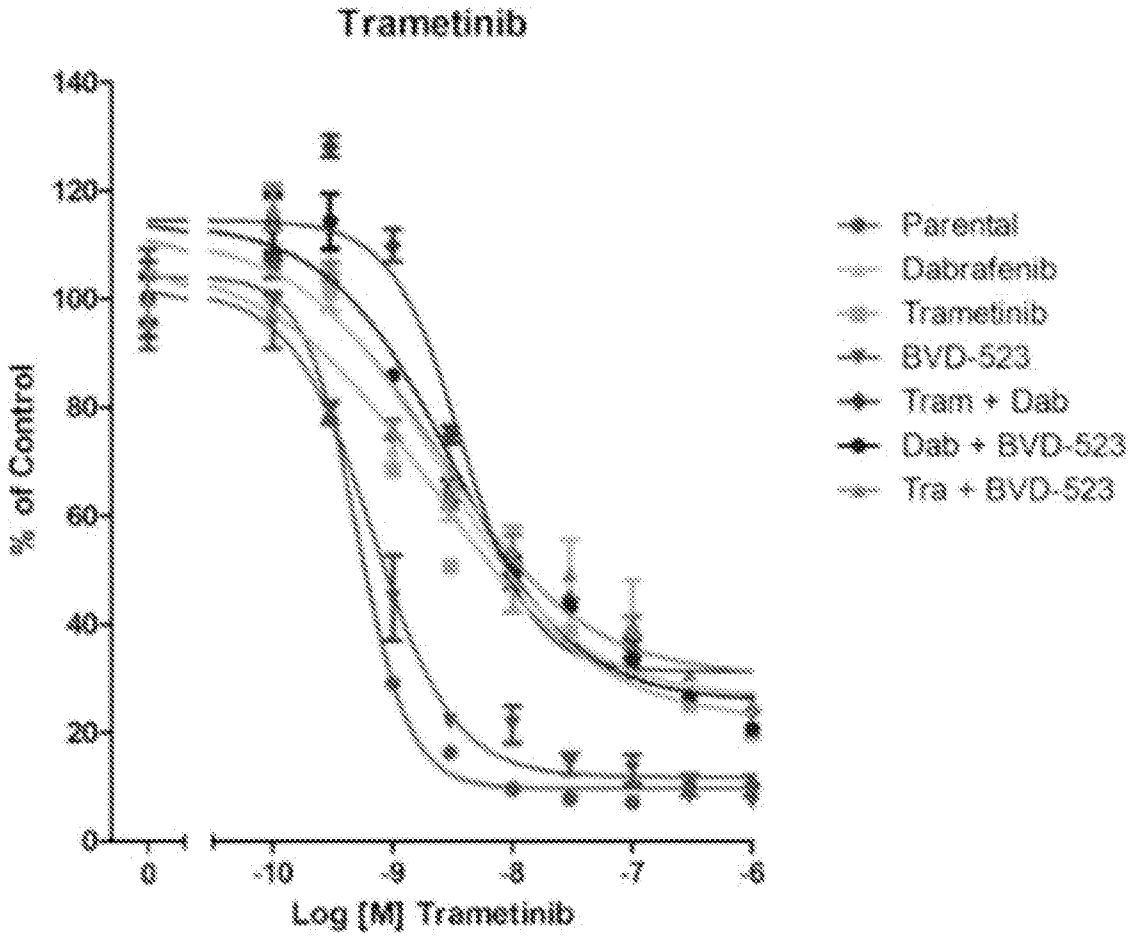

Trametinib
% of Control
140
120
100
80
60
40
20
0
0
-10
-9
-8
-7
-6
Log [M] Trametinib
Parental
Dabrafenib
Trametinib
BVD-523
Tram + Dab
Dab + BVD-523
Tra + BVD-523

FIG. 2, Con't

D

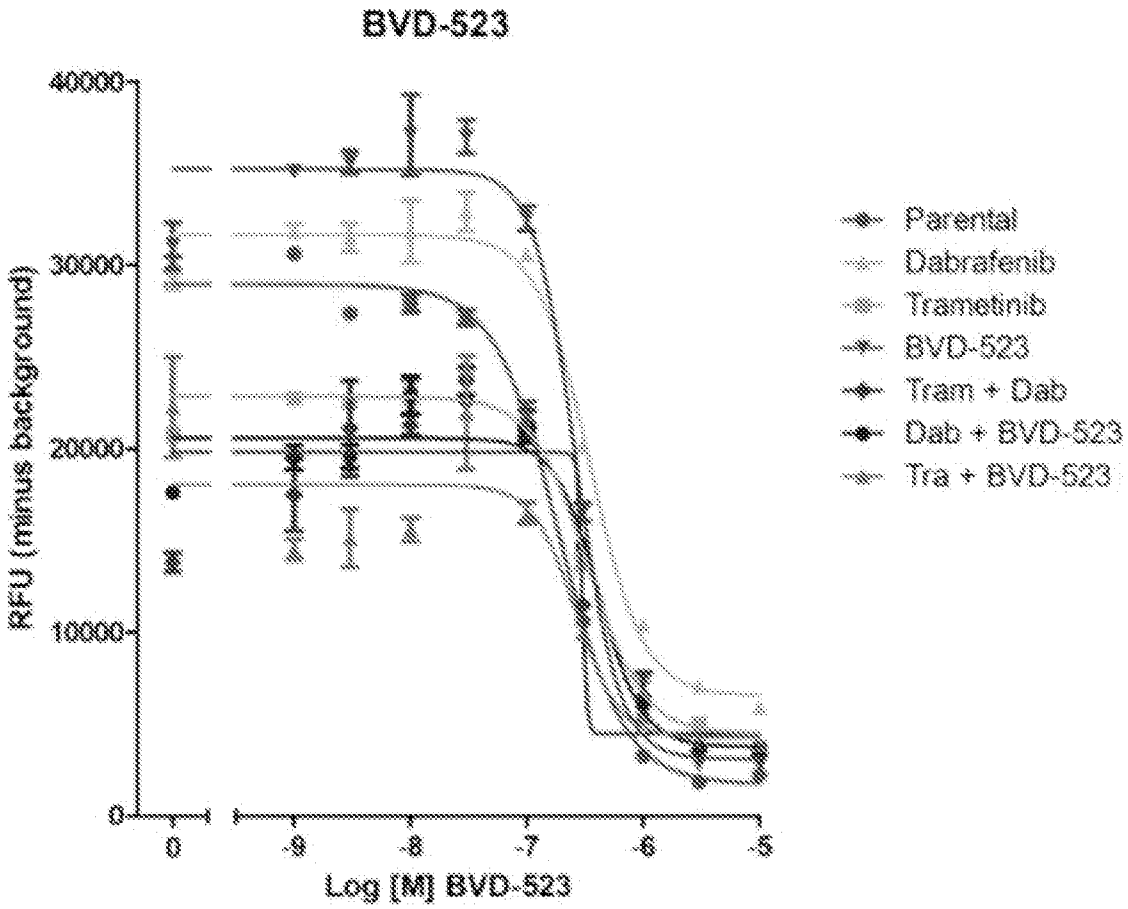

BVD-523
40000
30000
20000
10000
0
RFU (minus background)
0
-9
-8
-7
-6
-5
Log [M] BVD-523
Parental
Dabrafenib
Trametinib
BVD-523
Tram + Dab
Dab + BVD-523
Tra + BVD-523

FIG. 2, Con't

E

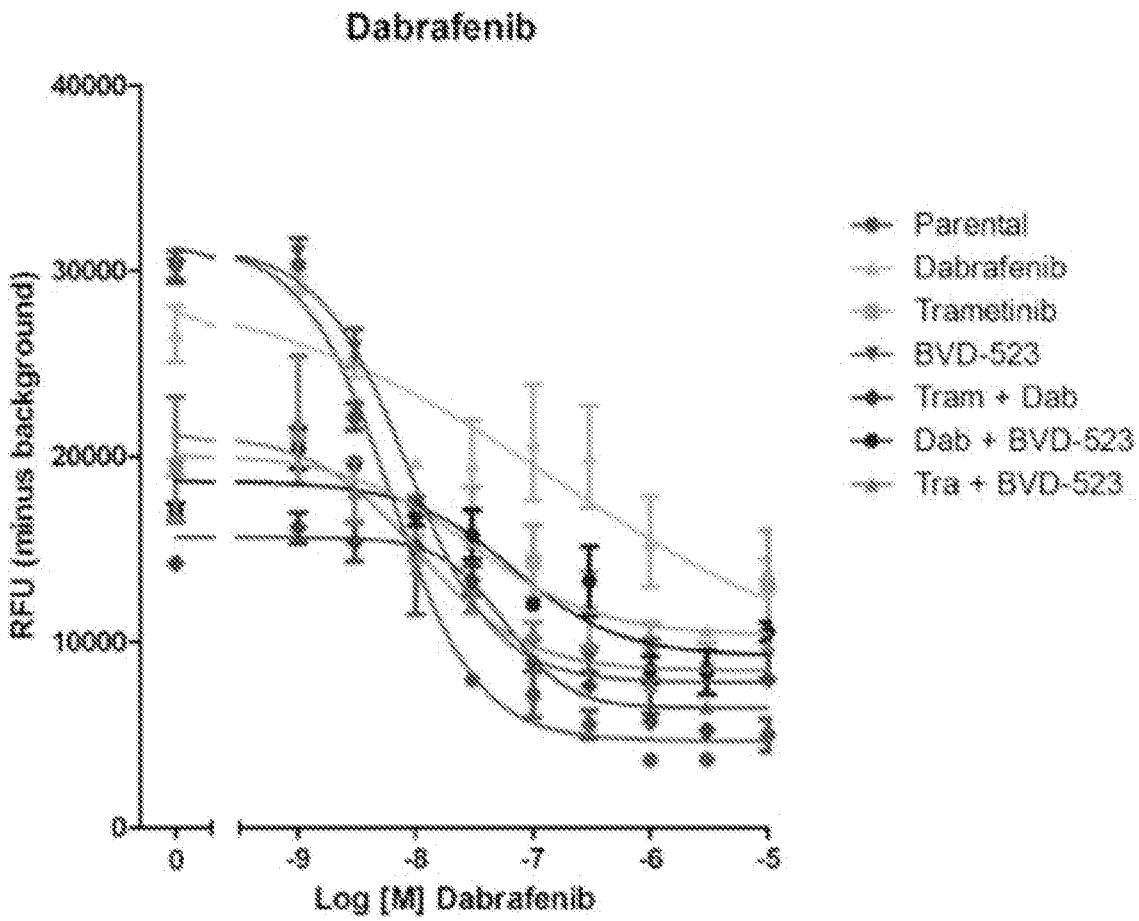

Dabrafenib
RFU (minus background)
40000
30000
20000
10000
0
0
-9
-8
-7
-6
-5
Log [M] Dabrafenib
Parental
Dabrafenib
Trametinib
BVD-523
Tram + Dab
Dab + BVD-523
Tra + BVD-523

FIG. 2, Con't

F

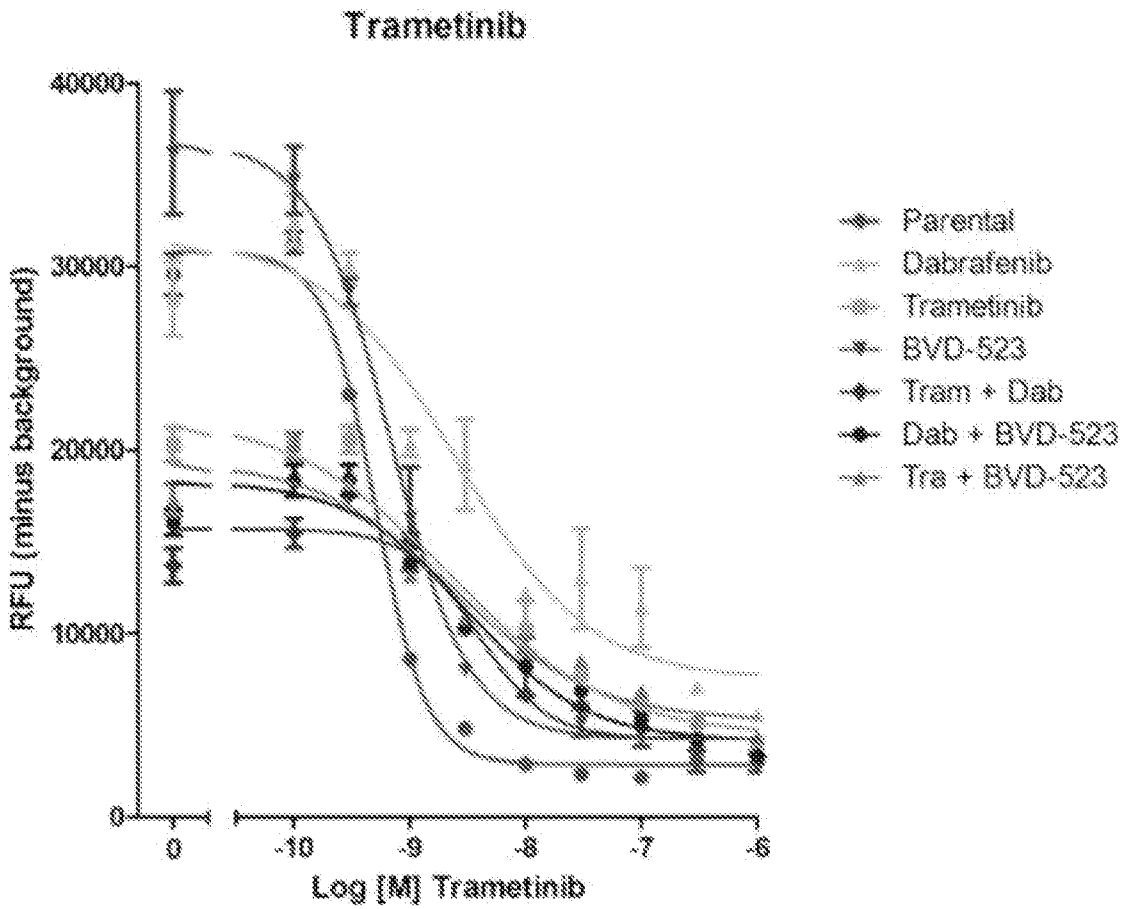

Trametinib
40000
30000
20000
10000
0
RFU (minus background)
0
-10
-9
-8
-7
-6
Log [M] Trametinib
Parental
Dabrafenib
Trametinib
BVD-523
Tram + Dab
Dab + BVD-523
Tra + BVD-523

FIG. 2, Con't

G

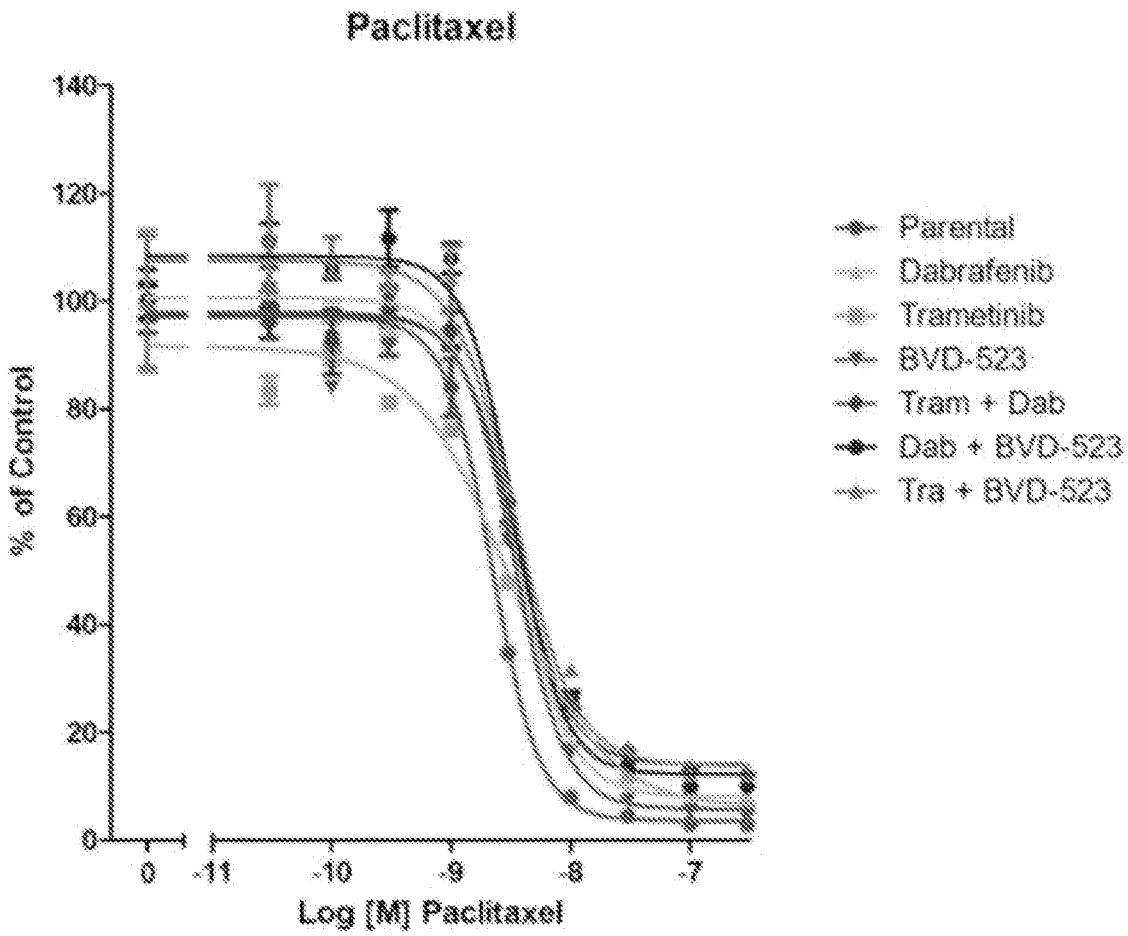

Paclitaxel
% of Control
140
120
100
80
60
40
20
0
0
-11
-10
-9
-8
-7
Log [M] Paclitaxel
Parental
Dabrafenib
Trametinib
BVD-523
Tram + Dab
Dab + BVD-523
Tra + BVD-523

FIG. 2, Con't

H

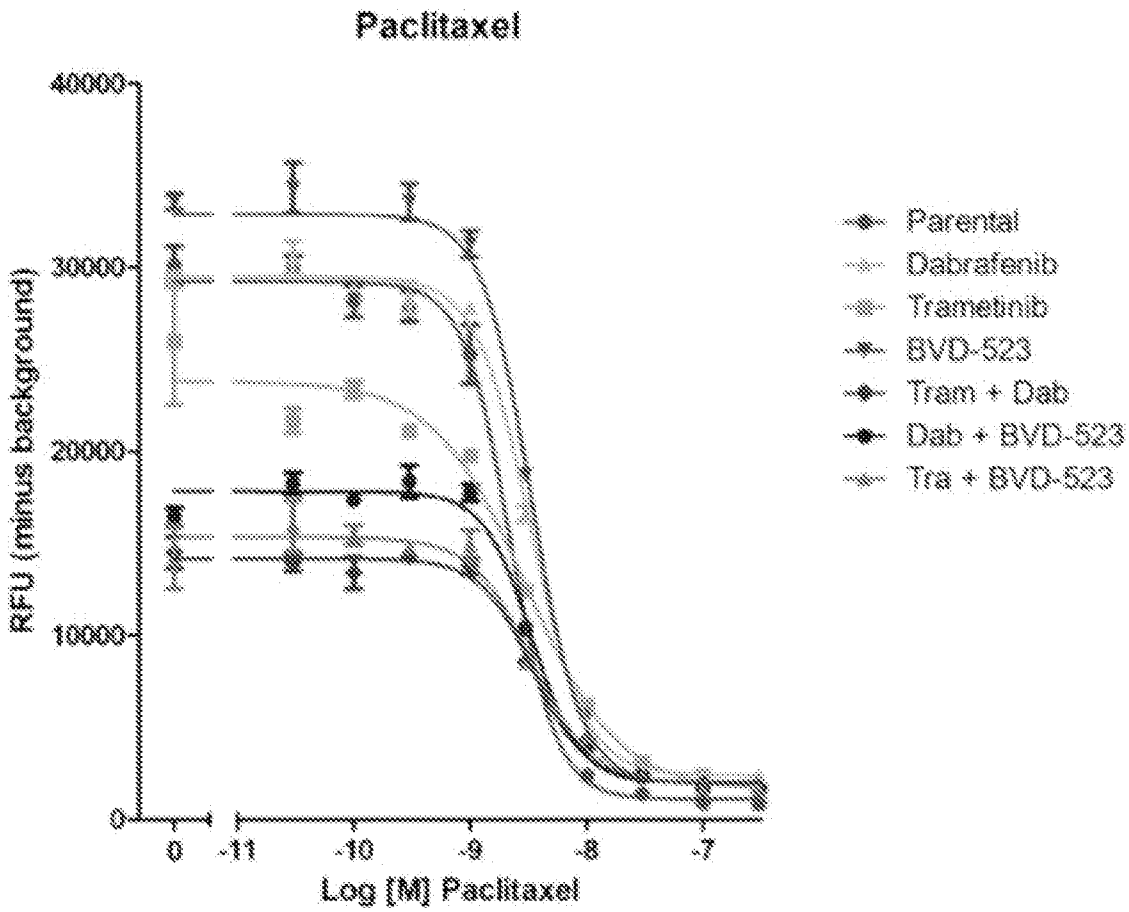

Paclitaxel
40000
30000
20000
10000
0
RFU (minus background)
0
-11
-10
-9
-8
-7
Log [M] Paclitaxel
Parental
Dabrafenib
Trametinib
BVD-523
Tram + Dab
Dab + BVD-523
Tra + BVD-523

FIG. 3

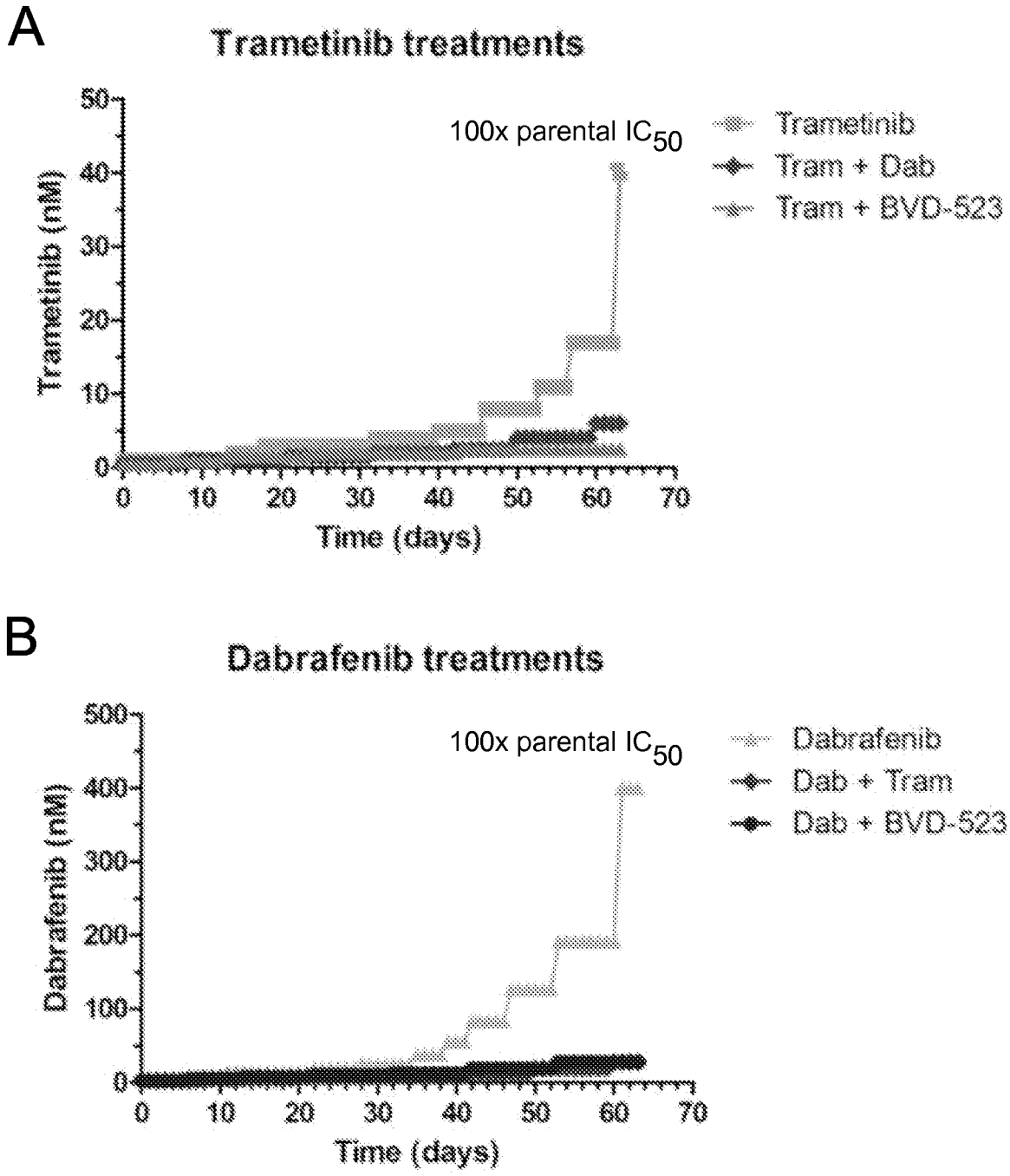
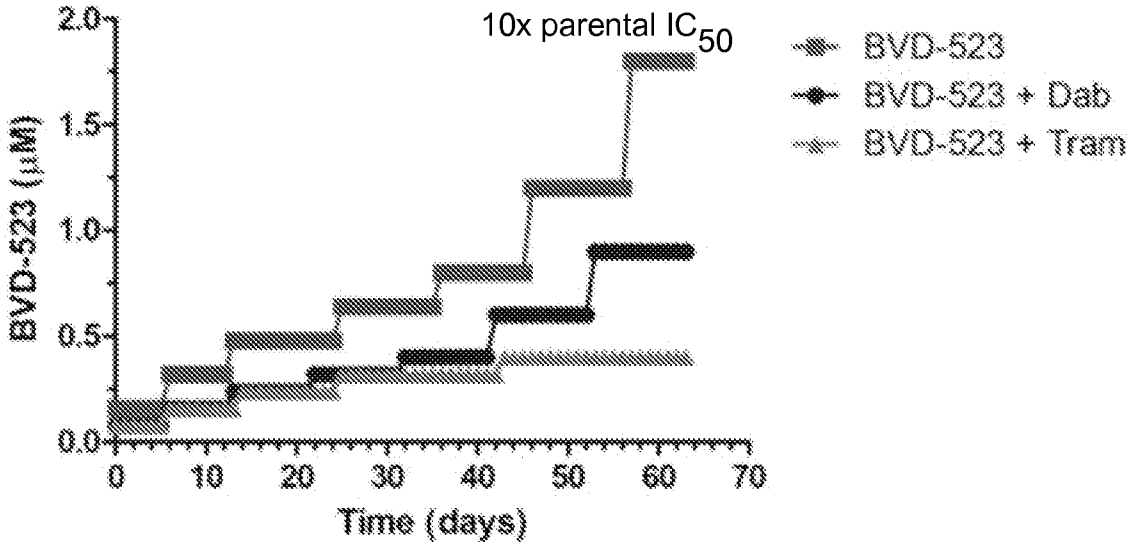

A
Trametinib treatments
100x parental IC50
Trametinib
Tram + Dab
Tram + BVD-523
Trametinib (nM)
Time (days)
B
Dabrafenib treatments
100x parental IC50
Dabrafenib
Dab + Tram
Dab + BVD-523
Dabrafenib (nM)
Time (days)

FIG. 3, Con't

C


BVD-523 treatments
10x parental IC50
BVD-523
BVD-523 + Dab
BVD-523 + Tram
BVD-523 (μM)
2.0
1.5
1.0
0.5
0.0
0
10
20
30
40
50
60
70
Time (days)

D

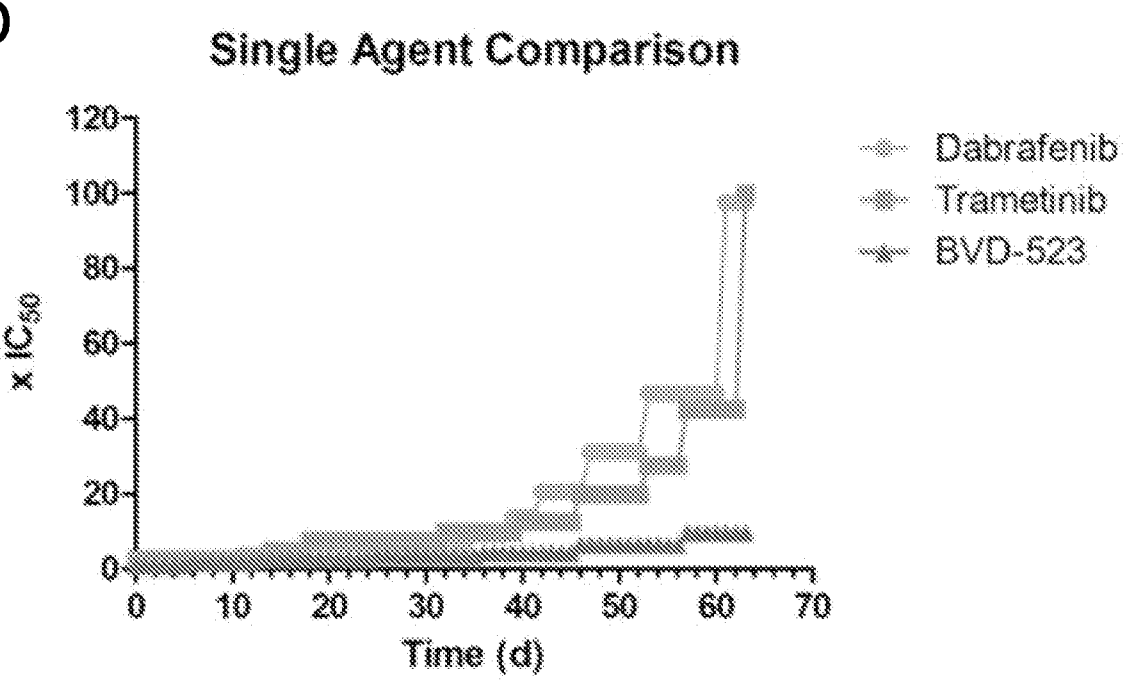

Single Agent Comparison
Dabrafenib
Trametinib
BVD-523
x IC50
120
100
80
60
40
20
0
0
10
20
30
40
50
60
70
Time (d)

FIG. 4

A

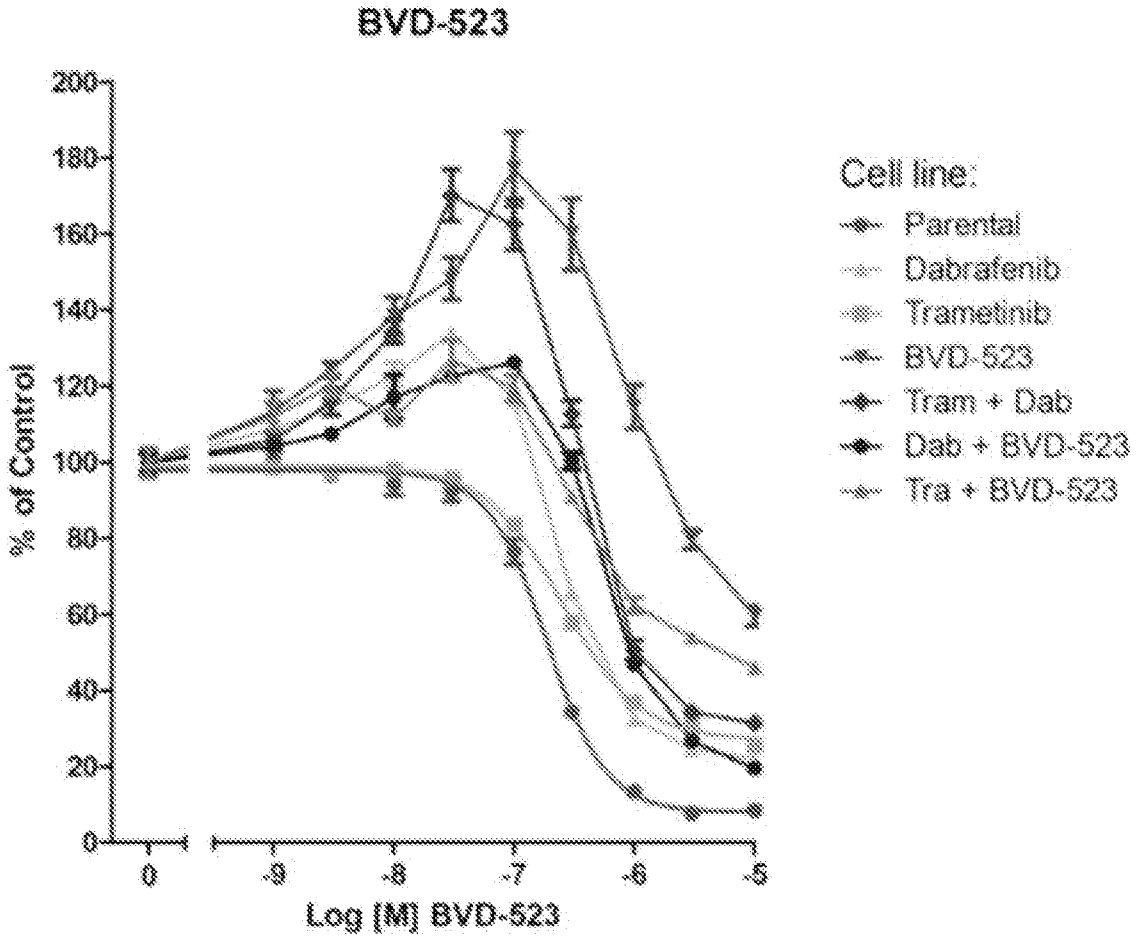

BVD-523
% of Control
200
180
160
140
120
100
80
60
40
20
0
0
-9
-8
-7
-6
-5
Log [M] BVD-523
Cell line:
Parental
Dabrafenib
Trametinib
BVD-523
Tram + Dab
Dab + BVD-523
Tra + BVD-523

FIG. 4, Con't

B

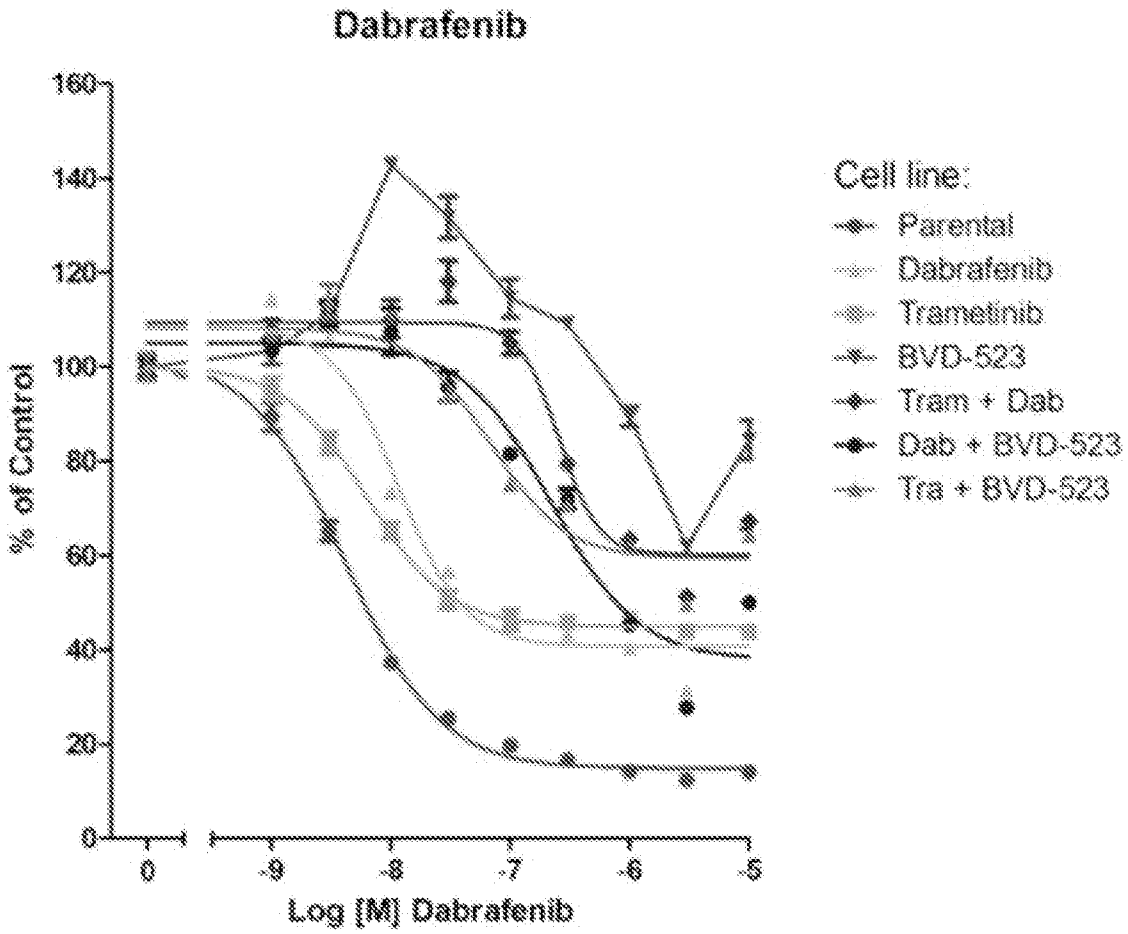

Dabrafenib
% of Control
160
140
120
100
80
60
40
20
0
0
-9
-8
-7
-6
-5
Log [M] Dabrafenib
Cell line:
Parental
Dabrafenib
Trametinib
BVD-523
Tram + Dab
Dab + BVD-523
Tra + BVD-523

FIG. 4, Con't

C

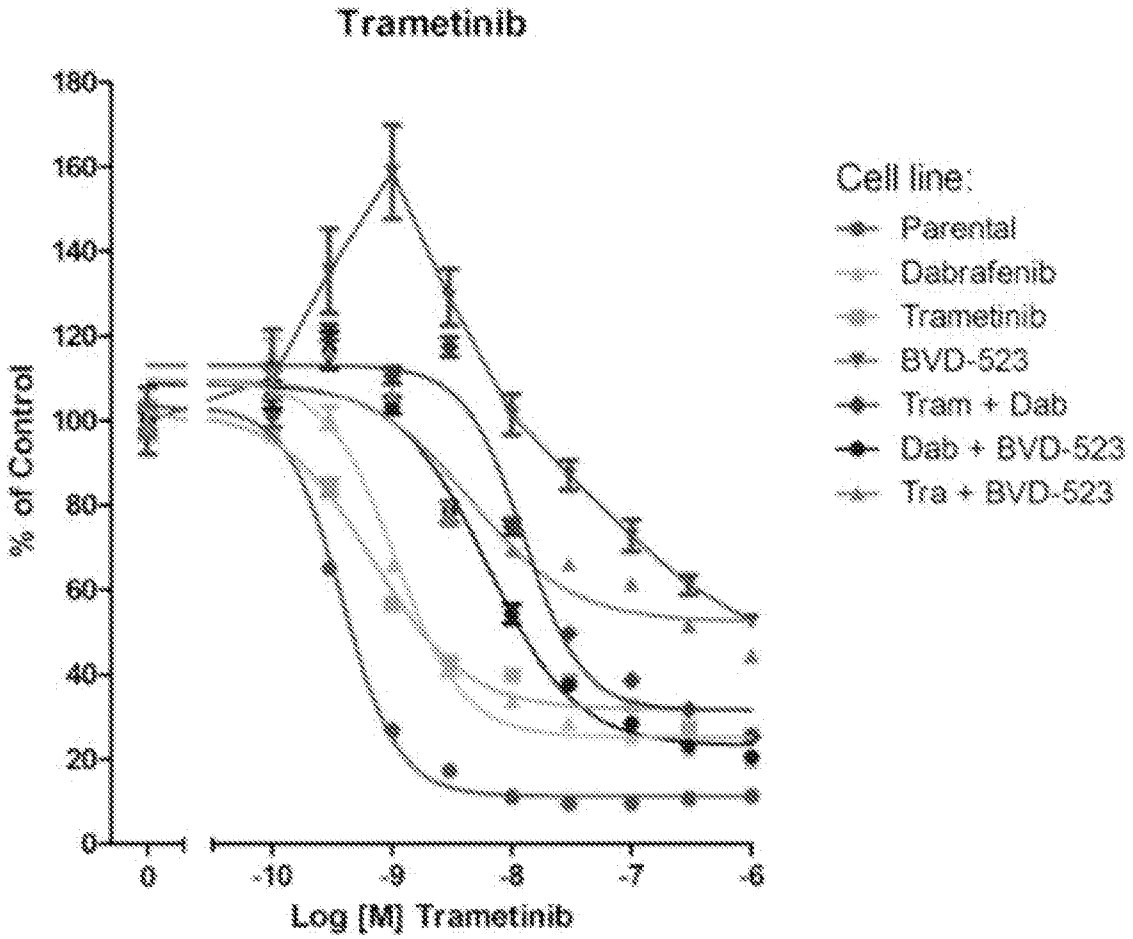

Trametinib
% of Control
180
160
140
120
100
80
60
40
20
0
0
-10
-9
-8
-7
-6
Log [M] Trametinib
Cell line:
Parental
Dabrafenib
Trametinib
BVD-523
Tram + Dab
Dab + BVD-523
Tra + BVD-523

FIG. 4, Con't

D

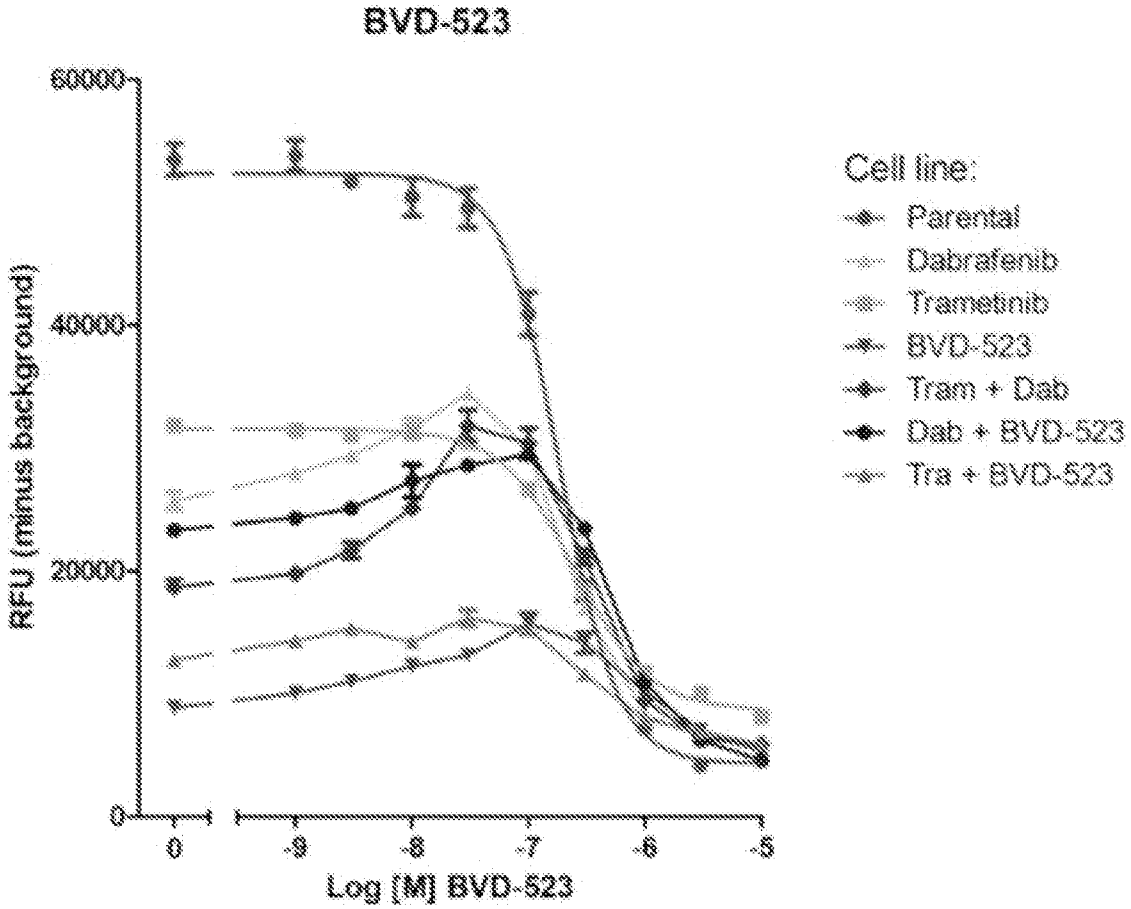

BVD-523
60000
40000
20000
0
RFU (minus background)
0
-9
-8
-7
-6
-5
Log [M] BVD-523
Cell line:
Parental
Dabrafenib
Trametinib
BVD-523
Tram + Dab
Dab + BVD-523
Tra + BVD-523

FIG. 4, Con't

E

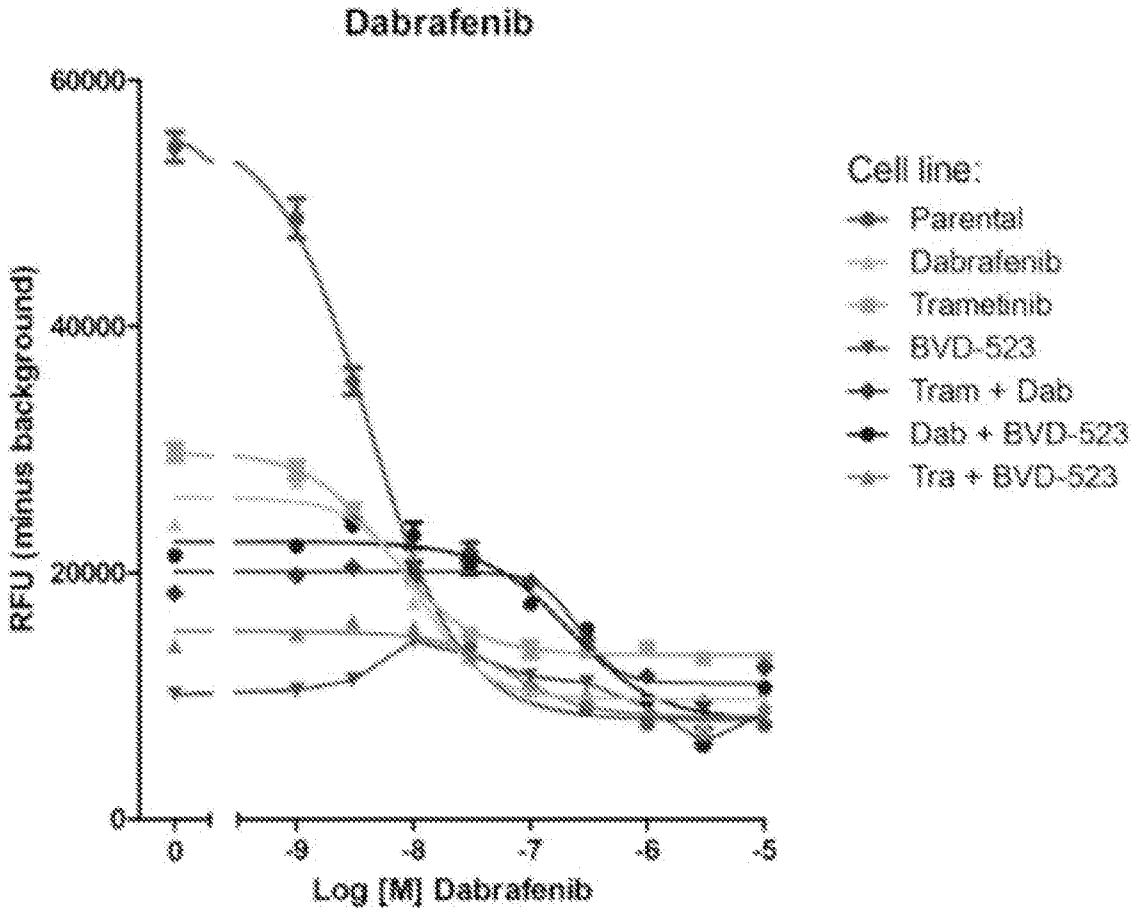

Dabrafenib
60000
40000
20000
0
RFU (minus background)
0
-9
-8
-7
-6
-5
Log [M] Dabrafenib
Cell line:
Parental
Dabrafenib
Trametinib
BVD-523
Tram + Dab
Dab + BVD-523
Tra + BVD-523

FIG. 4, Con't

F

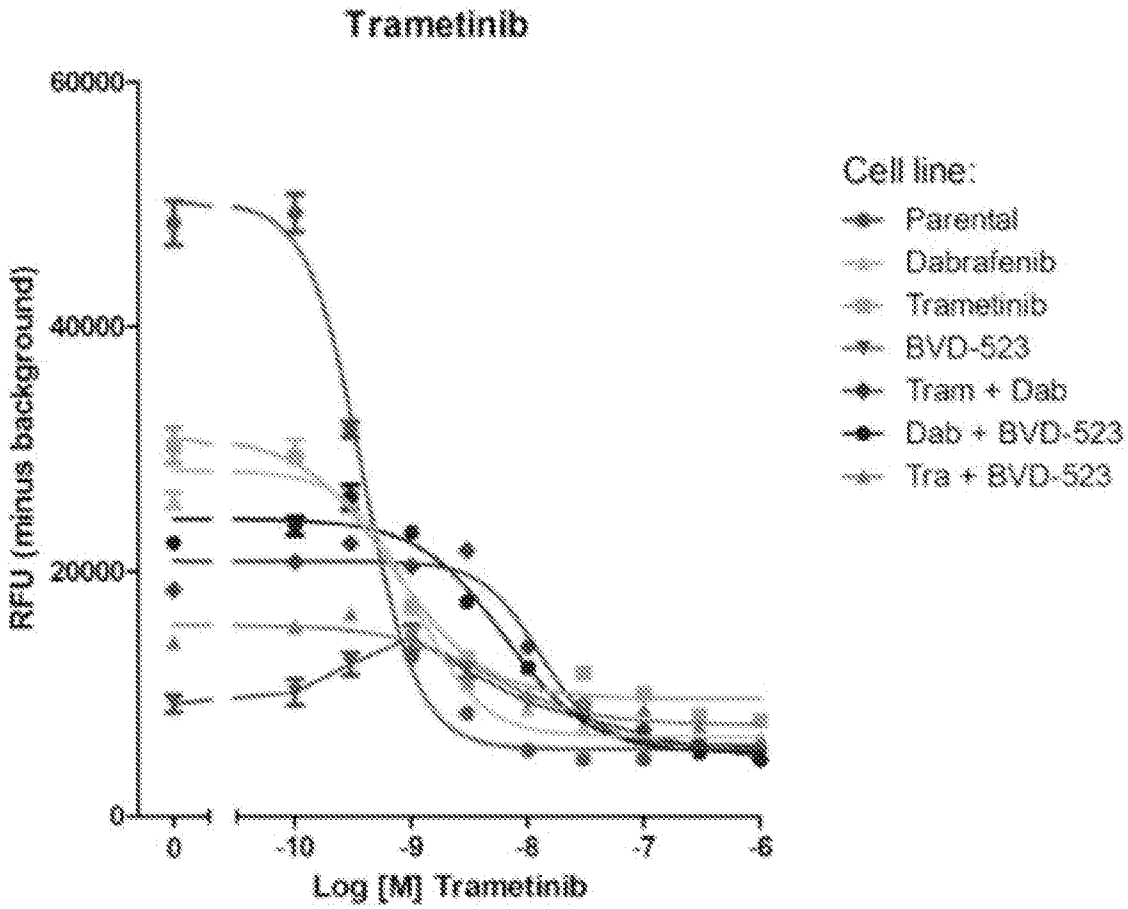

Trametinib
60000
40000
20000
0
RFU (minus background)
0
-10
-9
-8
-7
-6
Log [M] Trametinib
Cell line:
Parental
Dabrafenib
Trametinib
BVD-523
Tram + Dab
Dab + BVD-523
Tra + BVD-523

FIG. 4, Con't

G

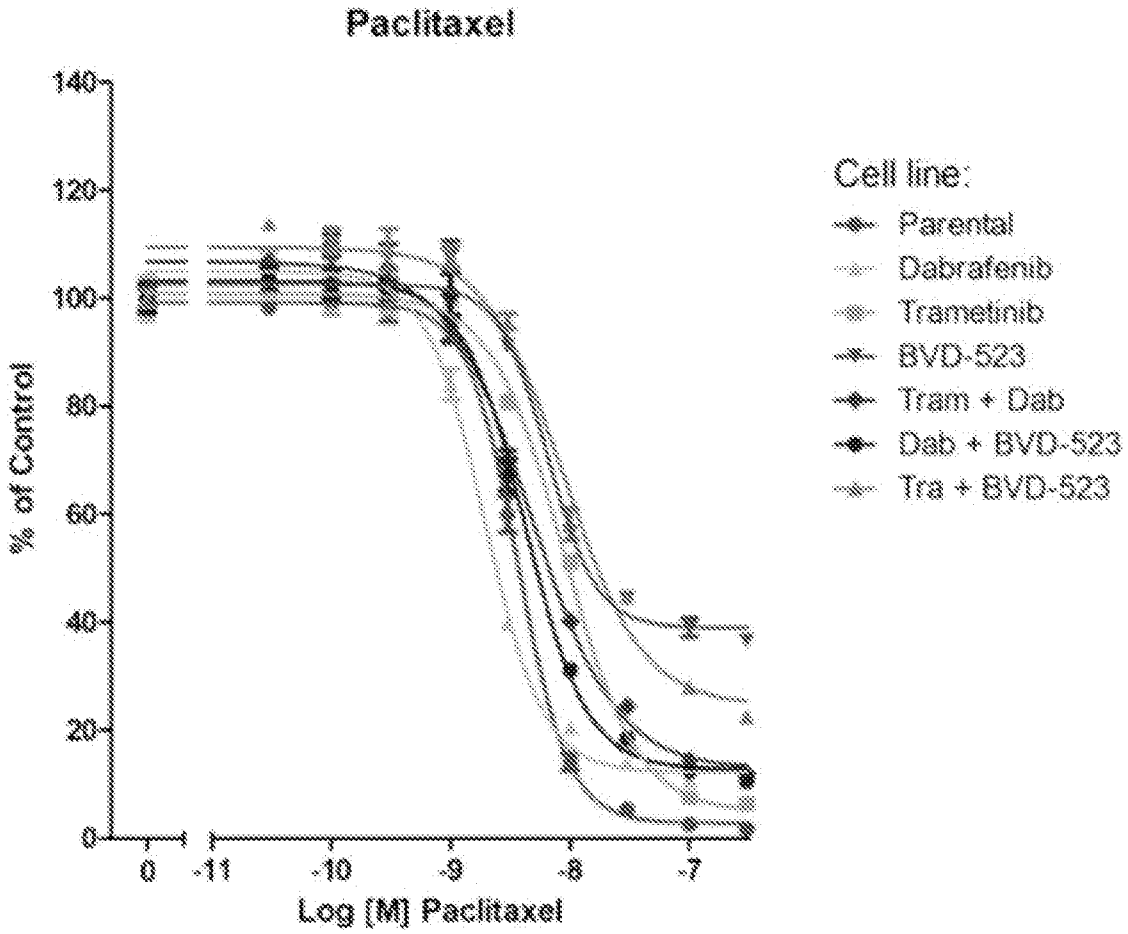

Paclitaxel
% of Control
140
120
100
80
60
40
20
0
0
-11
-10
-9
-8
-7
Log [M] Paclitaxel
Cell line:
Parental
Dabrafenib
Trametinib
BVD-523
Tram + Dab
Dab + BVD-523
Tra + BVD-523

FIG. 4, Con't

H

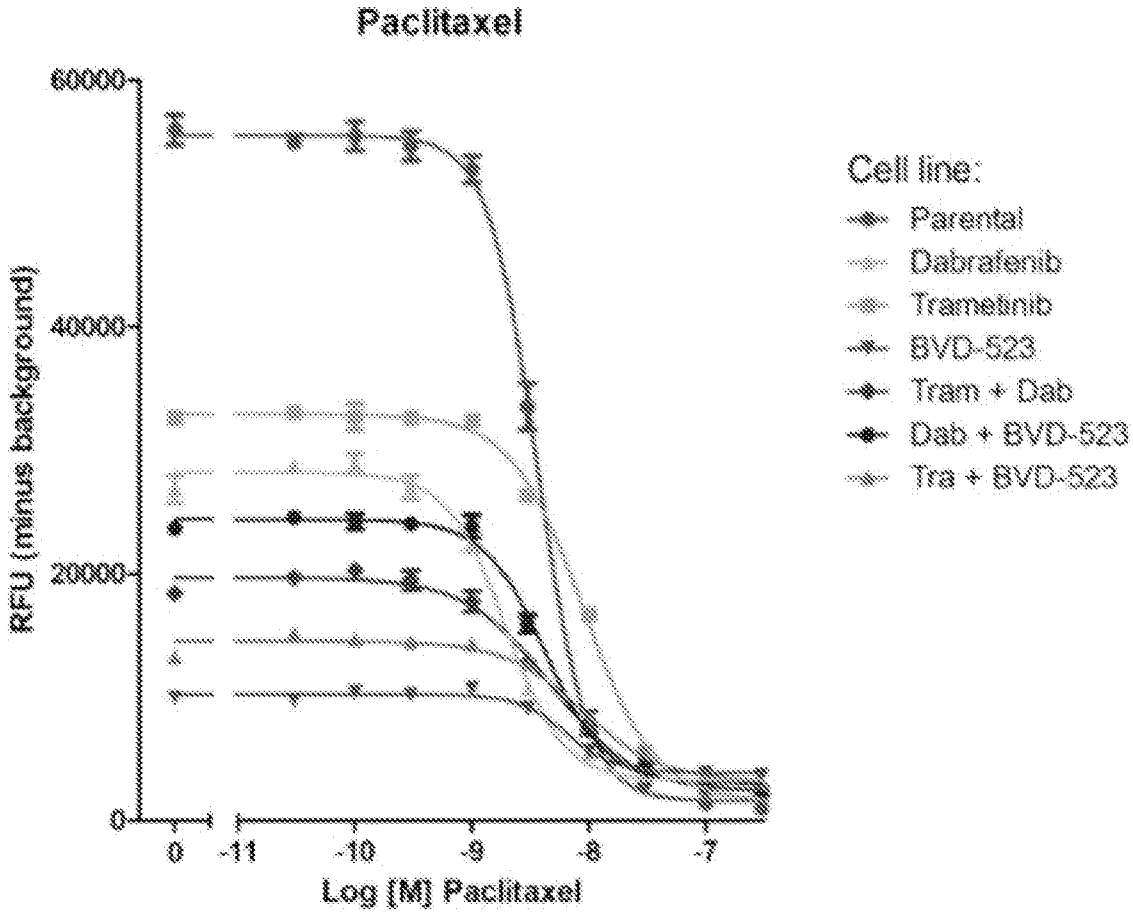

Paclitaxel
60000
40000
20000
0
RFU (minus background)
0
-11
-10
-9
-8
-7
Log [M] Paclitaxel
Cell line:
Parental
Dabrafenib
Trametinib
BVD-523
Tram + Dab
Dab + BVD-523
Tra + BVD-523

FIG. 5

A

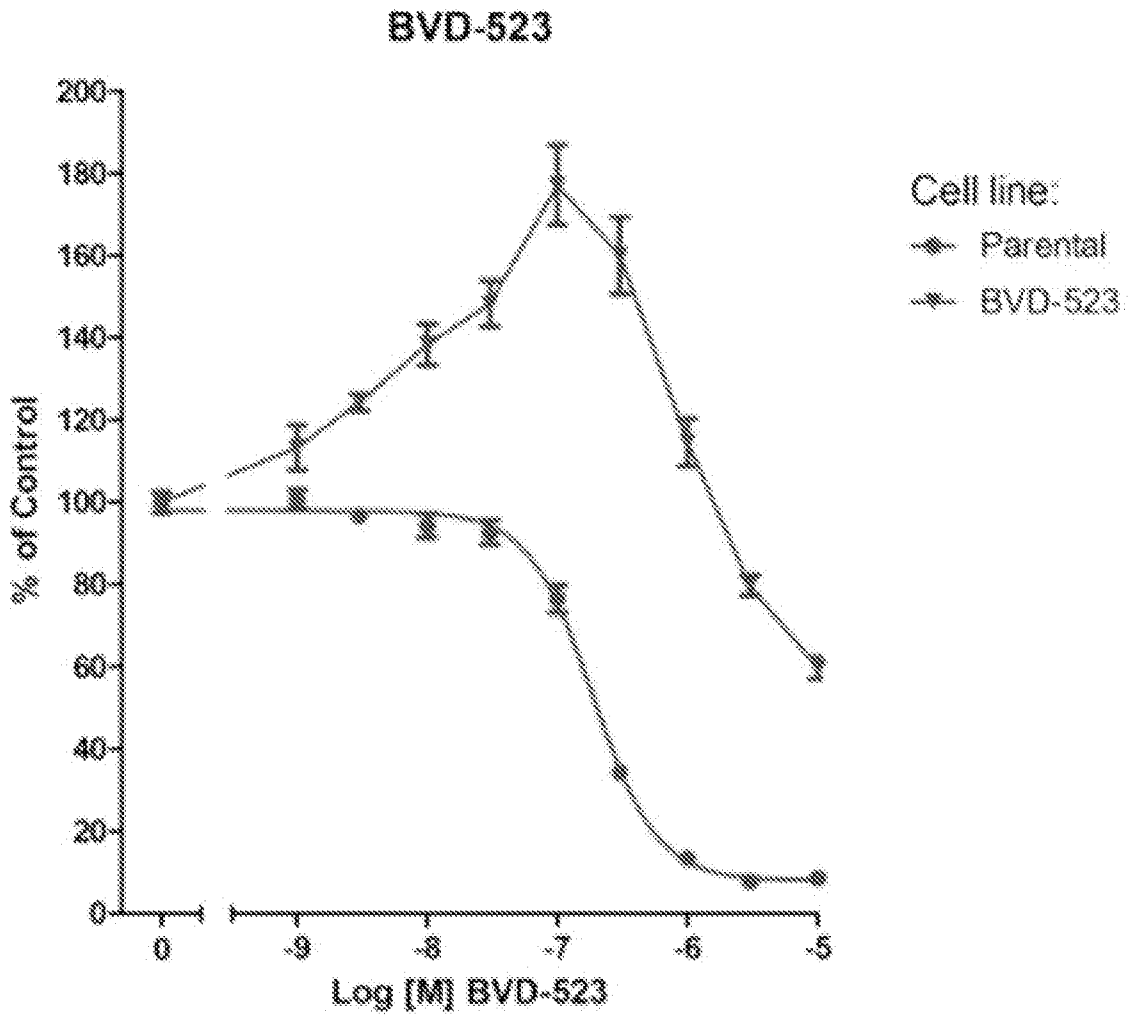

BVD-523
% of Control
200
180
160
140
120
100
80
60
40
20
0
0
-9
-8
-7
-6
-5
Log [M] BVD-523
Cell line:
Parental
BVD-523

FIG. 5, Con't

B

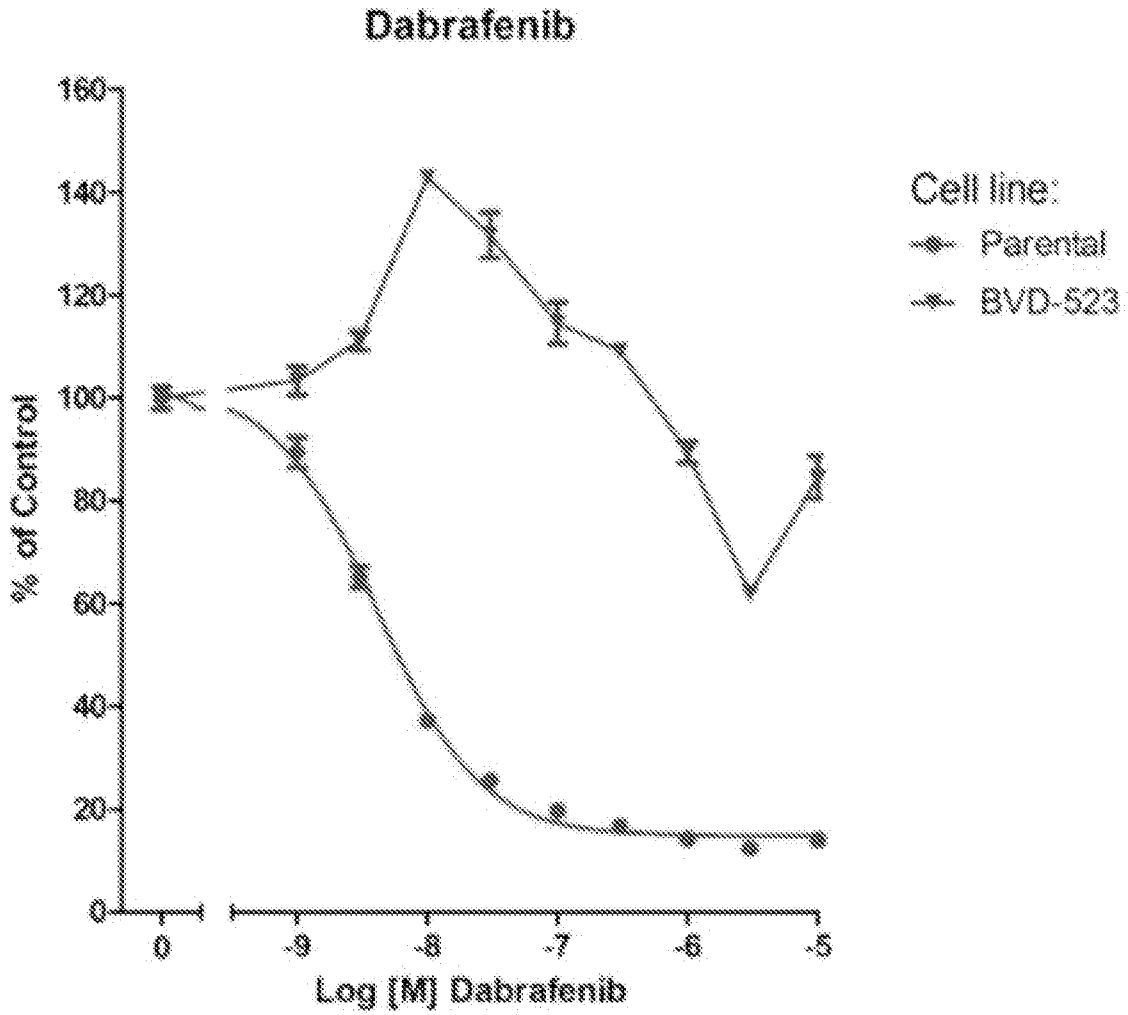

Dabrafenib
% of Control
160
140
120
100
80
60
40
20
0
0
-9
-8
-7
-6
-5
Log [M] Dabrafenib
Cell line:
Parental
BVD-523

FIG. 5, Con't

C

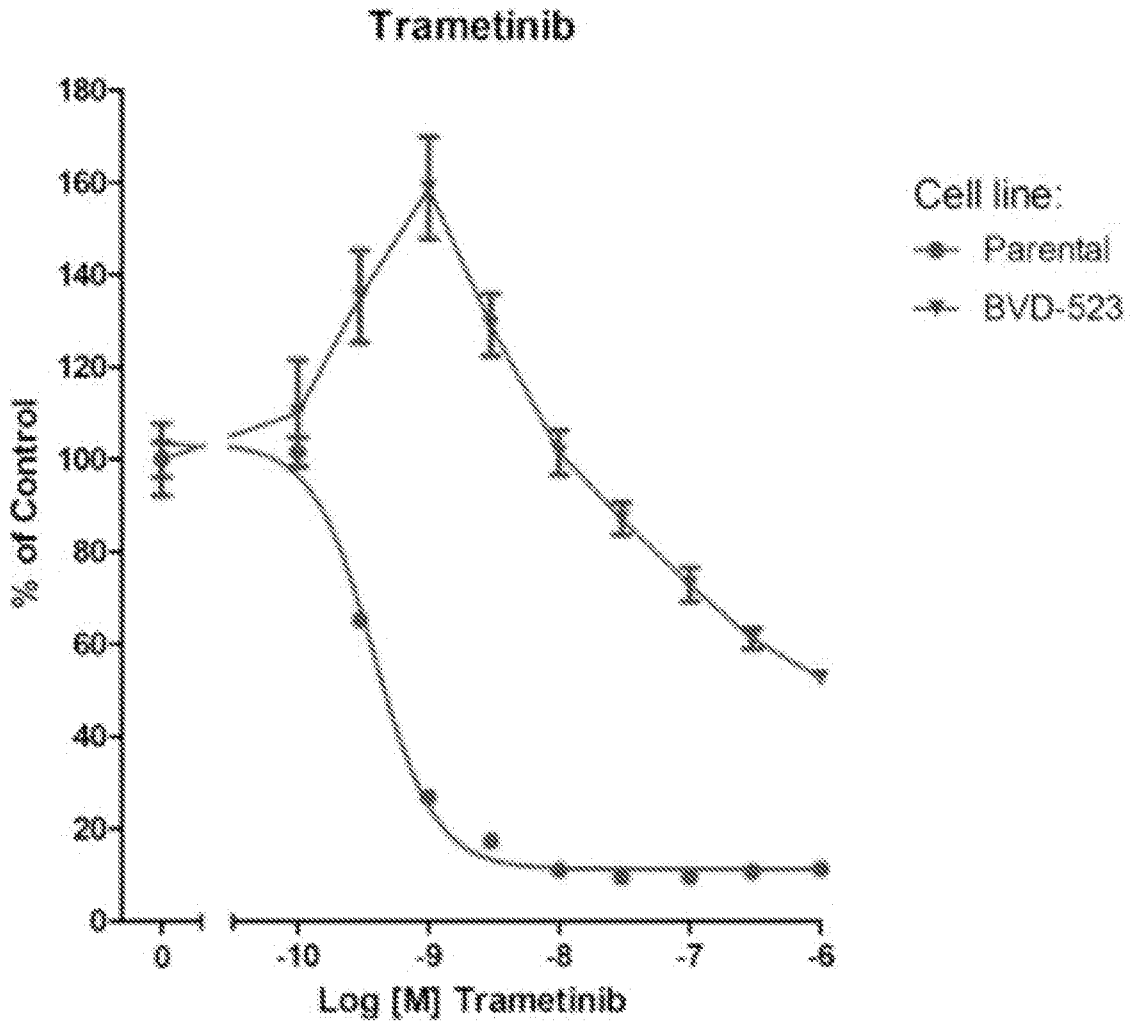

Trametinib
% of Control
180
160
140
120
100
80
60
40
20
0
0
-10
-9
-8
-7
-6
Log [M] Trametinib
Cell line:
Parental
BVD-523

FIG. 5, Con't

D

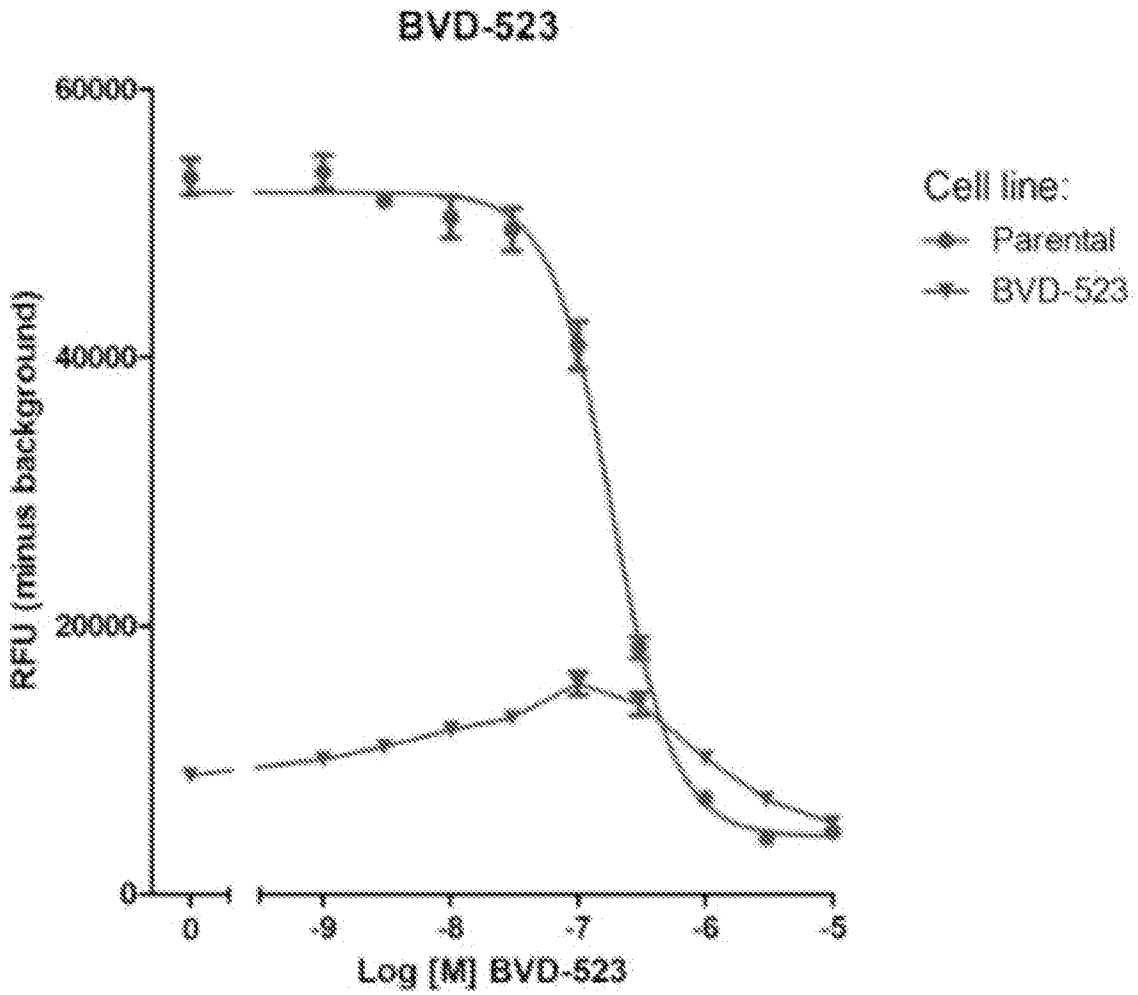

BVD-523
60000
40000
20000
0
RFU (minus background)
0
-9
-8
-7
-6
-5
Log [M] BVD-523
Cell line:
Parental
BVD-523

FIG. 5, Con't

E

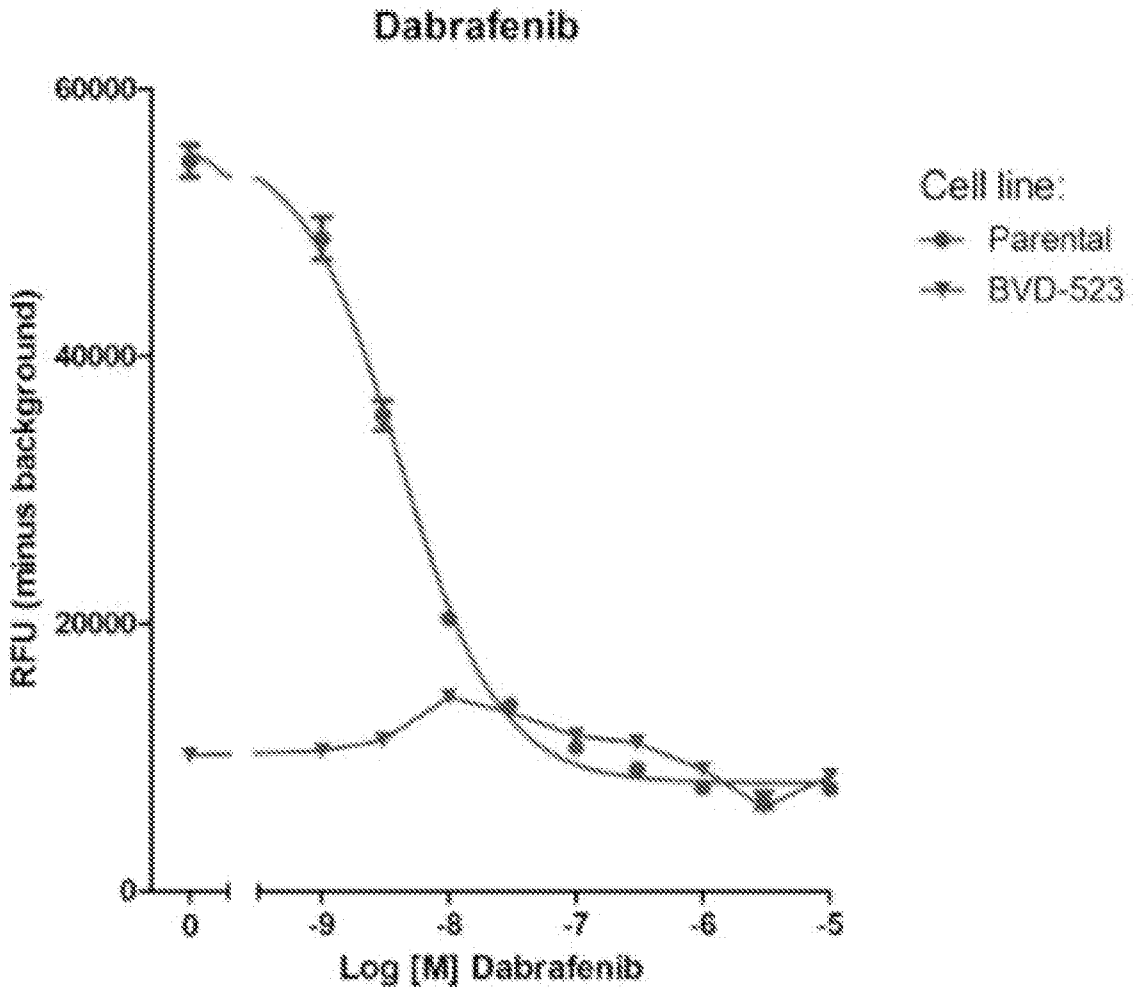

Dabrafenib
60000
40000
20000
0
RFU (minus background)
0
-9
-8
-7
-6
-5
Log [M] Dabrafenib
Cell line:
Parental
BVD-523

FIG. 5, Con't

F

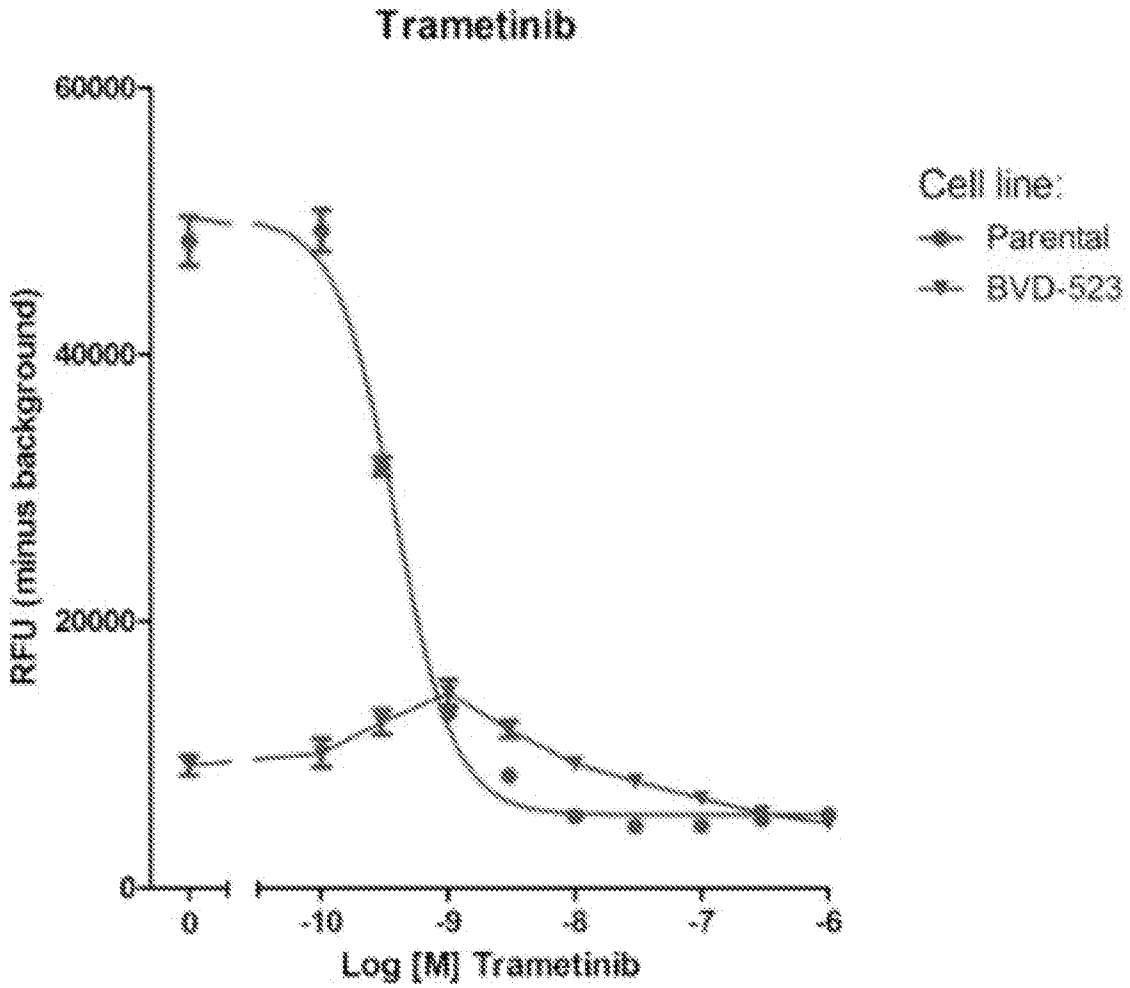
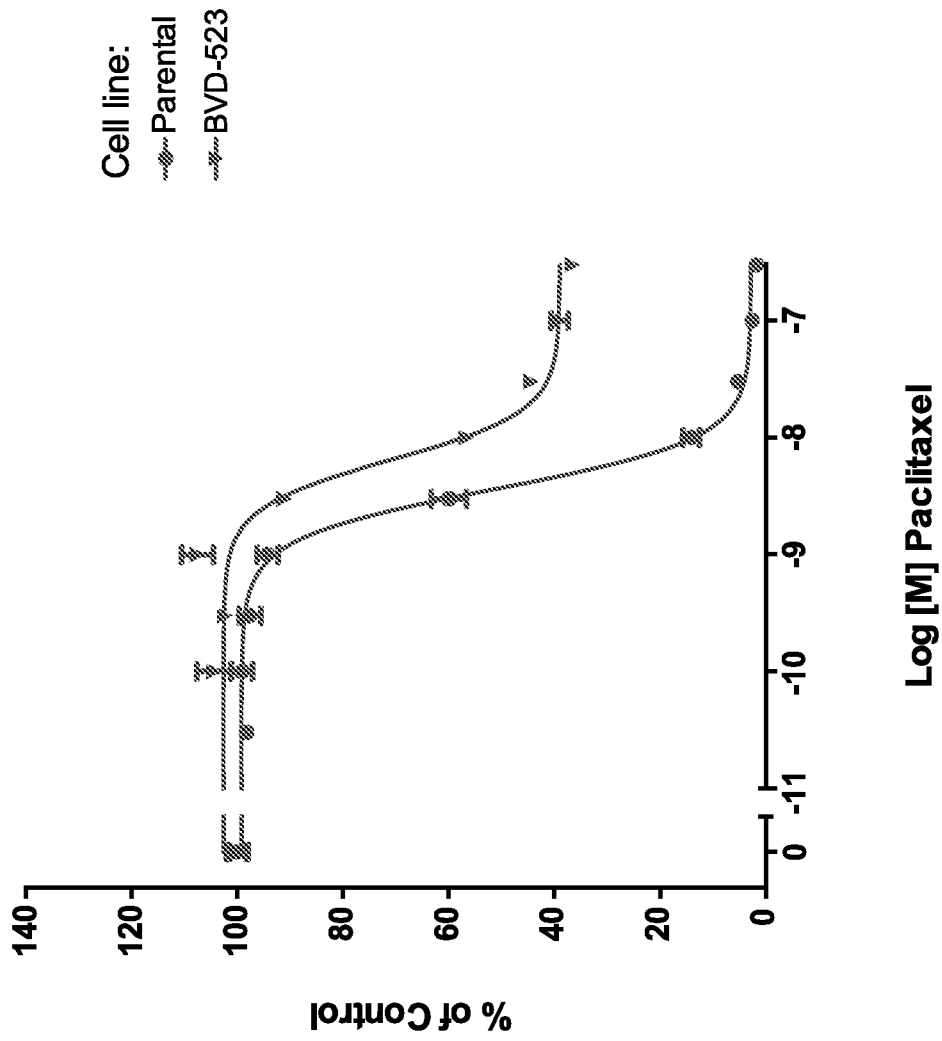

Trametinib
60000
40000
20000
0
RFU (minus background)
0
-10
-9
-8
-7
-6
Log [M] Trametinib
Cell line:
Parental
BVD-523

FIG. 5 Con't


Paclitaxel
Cell line:
Parental
BVD-523
% of Control
140
120
100
80
60
40
20
0
0
-11
-10
-9
-8
-7
Log [M] Paclitaxel

G

FIG. 5 Con't

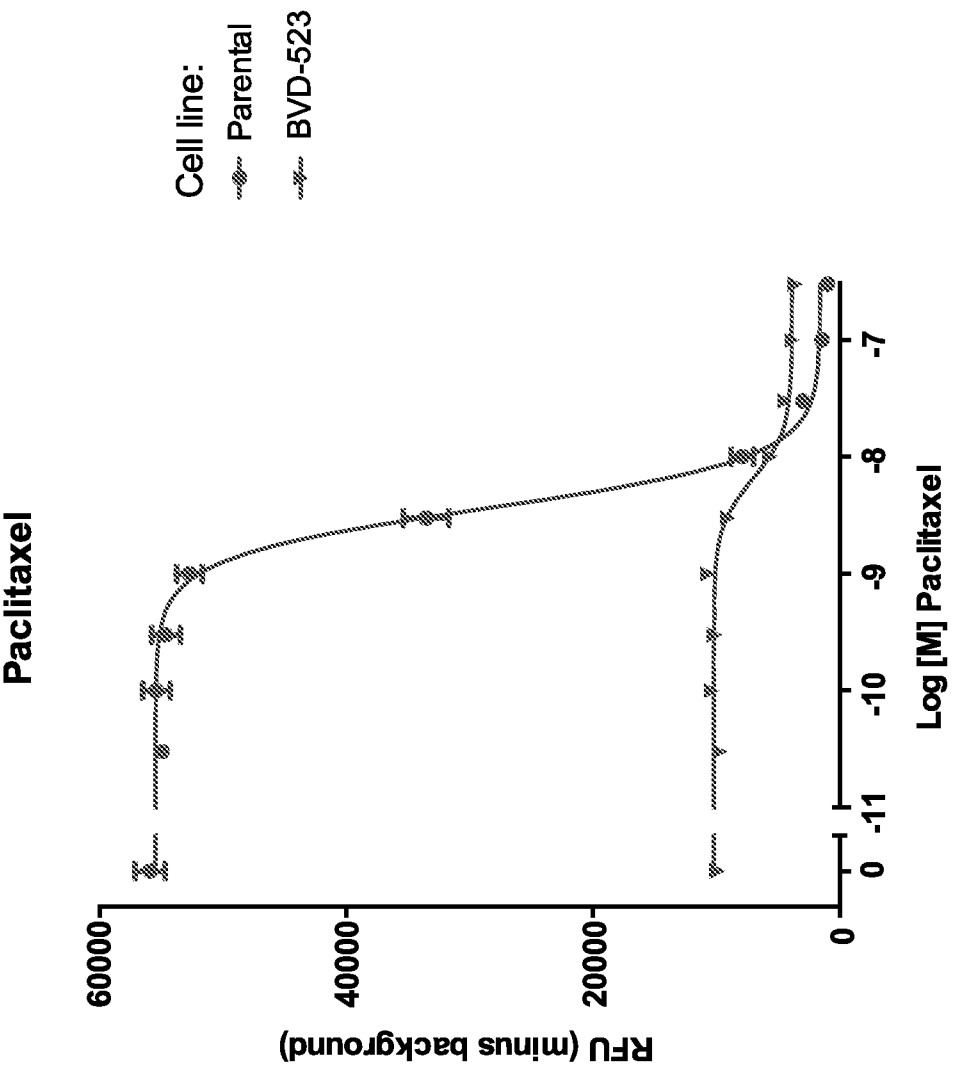
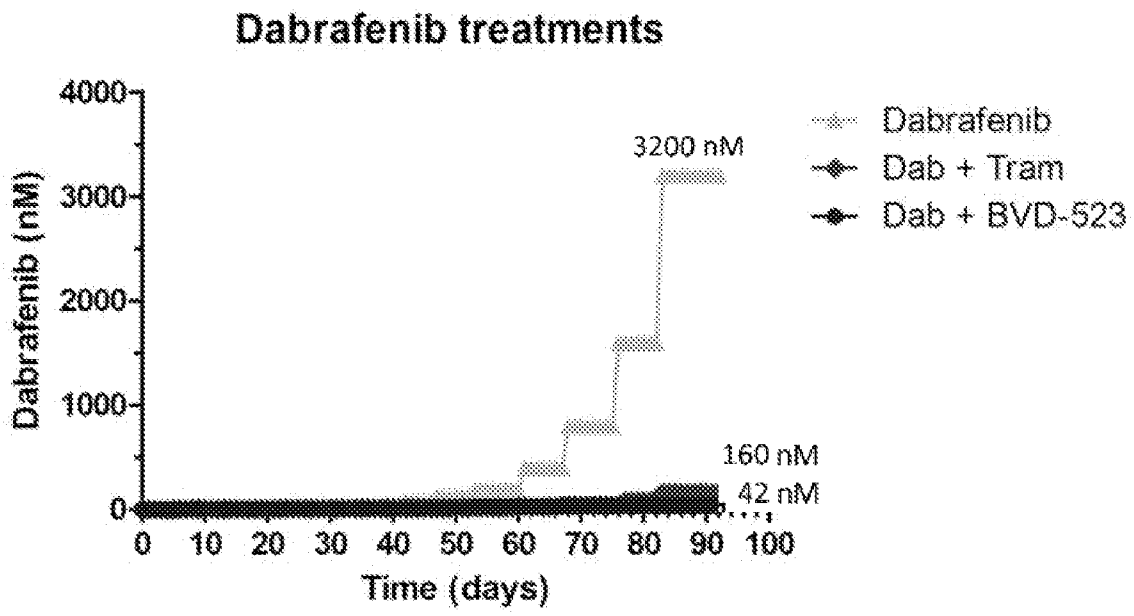

H
Paclitaxel
Cell line:
Parental
BVD-523
RFU (minus background)
60000
40000
20000
0
0
-11
-10
-9
-8
-7
Log [M] Paclitaxel

FIG. 6

A


Dabrafenib treatments
Dabrafenib (nM)
4000
3000
2000
1000
0
3200 nM
160 nM
42 nM
0 10 20 30 40 50 60 70 80 90 100
Time (days)
Dabrafenib
Dab + Tram
Dab + BVD-523

B

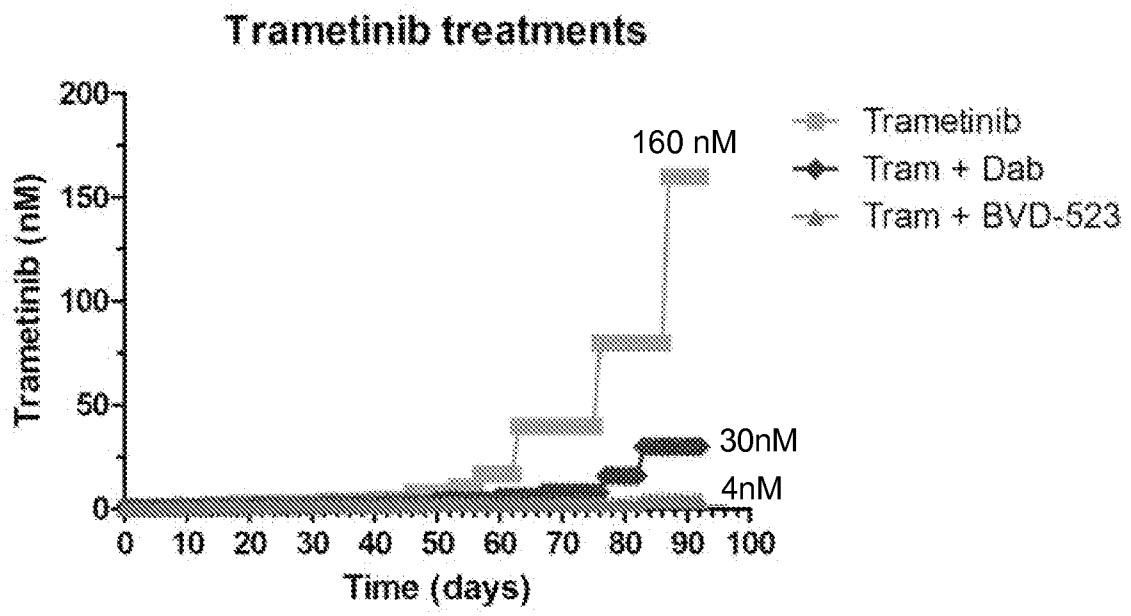
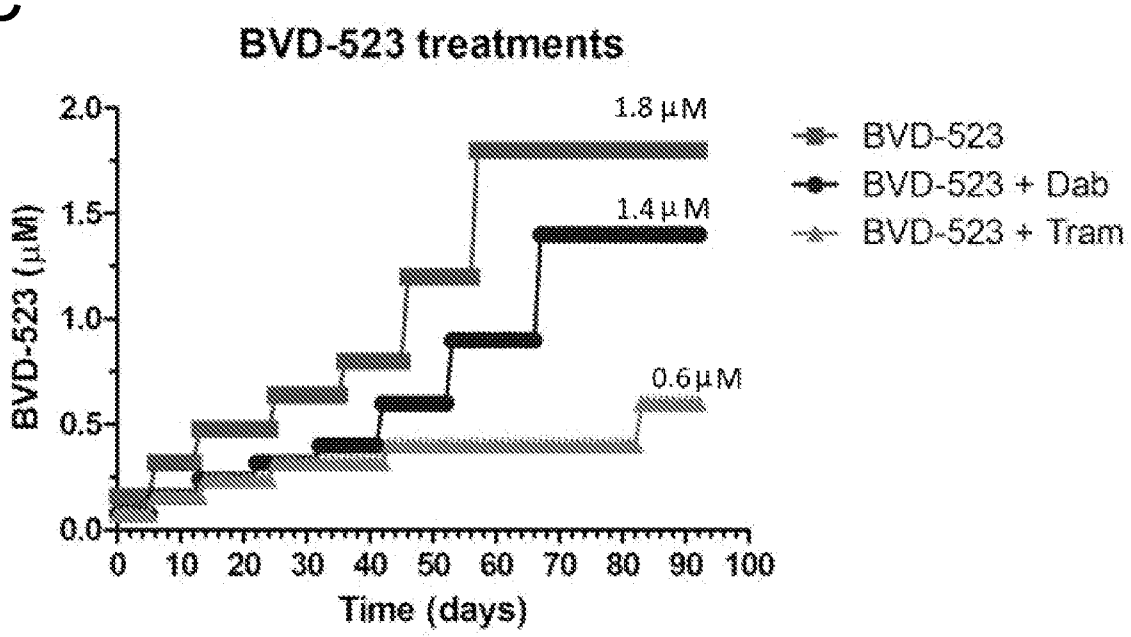

Trametinib treatments
Trametinib (nM)
200
150
100
50
0
160 nM
30nM
4nM
0 10 20 30 40 50 60 70 80 90 100
Time (days)
Trametinib
Tram + Dab
Tram + BVD-523

FIG. 6, Con't

C


BVD-523 treatments
BVD-523 (μM)
2.0
1.5
1.0
0.5
0.0
1.8 μM
1.4 μM
0.6 μM
0 10 20 30 40 50 60 70 80 90 100
Time (days)
BVD-523
BVD-523 + Dab
BVD-523 + Tram

D

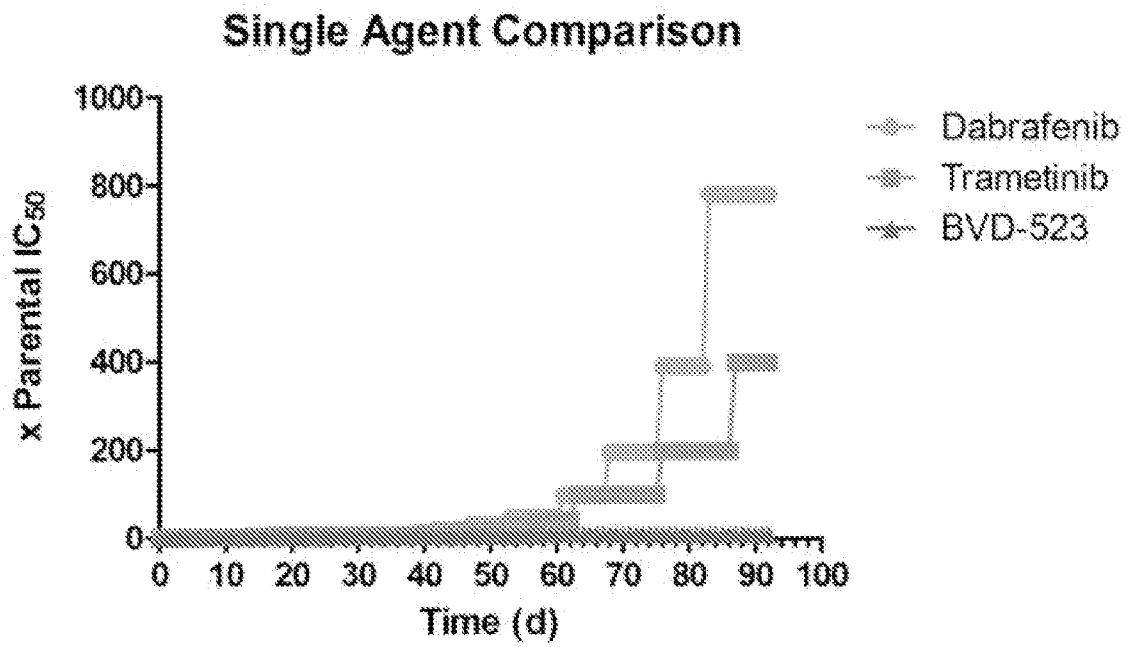

Single Agent Comparison
x Parental IC50
1000
800
600
400
200
0
0 10 20 30 40 50 60 70 80 90 100
Time (d)
Dabrafenib
Trametinib
BVD-523

FIG. 7

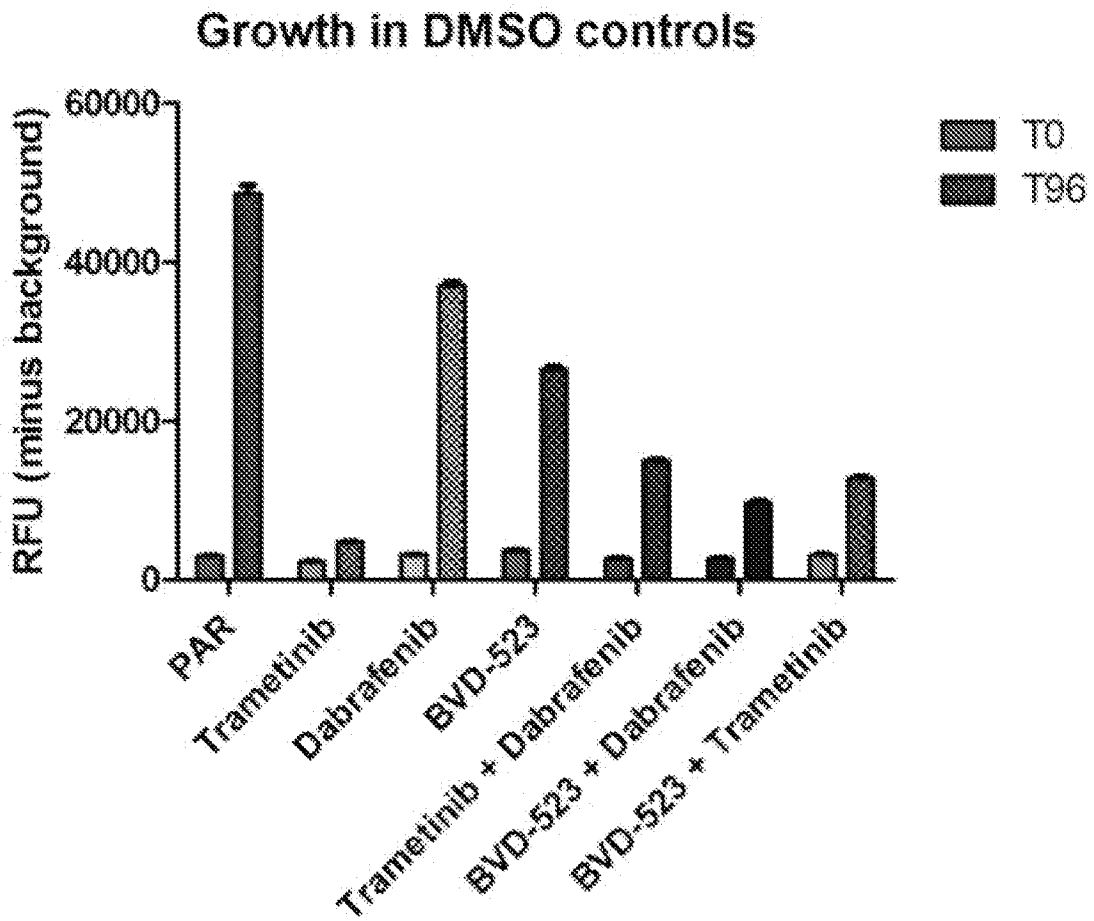

Growth in DMSO controls
RFU (minus background)
60000
40000
20000
0
T0
T96
PAR
Trametinib
Dabrafenib
BVD-523
Trametinib + Dabrafenib
BVD-523 + Dabrafenib
BVD-523 + Trametinib

FIG. 8

A

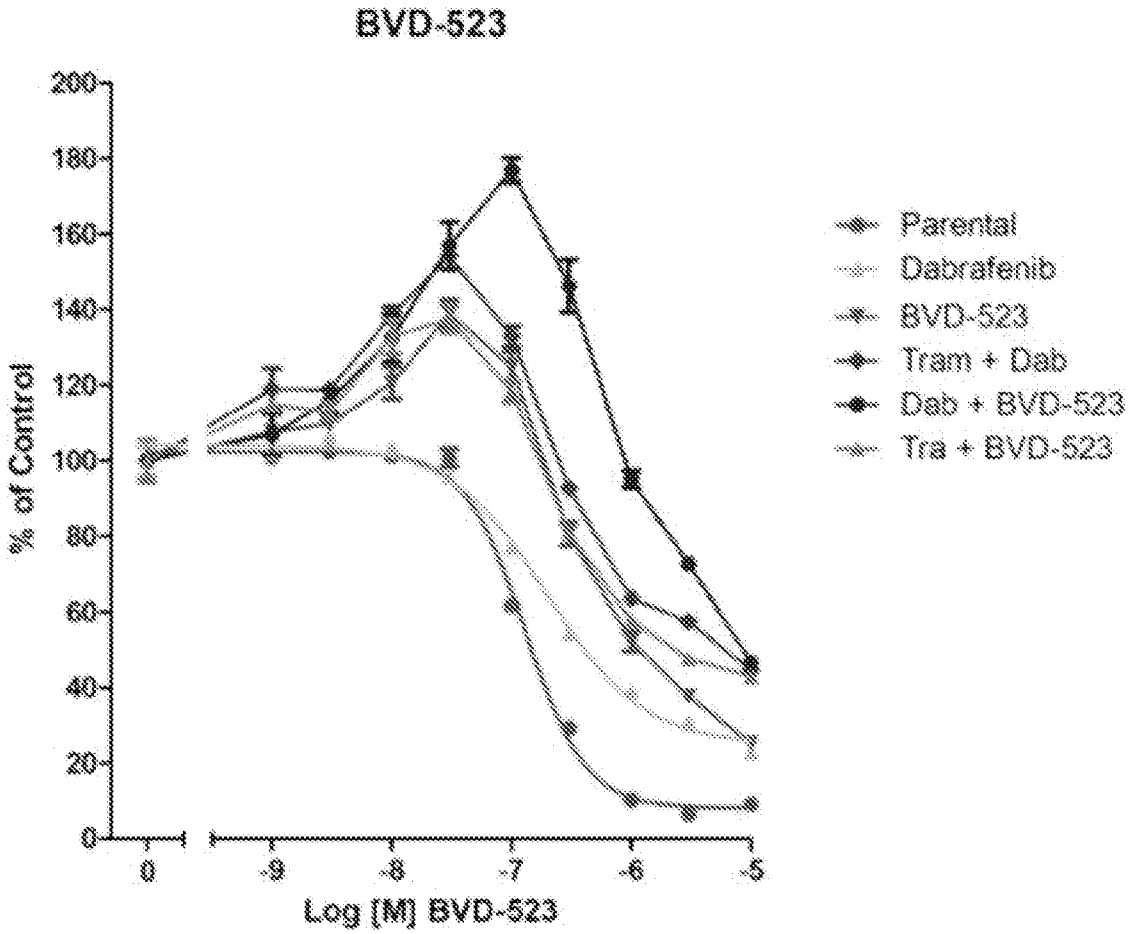

BVD-523
% of Control
200
180
160
140
120
100
80
60
40
20
0
0
-9
-8
-7
-6
-5
Log [M] BVD-523
Parental
Dabrafenib
BVD-523
Tram + Dab
Dab + BVD-523
Tra + BVD-523

FIG. 8, Con't

B

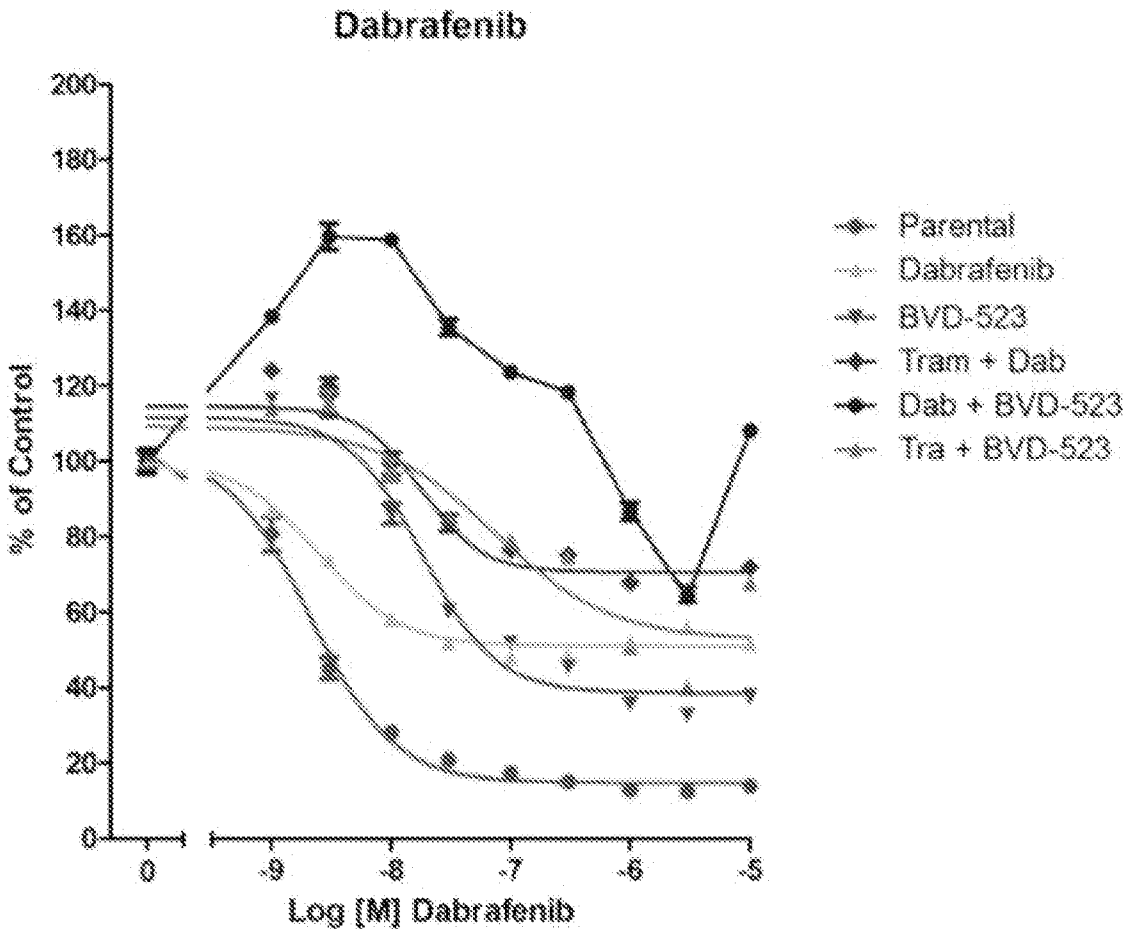

Dabrafenib
% of Control
200
180
160
140
120
100
80
60
40
20
0
0
-9
-8
-7
-6
-5
Log [M] Dabrafenib
Parental
Dabrafenib
BVD-523
Tram + Dab
Dab + BVD-523
Tra + BVD-523

FIG. 8, Con't

C

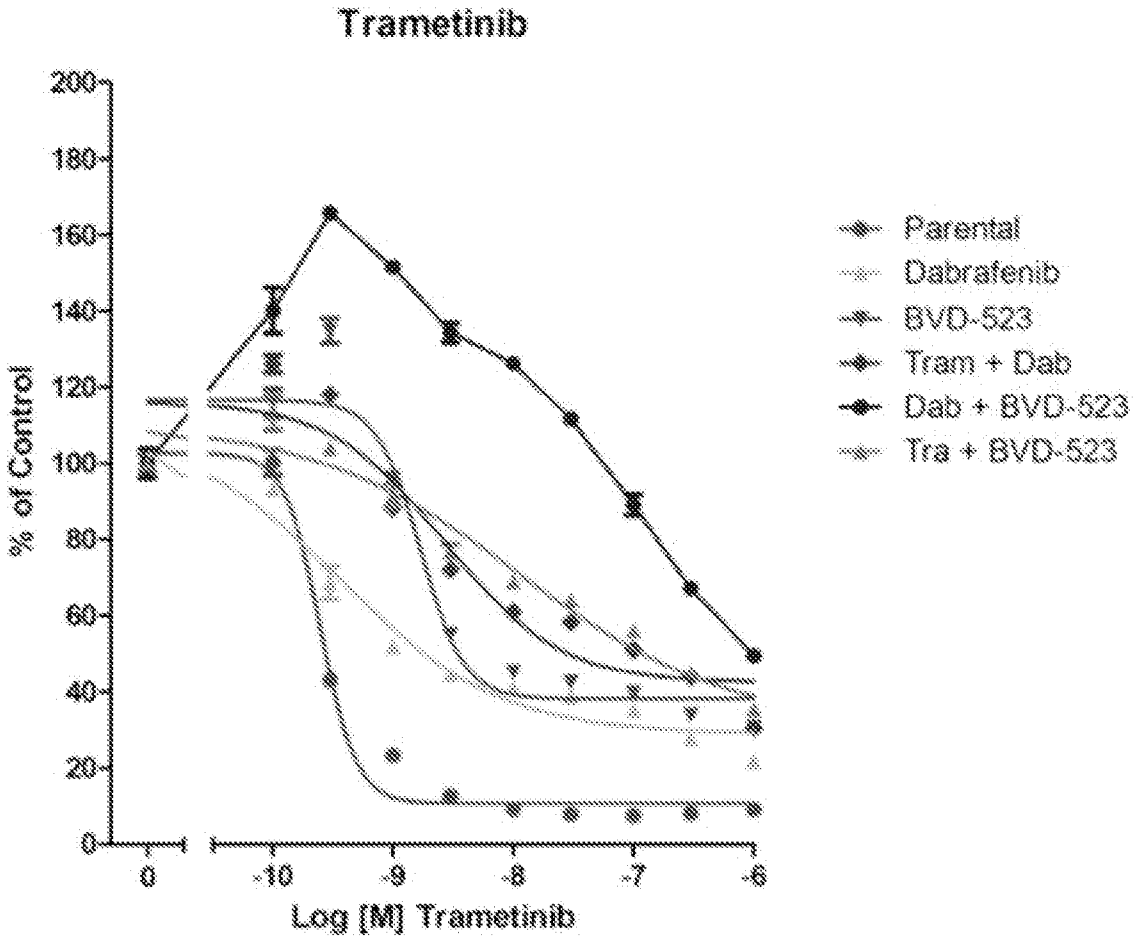

Trametinib
% of Control
200
180
160
140
120
100
80
60
40
20
0
0
-10
-9
-8
-7
-6
Log [M] Trametinib
Parental
Dabrafenib
BVD-523
Tram + Dab
Dab + BVD-523
Tra + BVD-523

FIG. 8, Con't

D

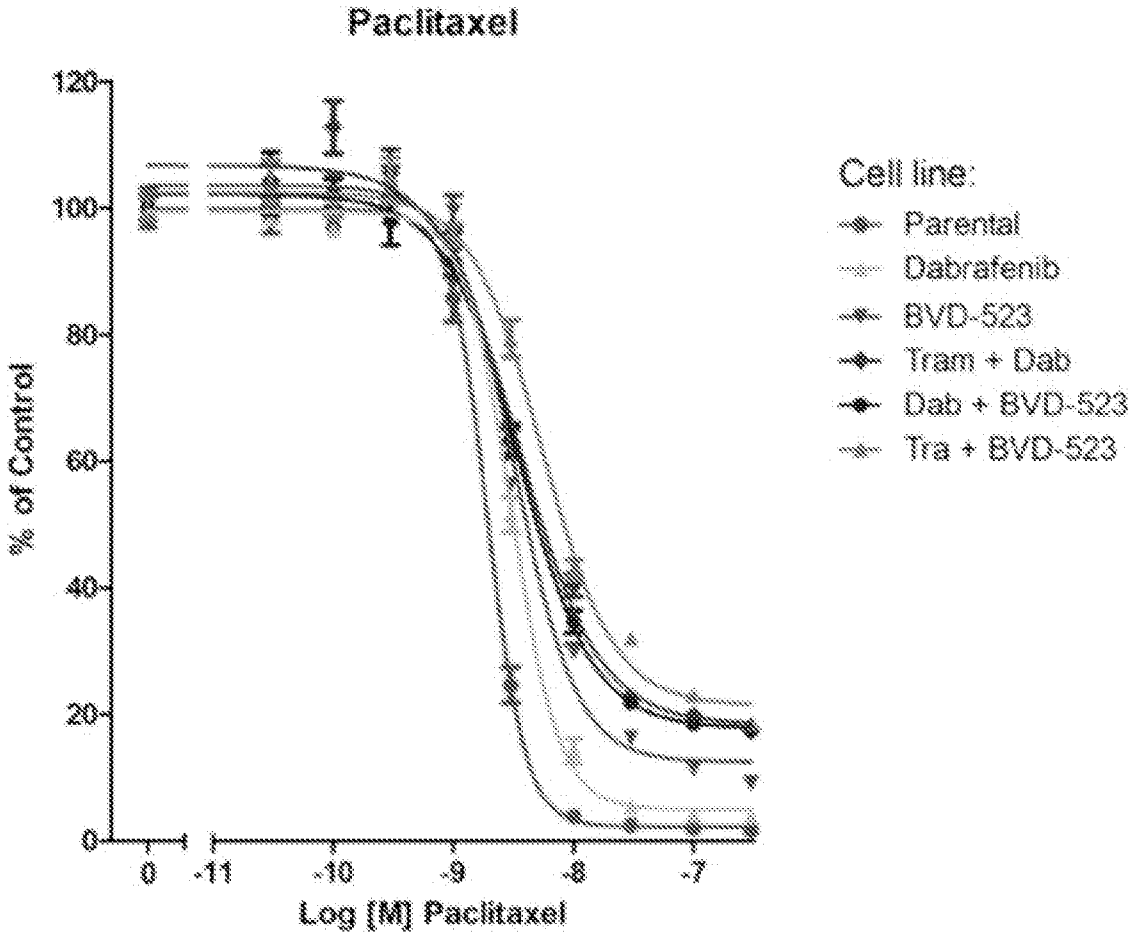

Paclitaxel
120
100
80
60
40
20
0
% of Control
0
-11
-10
-9
-8
-7
Log [M] Paclitaxel
Cell line:
Parental
Dabrafenib
BVD-523
Tram + Dab
Dab + BVD-523
Tra + BVD-523

FIG. 9

A

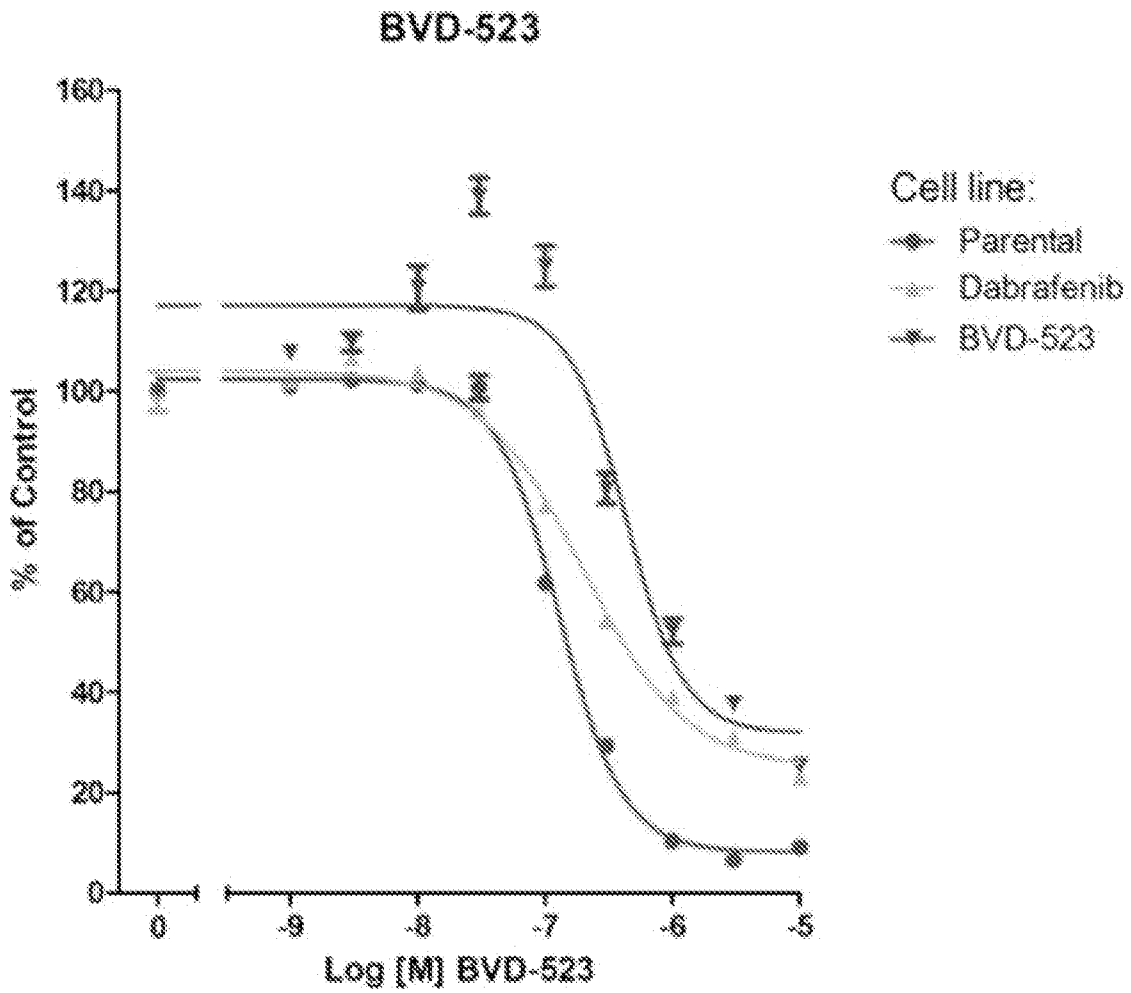

BVD-523
% of Control
160
140
120
100
80
60
40
20
0
0
-9
-8
-7
-6
-5
Log [M] BVD-523
Cell line:
Parental
Dabrafenib
BVD-523

FIG. 9, Con't

B

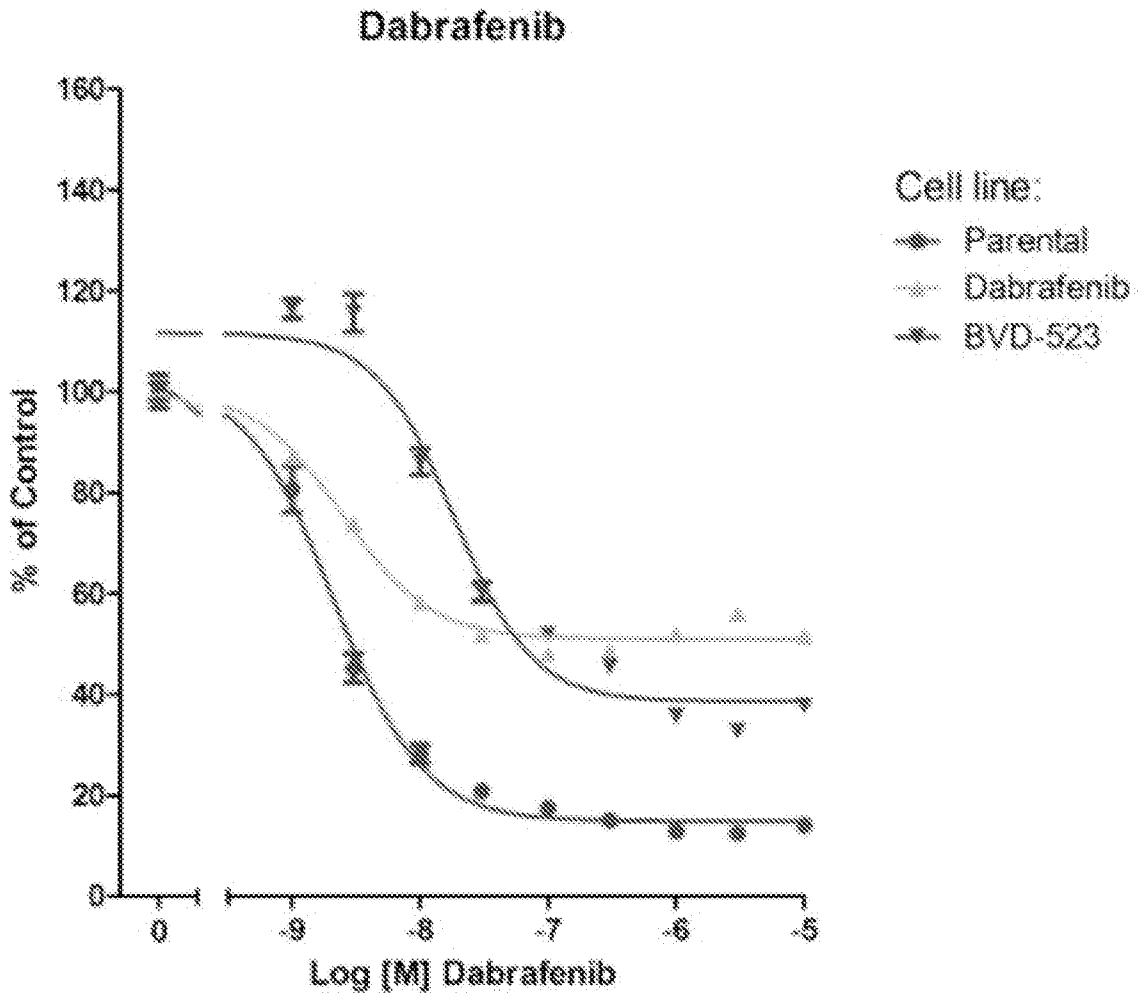

Dabrafenib
% of Control
160
140
120
100
80
60
40
20
0
0
-9
-8
-7
-6
-5
Log [M] Dabrafenib
Cell line:
Parental
Dabrafenib
BVD-523

FIG. 9, Con't

C

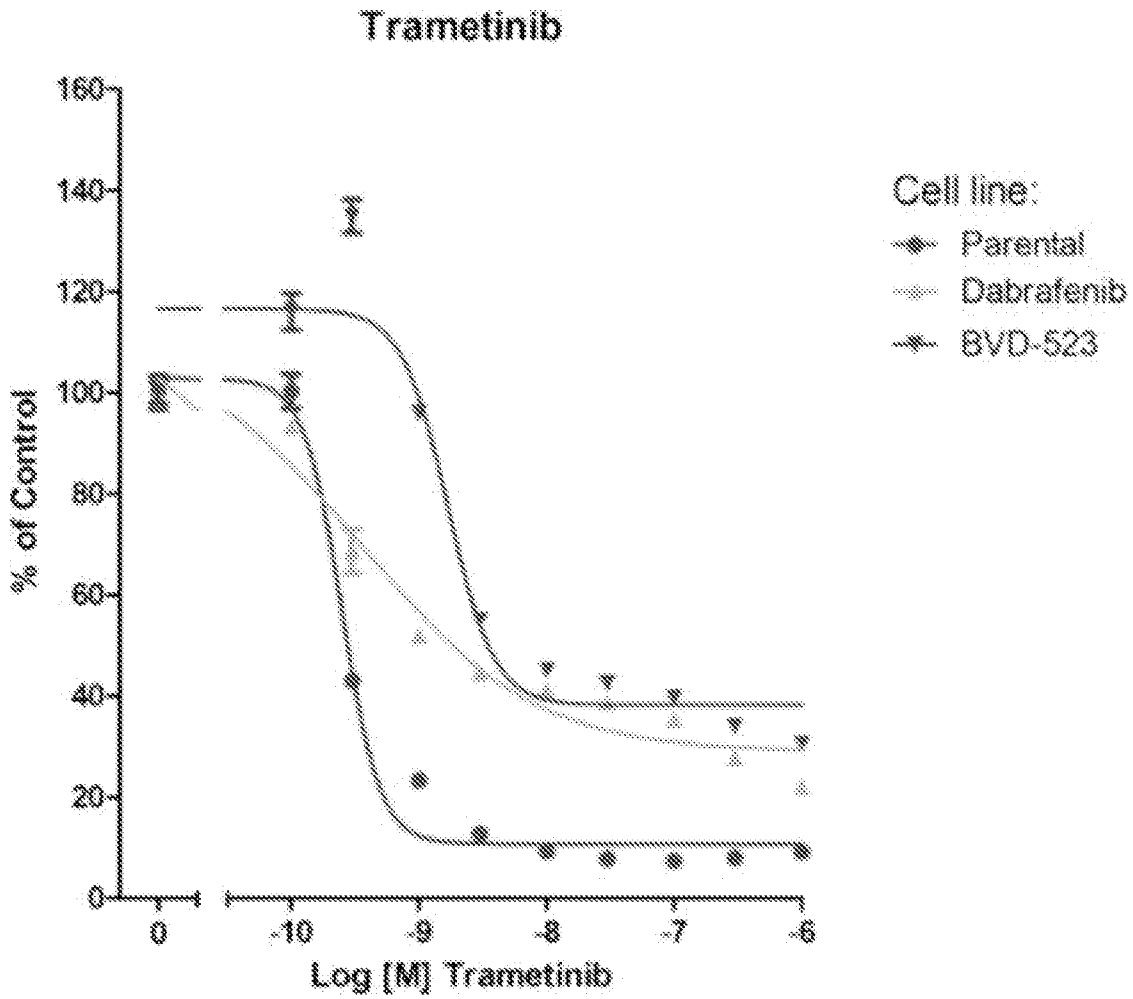

Trametinib
% of Control
160
140
120
100
80
60
40
20
0
0
-10
-9
-8
-7
-6
Log [M] Trametinib
Cell line:
Parental
Dabrafenib
BVD-523

FIG. 9, Con't

D

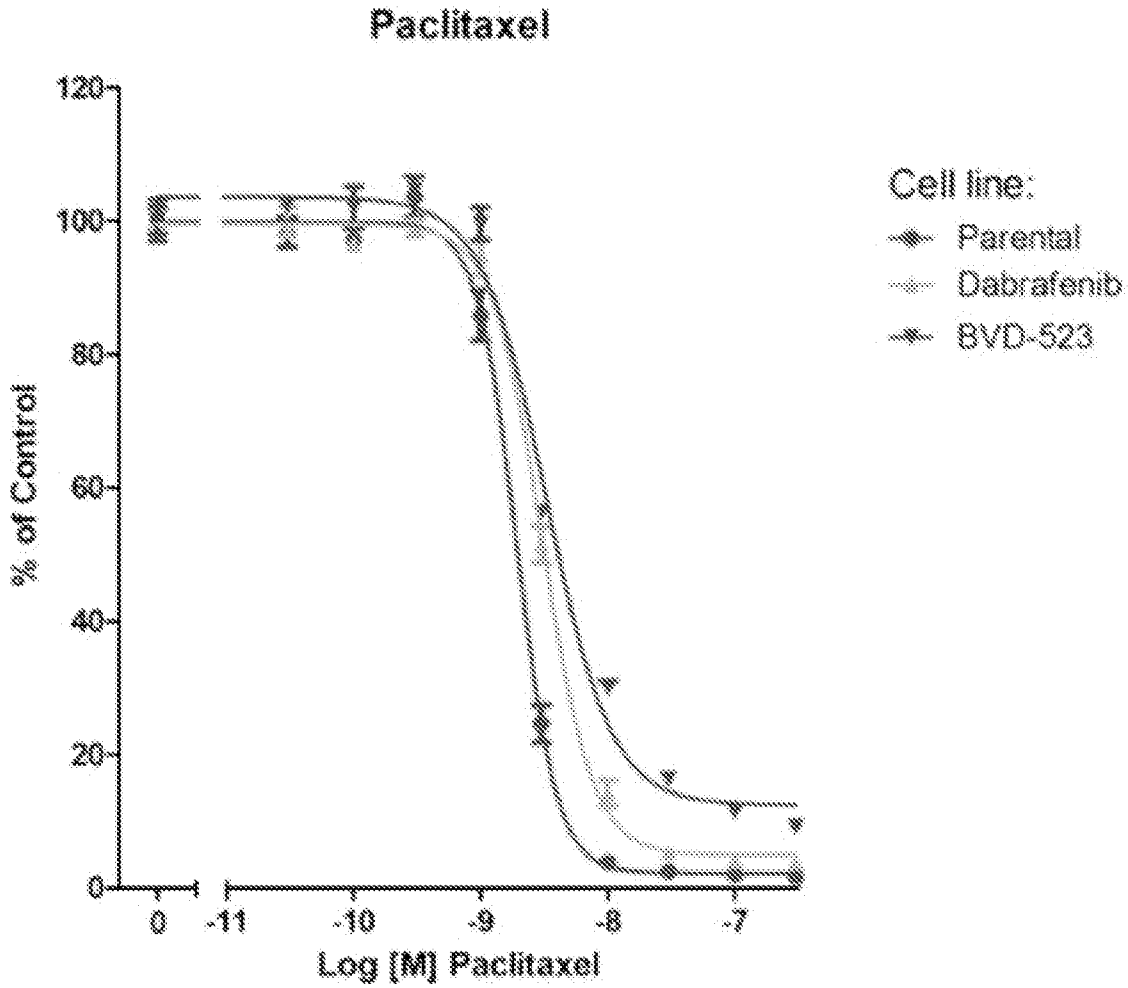

Paclitaxel
120
100
80
60
40
20
0
% of Control
0
-11
-10
-9
-8
-7
Log [M] Paclitaxel
Cell line:
Parental
Dabrafenib
BVD-523

FIG. 10

A

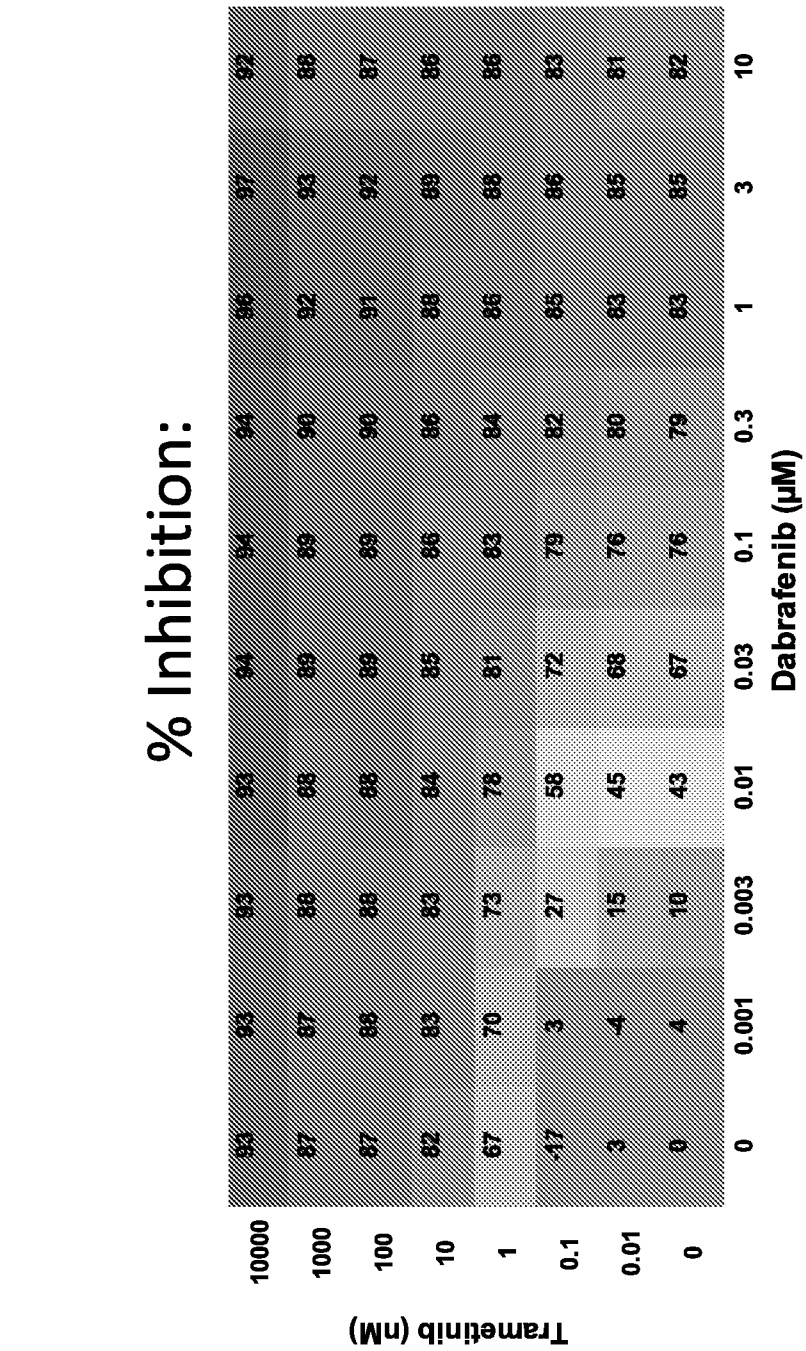

% Inhibition:
Trametinib (nM)
10000
1000
100
10
1
0.1
0.01
0
Dabrafenib (μM)
0
0.001
0.003
0.01
0.03
0.1
0.3
1
3
10
93 93 93 93 93 94 94 94 96 97 98
87 87 88 88 88 89 89 90 92 93 98
87 88 88 88 89 89 90 90 91 92 97
82 83 83 84 85 86 86 88 89 89 96
67 70 73 79 81 83 84 86 88 88 93
-17 3 27 58 72 79 82 85 86 86 93
3 -4 15 45 68 76 80 83 85 85 91
0 4 10 43 67 76 79 83 85 85 92

FIG. 10, Con't

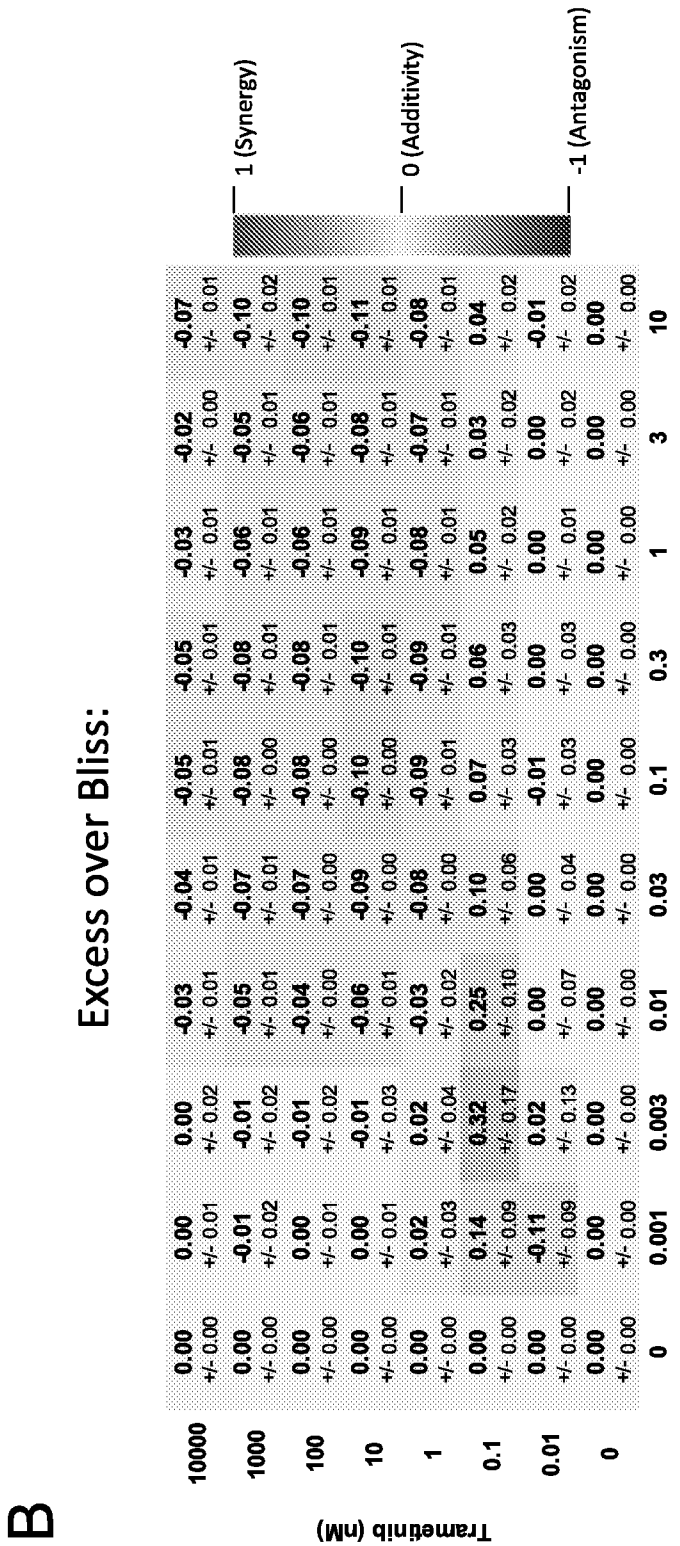

B
Excess over Bliss:
Trametinib (nM)
Dabrafenib (μM)
1 (Synergy)
0 (Additivity)
-1 (Antagonism)
10000
1000
100
10
1
0.1
0.01
0
0 0.001 0.003 0.01 0.03 0.1 0.3 1 3 10

FIG. 10, Con't

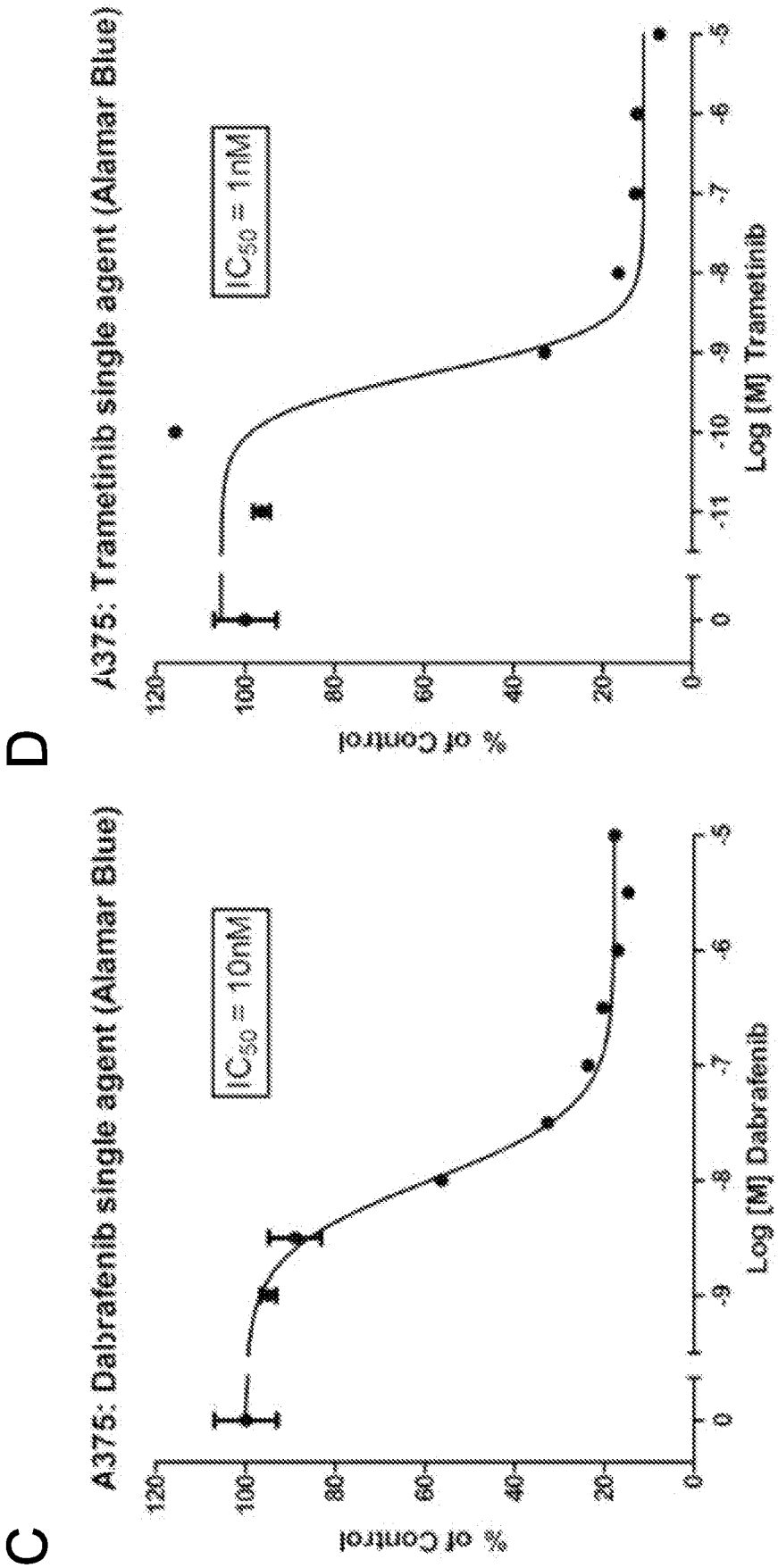

C
A375: Dabrafenib single agent (Alamar Blue)
IC50 = 10nM
% of Control
Log [M] Dabrafenib
D
A375: Trametinib single agent (Alamar Blue)
IC50 = 1nM
% of Control
Log [M] Trametinib

FIG. 10, Con't

E

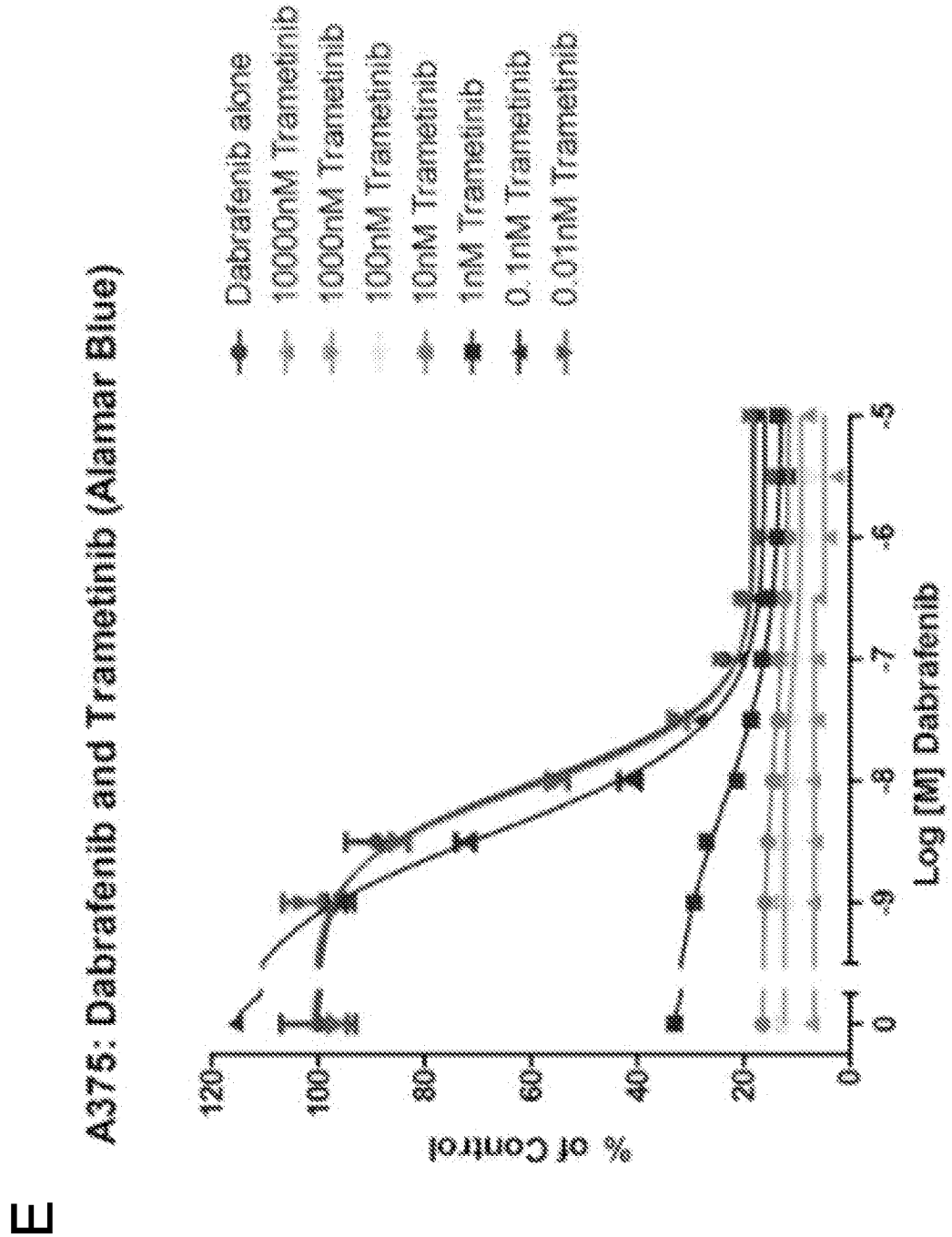

A375: Dabrafenib and Trametinib (Alamar Blue)
Dabrafenib alone
1000nM Trametinib
100nM Trametinib
10nM Trametinib
1nM Trametinib
0.1nM Trametinib
0.01nM Trametinib
% of Control
120
100
80
60
40
20
0
0
-5
-6
-7
-8
-9
Log [M] Dabrafenib

FIG. 11

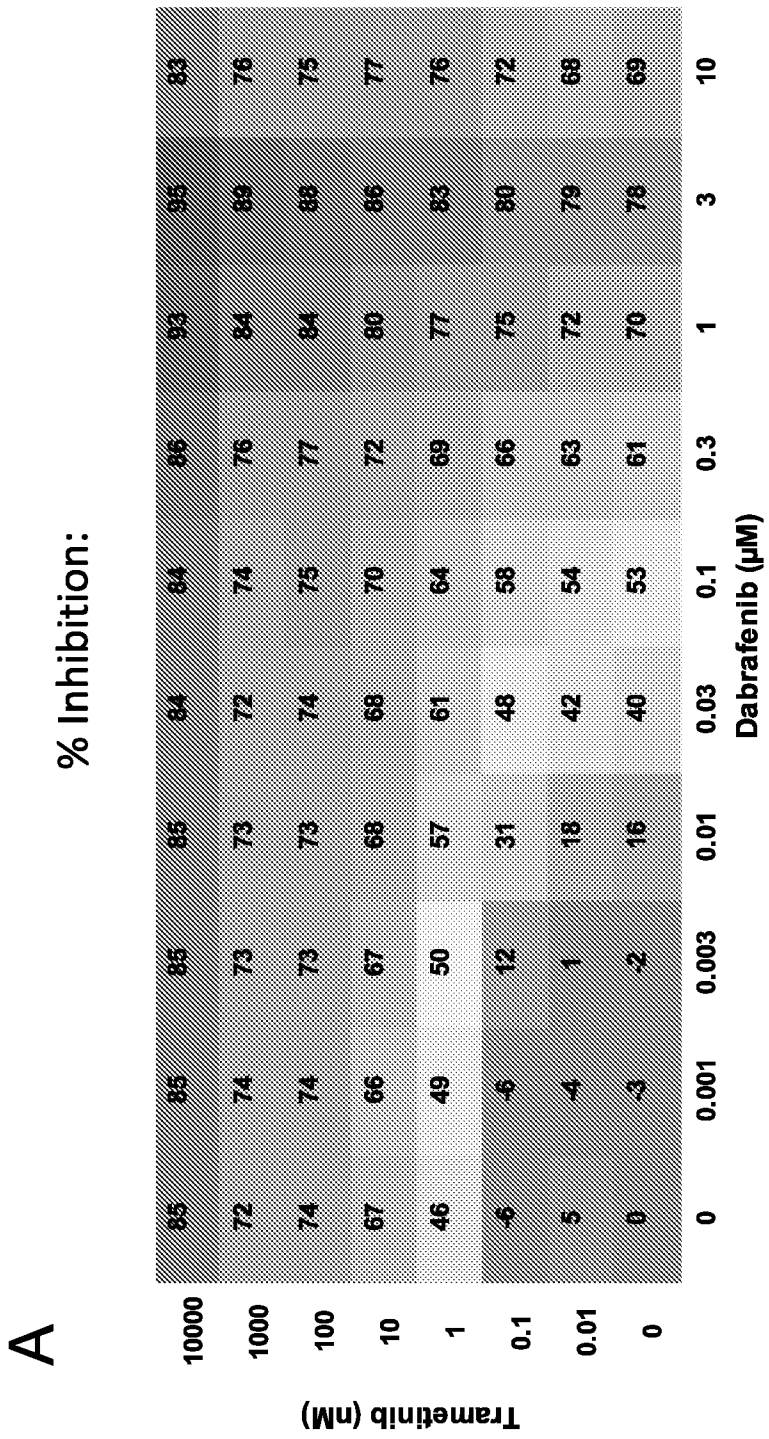

A
% Inhibition:
Trametinib (nM)
10000
1000
100
10
1
0.1
0.01
0
Dabrafenib (μM)
0
0.001
0.003
0.01
0.03
0.1
0.3
1
3
10
85 85 85 86 84 84 86 93 95 83
72 74 73 73 72 74 76 84 89 76
74 74 73 73 74 75 77 84 88 75
67 66 67 68 68 70 72 80 86 77
46 49 50 57 61 64 69 77 83 76
-6 -6 12 31 48 58 66 75 80 72
5 -4 1 18 42 54 63 72 79 68
0 -3 -2 16 40 53 61 70 78 69

FIG. 11, Con't

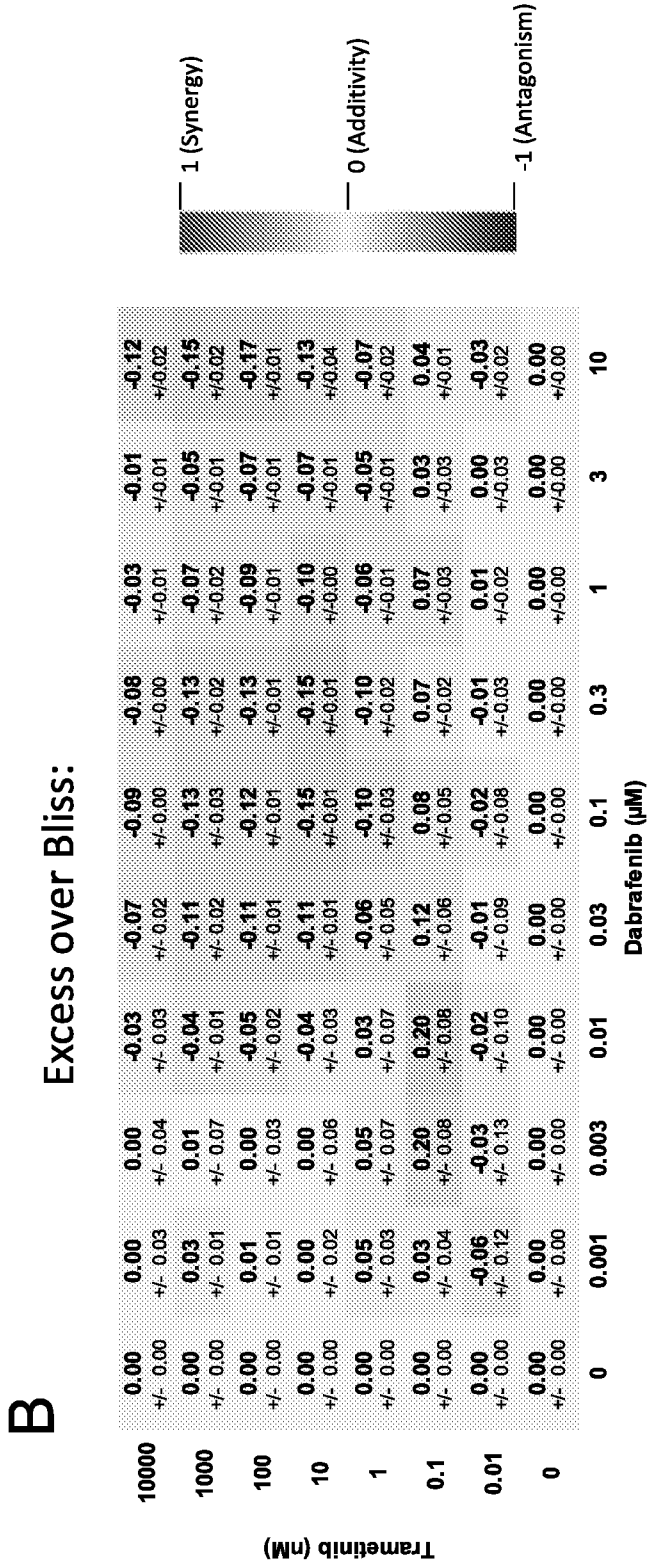

B
Excess over Bliss:
Trametinib (nM)
Dabrafenib (μM)
10000
1000
100
10
1
0.1
0.01
0
0 0.001 0.003 0.01 0.03 0.1 0.3 1 3 10
0.00 +/- 0.00 0.00 +/- 0.03 0.00 +/- 0.04 -0.03 +/- 0.03 -0.07 +/- 0.02 -0.09 +/-0.00 -0.08 +/-0.00 -0.03 +/-0.01 -0.01 +/-0.01 -0.12 +/-0.02
0.00 +/- 0.00 0.03 +/- 0.01 0.01 +/- 0.07 -0.04 +/- 0.01 -0.11 +/- 0.02 -0.13 +/-0.03 -0.13 +/-0.02 -0.07 +/-0.02 -0.05 +/-0.01 -0.15 +/-0.02
0.00 +/- 0.00 0.01 +/- 0.01 0.00 +/- 0.03 -0.05 +/- 0.02 -0.11 +/- 0.01 -0.12 +/-0.01 -0.13 +/-0.01 -0.09 +/-0.01 -0.07 +/-0.01 -0.17 +/-0.01
0.00 +/- 0.00 0.00 +/- 0.02 0.00 +/- 0.06 -0.04 +/- 0.03 -0.11 +/- 0.01 -0.15 +/-0.01 -0.15 +/-0.01 -0.10 +/-0.00 -0.07 +/-0.01 -0.13 +/-0.04
0.00 +/- 0.00 0.05 +/- 0.03 0.05 +/- 0.07 0.03 +/- 0.07 -0.06 +/- 0.05 -0.10 +/-0.03 -0.10 +/-0.02 -0.06 +/-0.01 -0.05 +/-0.01 -0.07 +/-0.02
0.00 +/- 0.00 0.03 +/- 0.04 0.20 +/- 0.08 0.20 +/- 0.08 0.12 +/- 0.06 0.08 +/- 0.05 0.07 +/-0.02 0.07 +/-0.03 0.03 +/-0.03 0.04 +/-0.01
0.00 +/- 0.00 -0.06 +/- 0.12 -0.03 +/- 0.13 -0.02 +/- 0.10 -0.01 +/- 0.09 -0.02 +/- 0.08 -0.01 +/-0.03 0.01 +/-0.02 0.00 +/-0.03 -0.03 +/-0.02
0.00 +/- 0.00 0.00 +/- 0.00 0.00 +/- 0.00 0.00 +/- 0.00 0.00 +/- 0.00 0.00 +/- 0.00 0.00 +/-0.00 0.00 +/-0.00 0.00 +/-0.00 0.00 +/-0.00
1 (Synergy)
0 (Additivity)
-1 (Antagonism)

FIG. 11, Con't

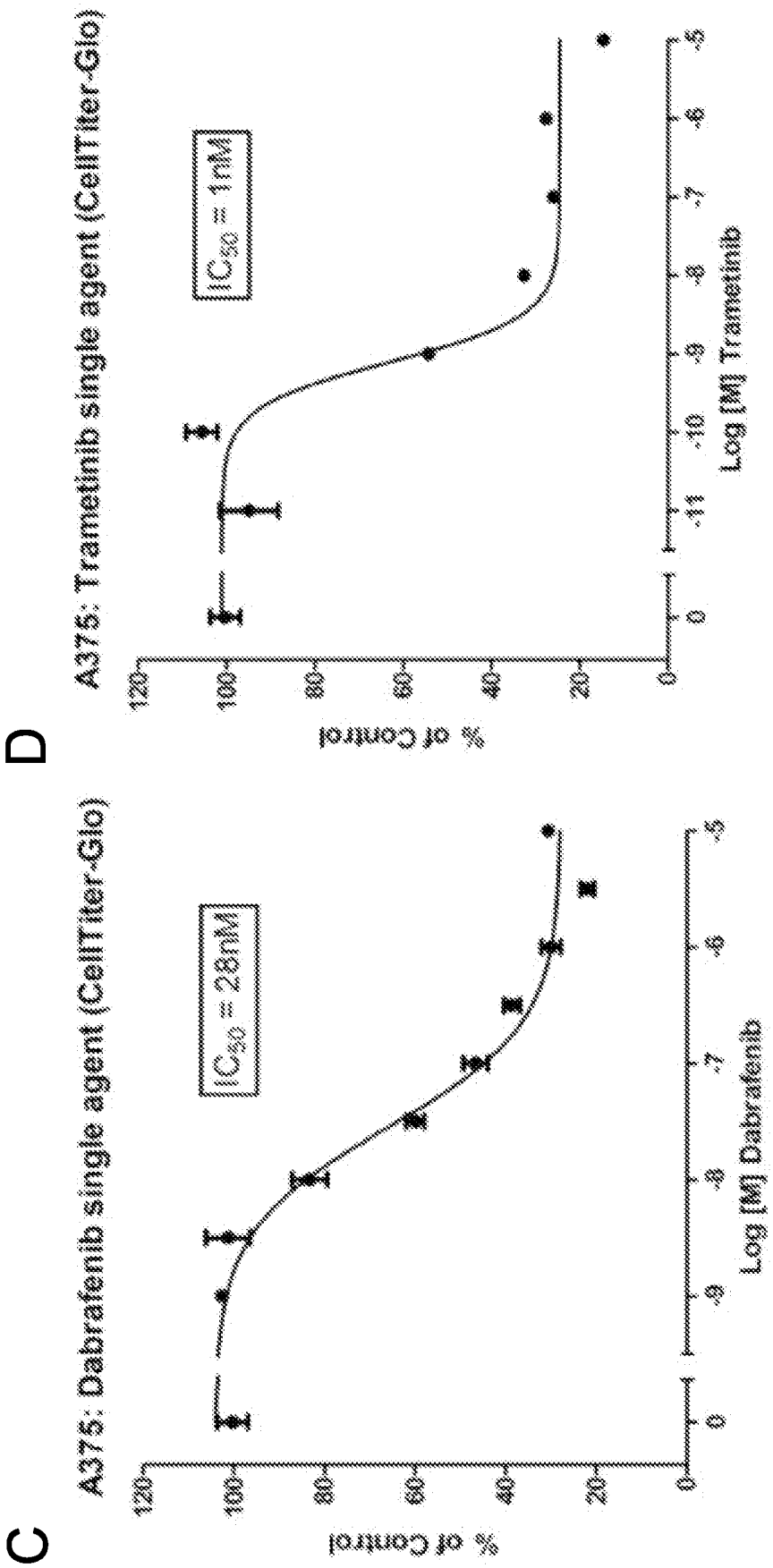

C
A375: Dabrafenib single agent (CellTiter-Glo)
IC50 = 28nM
% of Control
Log [M] Dabrafenib
D
A375: Trametinib single agent (CellTiter-Glo)
IC50 = 1nM
% of Control
Log [M] Trametinib

FIG. 11, Con't

E

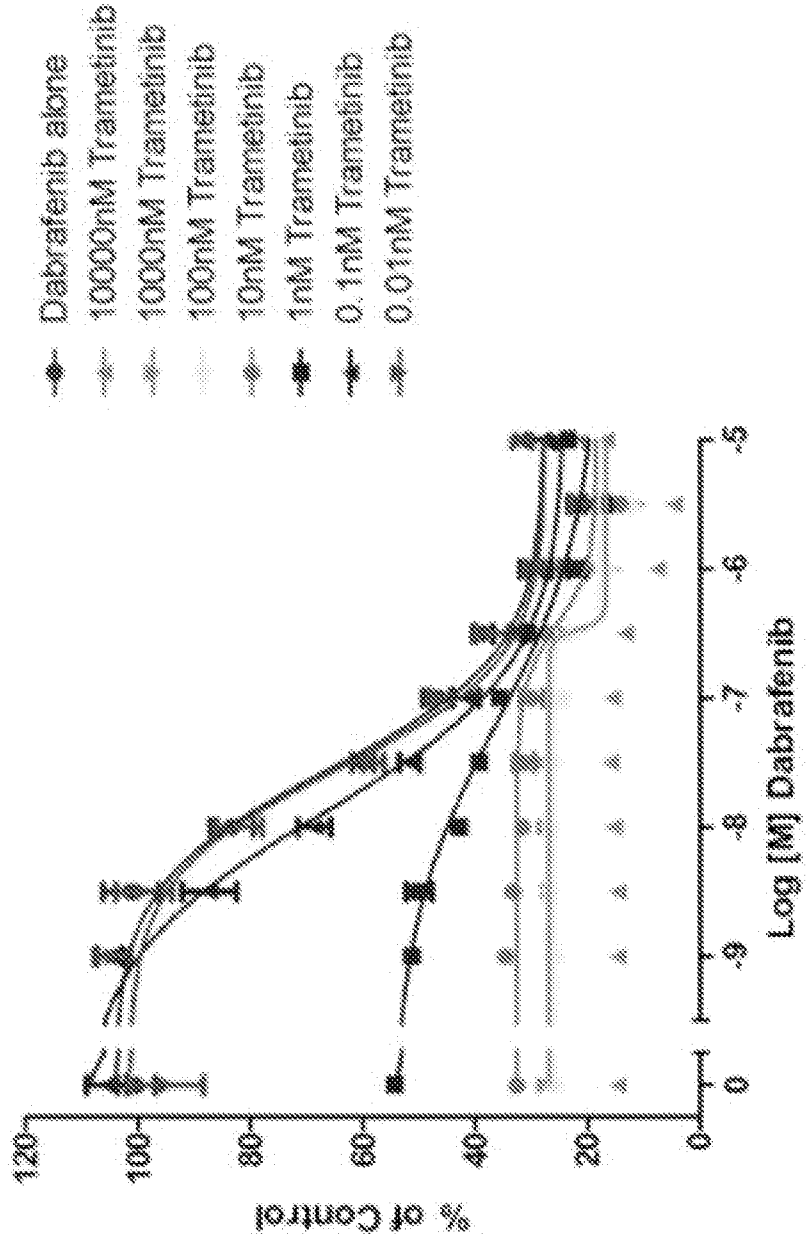

A375: Dabrafenib and Trametinib (CellTiter-Glo)
Dabrafenib alone
10000nM Trametinib
1000nM Trametinib
100nM Trametinib
10nM Trametinib
1nM Trametinib
0.1nM Trametinib
0.01nM Trametinib
% of Control
120
100
80
60
40
20
0
0
-5
-6
-7
-8
-9
Log [M] Dabrafenib

FIG. 12

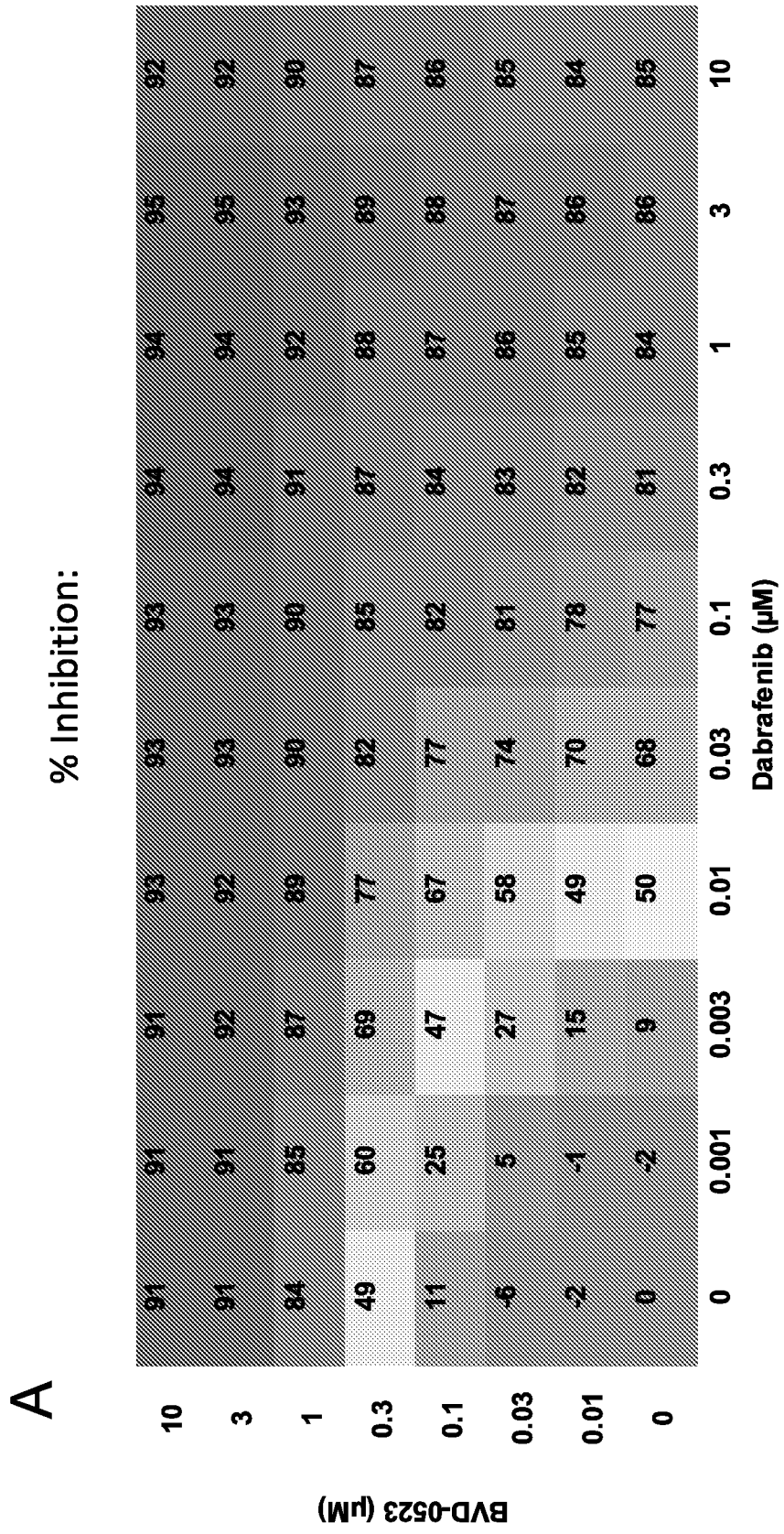

A
% Inhibition:
BVD-0523 (μM)
10
3
1
0.3
0.1
0.03
0.01
0
0
0.001
0.003
0.01
0.03
0.1
0.3
1
3
10
Dabrafenib (μM)
91 91 91 93 93 93 94 94 95 92
91 91 92 92 93 93 94 94 95 92
84 85 87 89 90 90 91 92 93 90
49 60 69 77 82 85 87 88 89 87
11 25 47 67 77 82 84 87 88 86
-6 5 27 58 74 81 83 86 87 85
-2 -1 15 49 70 78 82 85 86 84
0 -2 9 50 68 77 81 84 86 85

FIG. 12, Con't

B

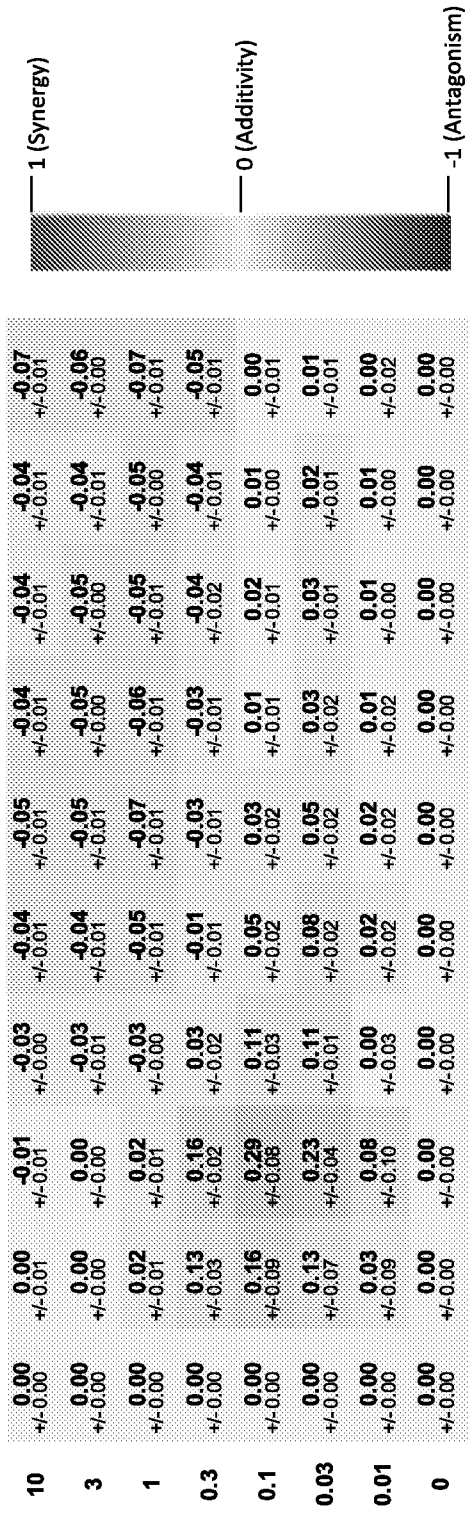
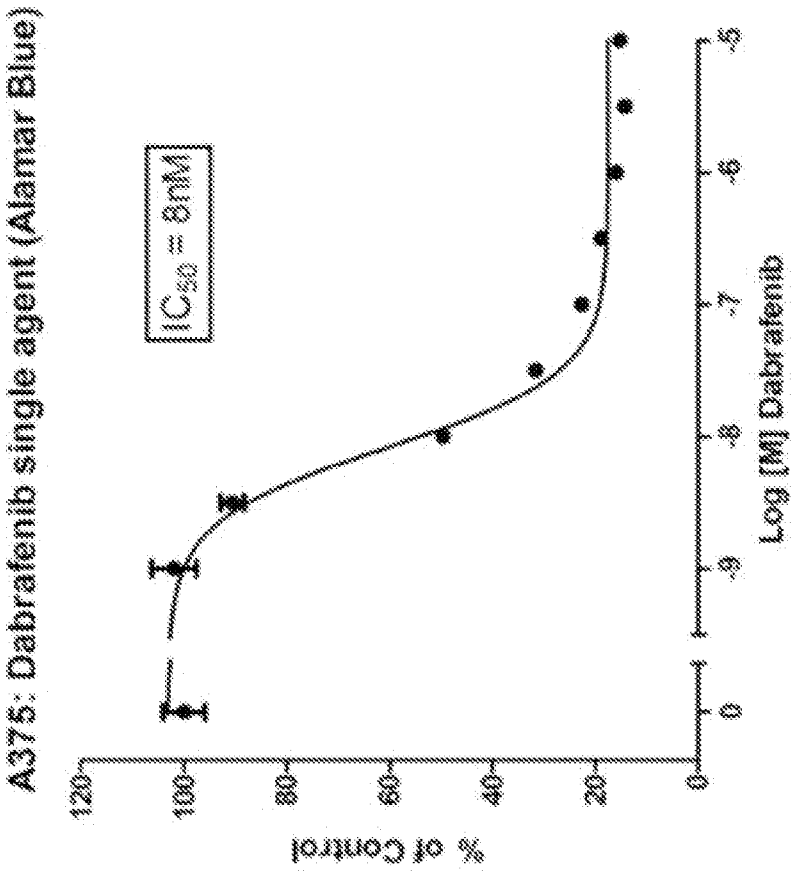

Excess over Bliss:
1 (Synergy)
0 (Additivity)
-1 (Antagonism)
BVD-523 (μM)
Dabrafenib (μM)
10 3 1 0.3 0.1 0.03 0.01 0
0 0.001 0.003 0.01 0.03 0.1 0.3 1 3 10
0.00 +/-0.00 0.00 +/-0.01 -0.01 +/-0.01 -0.03 +/-0.00 -0.04 +/-0.01 -0.05 +/-0.01 -0.04 +/-0.01 -0.04 +/-0.01 -0.04 +/-0.01 -0.07 +/-0.01
0.00 +/-0.00 0.00 +/-0.00 0.00 +/-0.00 -0.03 +/-0.01 -0.04 +/-0.01 -0.05 +/-0.01 -0.05 +/-0.00 -0.04 +/-0.01 -0.04 +/-0.01 -0.05 +/-0.00 -0.06 +/-0.00
0.00 +/-0.00 0.02 +/-0.01 0.02 +/-0.01 -0.03 +/-0.00 -0.05 +/-0.01 -0.07 +/-0.01 -0.06 +/-0.01 -0.05 +/-0.01 -0.04 +/-0.01 -0.05 +/-0.00 -0.07 +/-0.01
0.00 +/-0.00 0.13 +/-0.03 0.16 +/-0.02 0.03 +/-0.02 -0.01 +/-0.01 -0.03 +/-0.01 -0.03 +/-0.01 -0.04 +/-0.02 -0.04 +/-0.01 -0.05 +/-0.01
0.00 +/-0.00 0.16 +/-0.09 0.29 +/-0.08 0.11 +/-0.03 0.05 +/-0.02 0.03 +/-0.02 0.01 +/-0.01 0.02 +/-0.01 0.01 +/-0.00 0.00 +/-0.01
0.00 +/-0.00 0.13 +/-0.07 0.23 +/-0.04 0.11 +/-0.01 0.08 +/-0.02 0.05 +/-0.02 0.03 +/-0.02 0.03 +/-0.01 0.02 +/-0.01 0.01 +/-0.01
0.00 +/-0.00 0.03 +/-0.09 0.08 +/-0.10 0.00 +/-0.03 0.02 +/-0.02 0.02 +/-0.02 0.01 +/-0.02 0.01 +/-0.00 0.01 +/-0.00 0.00 +/-0.02
0.00 +/-0.00 0.00 +/-0.00 0.00 +/-0.00 0.00 +/-0.00 0.00 +/-0.00 0.00 +/-0.00 0.00 +/-0.00 0.00 +/-0.00 0.00 +/-0.00 0.00 +/-0.00

FIG. 12, Con't

C



D

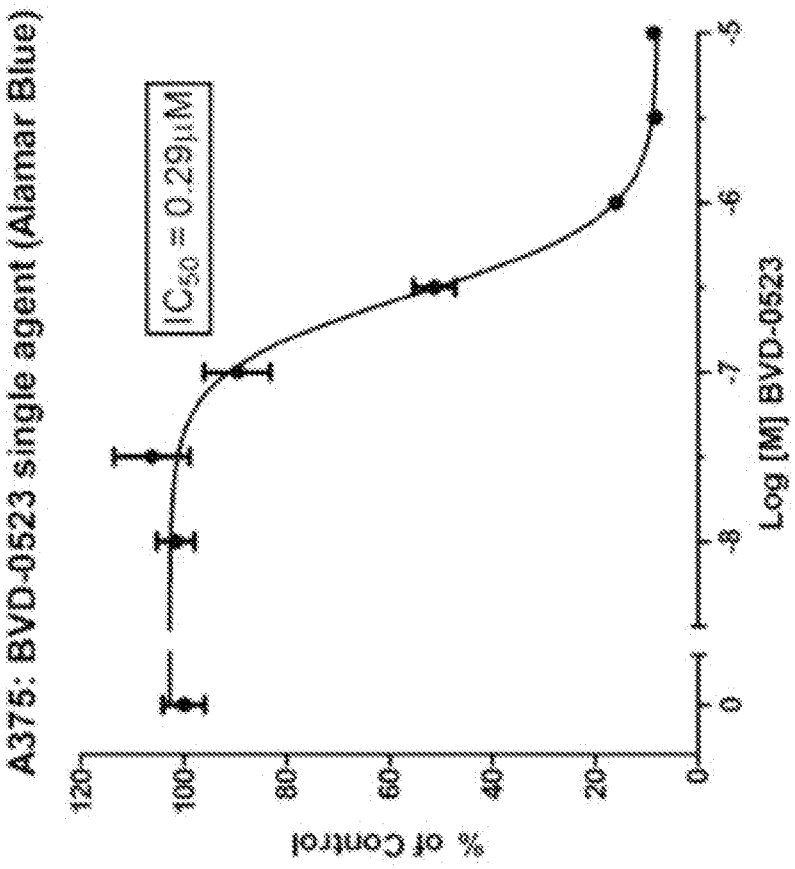


FIG. 12, Con't

E

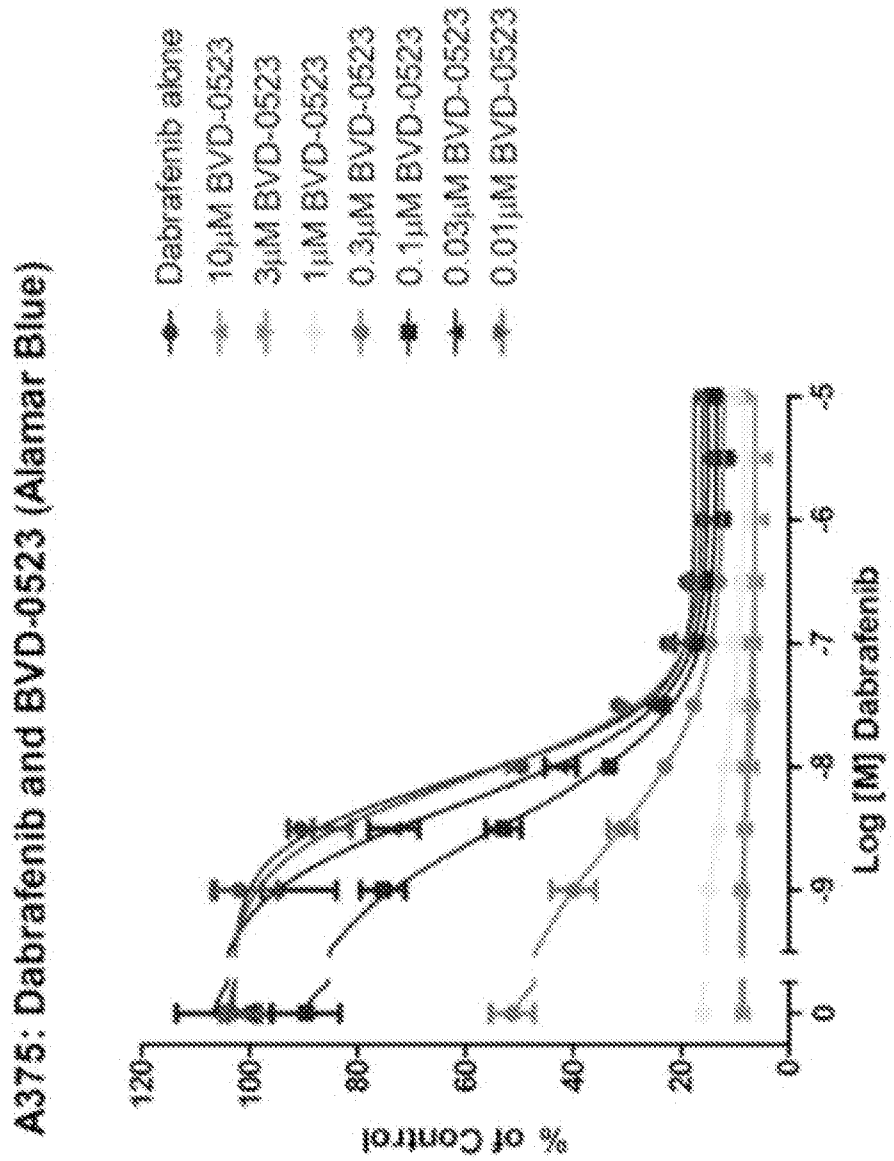

A375: Dabrafenib and BVD-0523 (Alamar Blue)
Dabrafenib alone
10μM BVD-0523
3μM BVD-0523
1μM BVD-0523
0.3μM BVD-0523
0.1μM BVD-0523
0.03μM BVD-0523
0.01μM BVD-0523
% of Control
120
100
80
60
40
20
0
0
-5
-6
-7
-8
-9
Log [M] Dabrafenib

FIG. 13

A

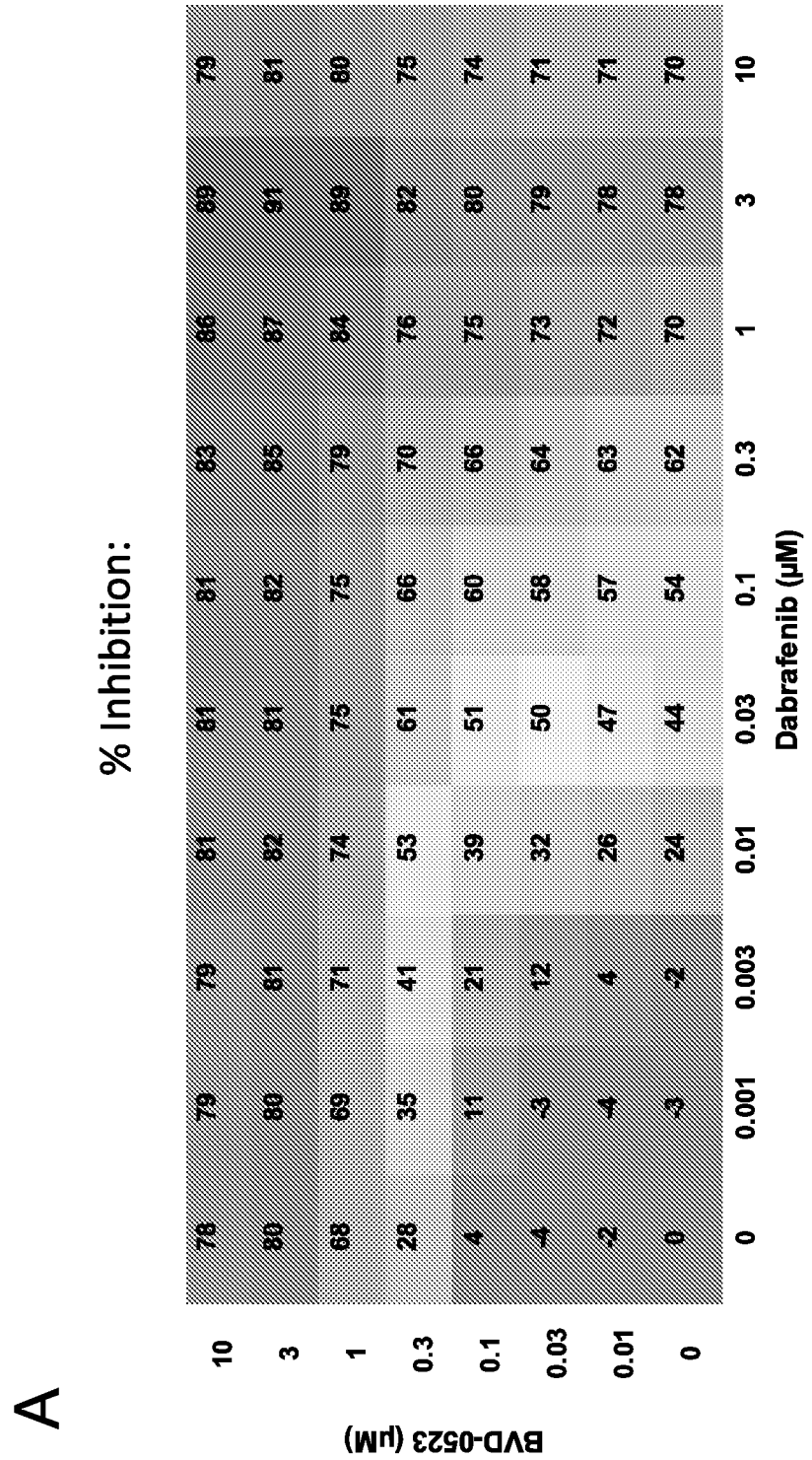

% Inhibition:
BVD-0523 (μM)
10 78 79 79 81 81 81 83 86 89 79
3 80 80 81 82 81 82 85 87 91 81
1 68 69 71 74 75 75 79 84 89 80
0.3 28 35 41 53 61 66 70 76 82 75
0.1 4 11 21 39 51 60 66 75 80 74
0.03 -4 -3 12 32 50 58 64 73 79 71
0.01 -2 -4 4 26 47 57 63 72 78 71
0 0 -3 -2 24 44 54 62 70 78 70
0 0.001 0.003 0.01 0.03 0.1 0.3 1 3 10
Dabrafenib (μM)

FIG. 13, Con't

B

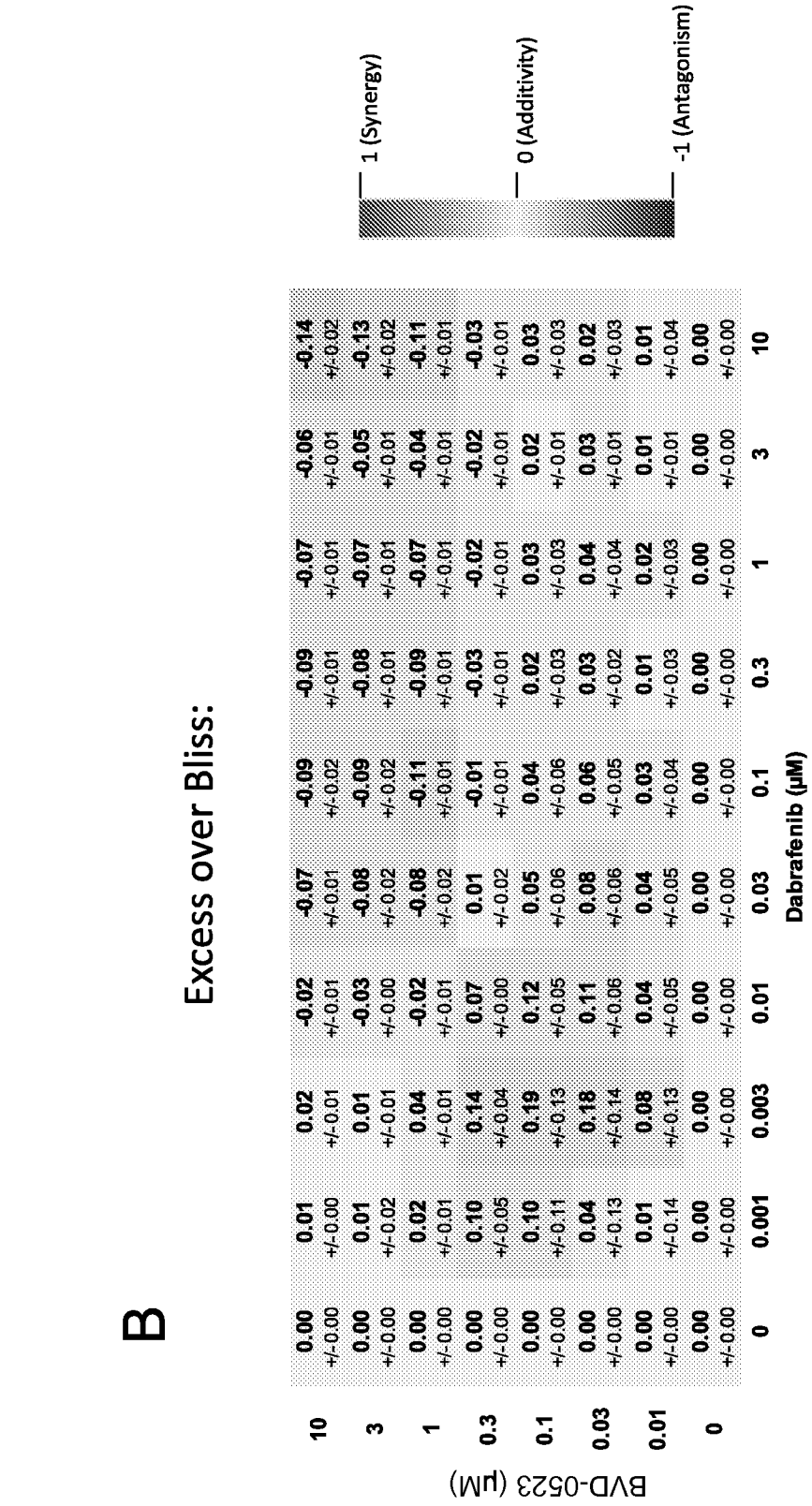

Excess over Bliss:
1 (Synergy)
0 (Additivity)
-1 (Antagonism)
BVD-523 (μM)
Dabrafenib (μM)
10 0.00 +/-0.00 0.01 +/-0.00 0.02 +/-0.01 -0.02 +/-0.01 -0.07 +/-0.01 -0.09 +/-0.02 -0.09 +/-0.01 -0.09 +/-0.01 -0.07 +/-0.01 -0.06 +/-0.01 -0.14 +/-0.02
3 0.00 +/-0.00 0.01 +/-0.02 0.01 +/-0.01 -0.03 +/-0.00 -0.08 +/-0.02 -0.09 +/-0.02 -0.08 +/-0.01 -0.07 +/-0.01 -0.05 +/-0.01 -0.13 +/-0.02
1 0.00 +/-0.00 0.02 +/-0.01 0.04 +/-0.01 -0.02 +/-0.01 -0.08 +/-0.02 -0.11 +/-0.01 -0.09 +/-0.01 -0.07 +/-0.01 -0.04 +/-0.01 -0.11 +/-0.01
0.3 0.00 +/-0.00 0.10 +/-0.05 0.14 +/-0.04 0.07 +/-0.00 0.01 +/-0.02 -0.01 +/-0.01 -0.03 +/-0.01 -0.02 +/-0.01 -0.02 +/-0.01 -0.03 +/-0.01
0.1 0.00 +/-0.00 0.10 +/-0.11 0.19 +/-0.13 0.12 +/-0.05 0.05 +/-0.06 0.04 +/-0.06 0.02 +/-0.03 0.03 +/-0.03 0.02 +/-0.01 0.03 +/-0.03
0.03 0.00 +/-0.00 0.04 +/-0.13 0.18 +/-0.14 0.11 +/-0.06 0.08 +/-0.06 0.06 +/-0.05 0.03 +/-0.02 0.04 +/-0.04 0.03 +/-0.01 0.02 +/-0.03
0.01 0.00 +/-0.00 0.01 +/-0.14 0.08 +/-0.13 0.04 +/-0.05 0.04 +/-0.05 0.03 +/-0.04 0.01 +/-0.03 0.02 +/-0.03 0.01 +/-0.01 0.01 +/-0.04
0 0.00 +/-0.00 0.00 +/-0.00 0.00 +/-0.00 0.00 +/-0.00 0.00 +/-0.00 0.00 +/-0.00 0.00 +/-0.00 0.00 +/-0.00 0.00 +/-0.00 0.00 +/-0.00
0 0.001 0.003 0.01 0.03 0.1 0.3 1 3 10

FIG. 13, Con't

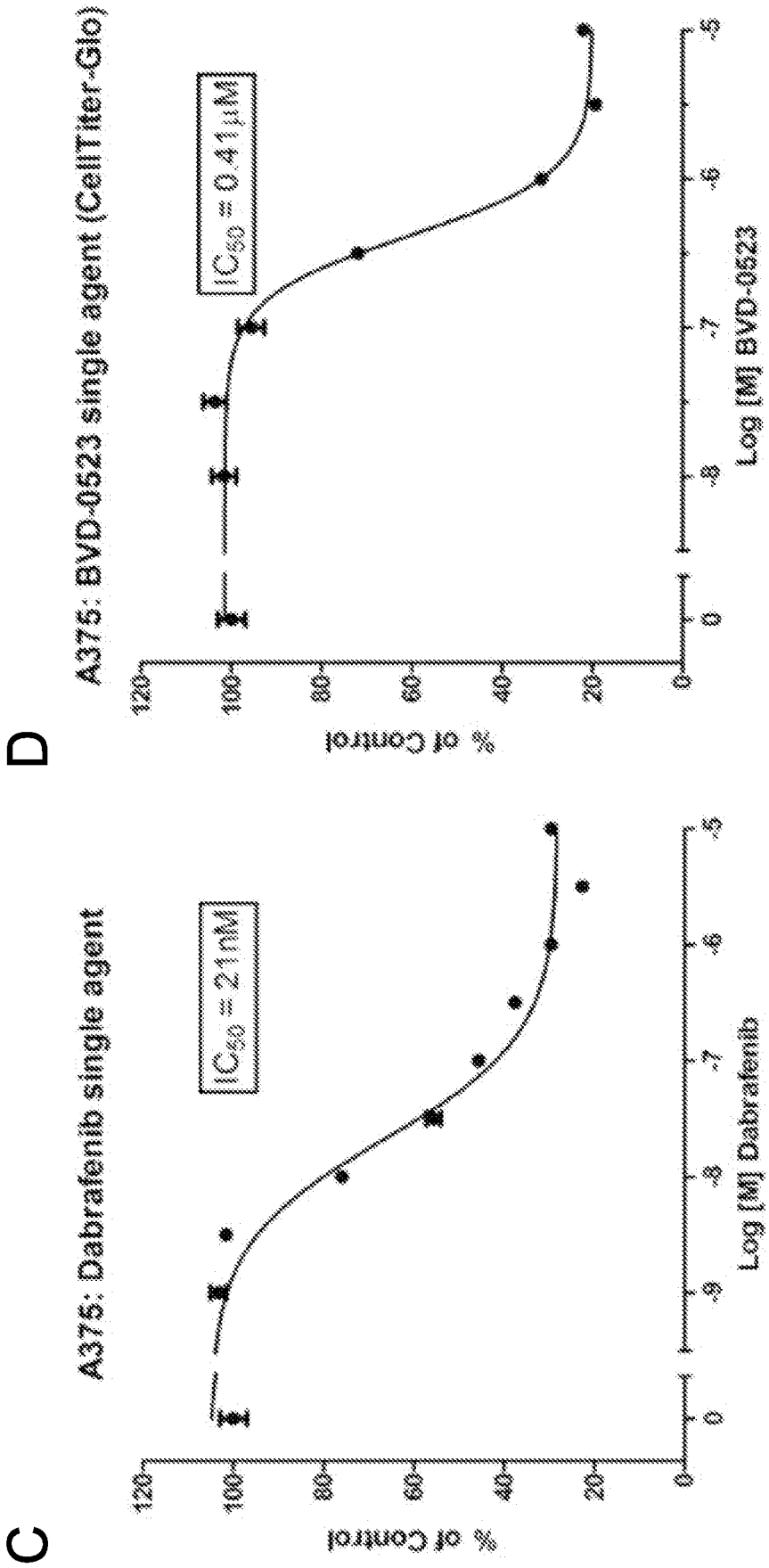

C
A375: Dabrafenib single agent
IC50 = 21nM
% of Control
120
100
80
60
40
20
0
0
-5
-6
-7
-8
-9
Log [M] Dabrafenib
D
A375: BVD-0523 single agent (CellTiter-Glo)
IC50 = 0.41μM
% of Control
120
100
80
60
40
20
0
0
-5
-6
-7
-8
Log [M] BVD-0523

FIG. 13, Con't

E

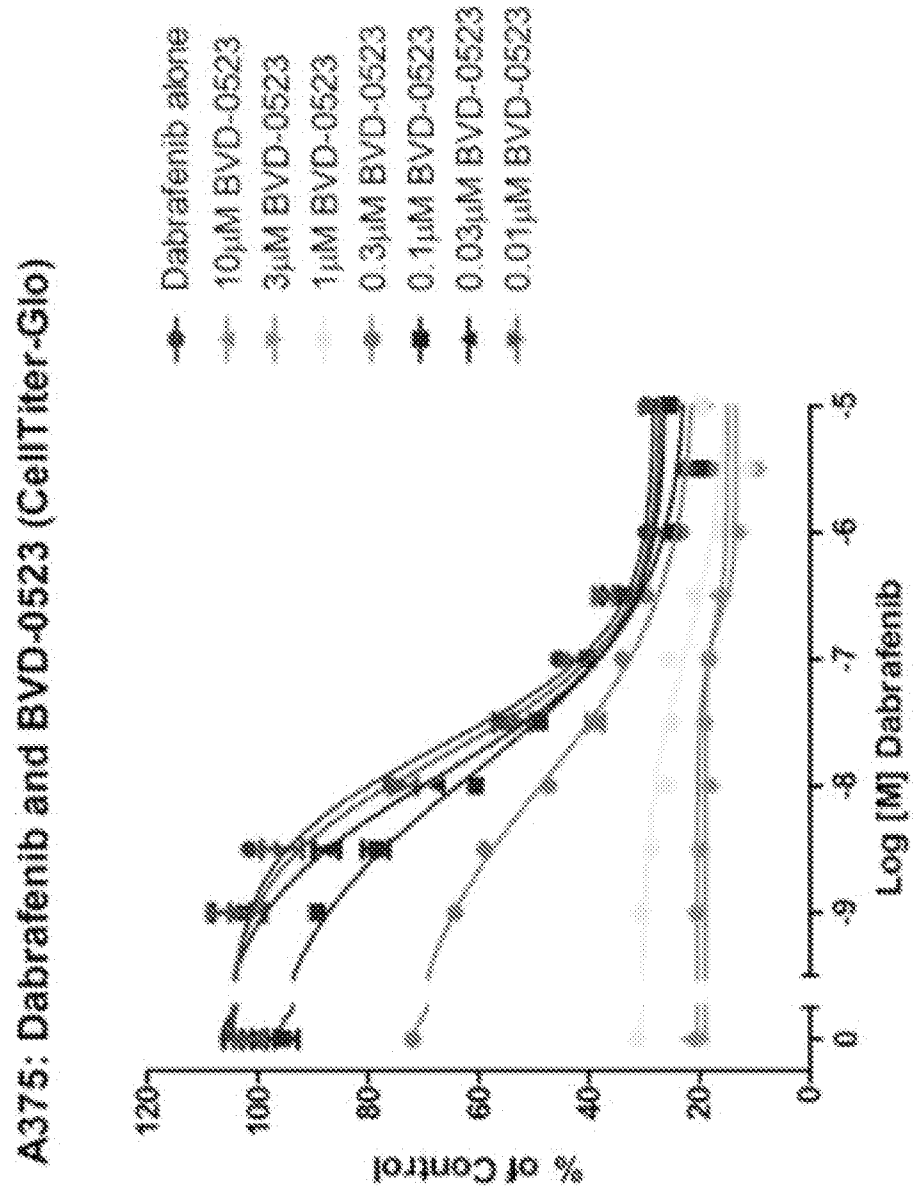

A375; Dabrafenib and BVD-0523 (CellTiter-Glo)
Dabrafenib alone
10μM BVD-0523
3μM BVD-0523
1μM BVD-0523
0.3μM BVD-0523
0.1μM BVD-0523
0.03μM BVD-0523
0.01μM BVD-0523
% of Control
120
100
80
60
40
20
0
0
-9
-8
-7
-6
-5
Log [M] Dabrafenib

FIG. 14

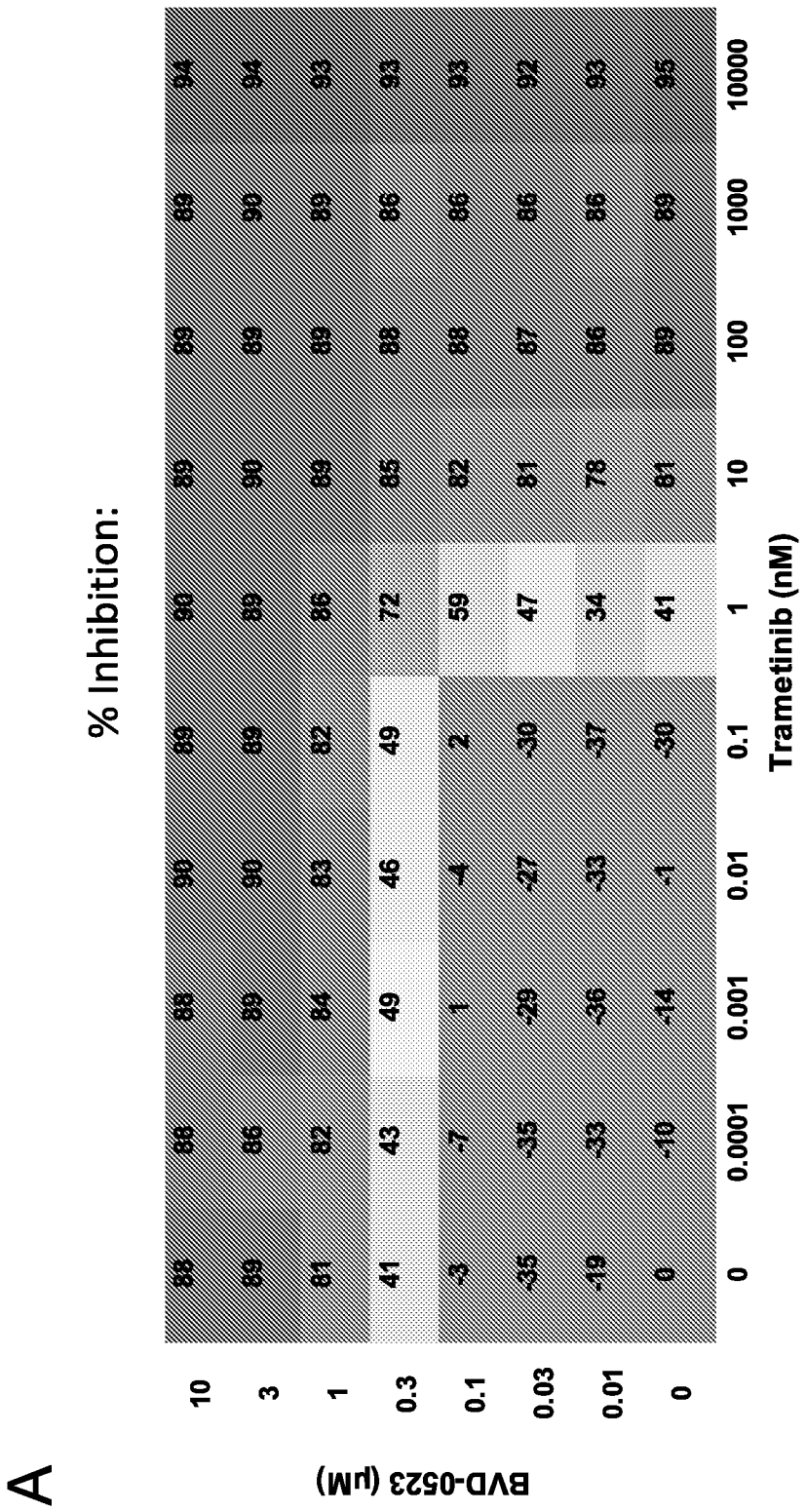

A
% Inhibition:
BVD-523 (μM)
10
3
1
0.3
0.1
0.03
0.01
0
Trametinib (nM)
0
0.0001
0.001
0.01
0.1
1
10
100
1000
10000
88 88 88 90 89 90 89 89 89 89 89
89 89 86 89 89 90 89 89 90 90 90
81 82 84 83 82 86 89 89 89 86 92
41 43 49 46 49 72 85 88 86 88 93
-3 -7 1 -4 2 59 82 88 86 86 93
-35 -35 -29 -27 -30 47 81 87 86 86 92
-19 -33 -36 -33 -37 34 78 86 86 86 93
0 -10 -14 -1 -30 41 81 89 89 89 95
84 86 89 90 89 89 89 89 89 89 94
89 86 90 89 90 89 90 89 90 89 94
82 82 84 83 82 86 89 89 89 89 92
43 43 49 46 49 72 85 88 86 86 93
-7 -7 1 -4 2 59 82 88 86 86 93
-35 -35 -29 -27 -30 47 81 87 86 86 92
-33 -33 -36 -33 -37 34 78 86 86 86 93
-10 -10 -14 -1 -30 41 81 89 89 89 95

FIG. 14, Con't

B

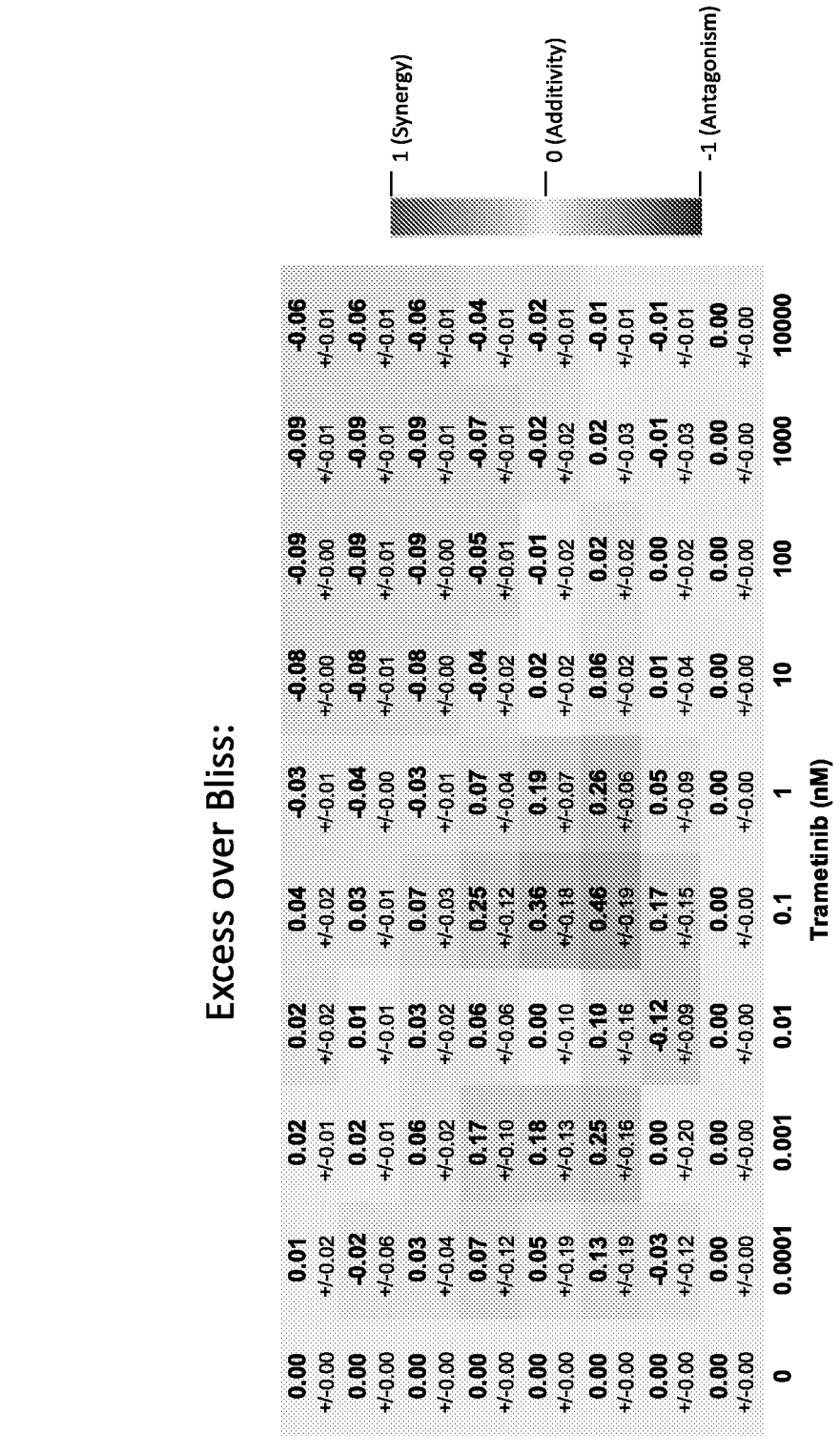

Excess over Bliss:
1 (Synergy)
0 (Additivity)
-1 (Antagonism)
BVD-0523 (μM)
Trametinib (nM)
10 0.00 +/-0.00 0.01 +/-0.02 0.02 +/-0.01 0.02 +/-0.02 0.04 +/-0.02 -0.03 +/-0.01 -0.08 +/-0.00 -0.09 +/-0.00 -0.09 +/-0.01 -0.06 +/-0.01
3 0.00 +/-0.00 -0.02 +/-0.06 0.02 +/-0.01 0.01 +/-0.01 0.03 +/-0.01 -0.04 +/-0.00 -0.08 +/-0.01 -0.09 +/-0.01 -0.09 +/-0.01 -0.06 +/-0.01
1 0.00 +/-0.00 0.03 +/-0.04 0.06 +/-0.02 0.03 +/-0.02 0.07 +/-0.03 -0.03 +/-0.01 -0.08 +/-0.00 -0.09 +/-0.00 -0.09 +/-0.01 -0.06 +/-0.01
0.3 0.00 +/-0.00 0.07 +/-0.12 0.17 +/-0.10 0.06 +/-0.06 0.25 +/-0.12 0.07 +/-0.04 -0.04 +/-0.02 -0.05 +/-0.01 -0.07 +/-0.01 -0.04 +/-0.01
0.1 0.00 +/-0.00 0.05 +/-0.19 0.18 +/-0.13 0.00 +/-0.10 0.36 +/-0.18 0.19 +/-0.07 0.02 +/-0.02 -0.01 +/-0.02 -0.02 +/-0.02 -0.02 +/-0.01
0.03 0.00 +/-0.00 0.13 +/-0.19 0.25 +/-0.16 0.10 +/-0.16 0.46 +/-0.19 0.26 +/-0.06 0.06 +/-0.02 0.02 +/-0.02 0.02 +/-0.03 -0.01 +/-0.01
0.01 0.00 +/-0.00 -0.03 +/-0.12 0.00 +/-0.20 -0.12 +/-0.09 0.17 +/-0.15 0.05 +/-0.09 0.01 +/-0.04 0.00 +/-0.02 -0.01 +/-0.03 -0.01 +/-0.01
0 0.00 +/-0.00 0.00 +/-0.00 0.00 +/-0.00 0.00 +/-0.00 0.00 +/-0.00 0.00 +/-0.00 0.00 +/-0.00 0.00 +/-0.00 0.00 +/-0.00 0.00 +/-0.00
0 0.0001 0.001 0.01 0.1 1 10 100 1000 10000

FIG. 14, Con't

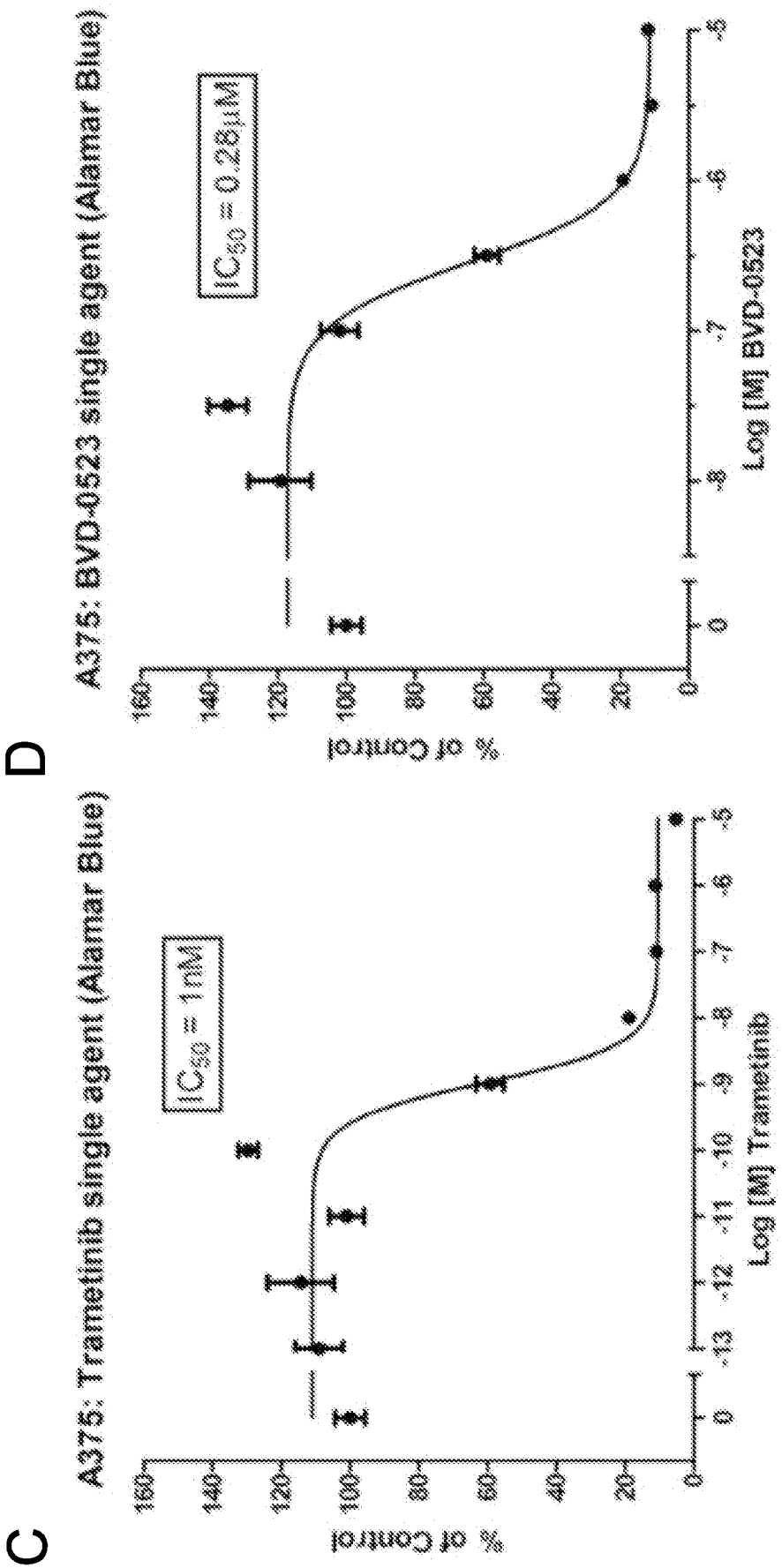

C
A375: Trametinib single agent (Alamar Blue)
IC50 = 1nM
% of Control
Log [M] Trametinib
D
A375: BVD-0523 single agent (Alamar Blue)
IC50 = 0.28μM
% of Control
Log [M] BVD-0523

FIG. 14, Con't

E

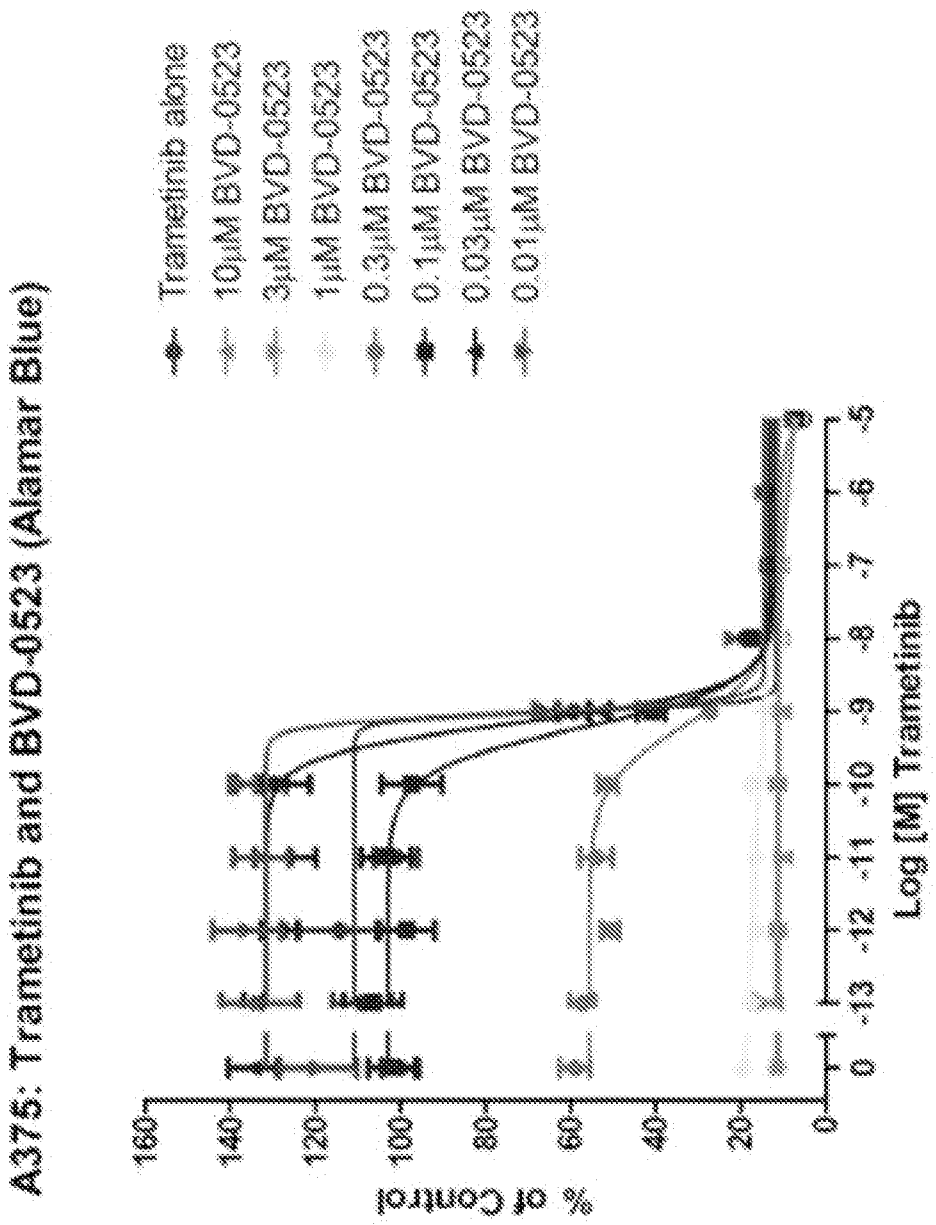

A375: Trametinib and BVD-0523 (Alamar Blue)
Trametinib alone
10μM BVD-0523
3μM BVD-0523
1μM BVD-0523
0.3μM BVD-0523
0.1μM BVD-0523
0.03μM BVD-0523
0.01μM BVD-0523
% of Control
Log [M] Trametinib

FIG. 15

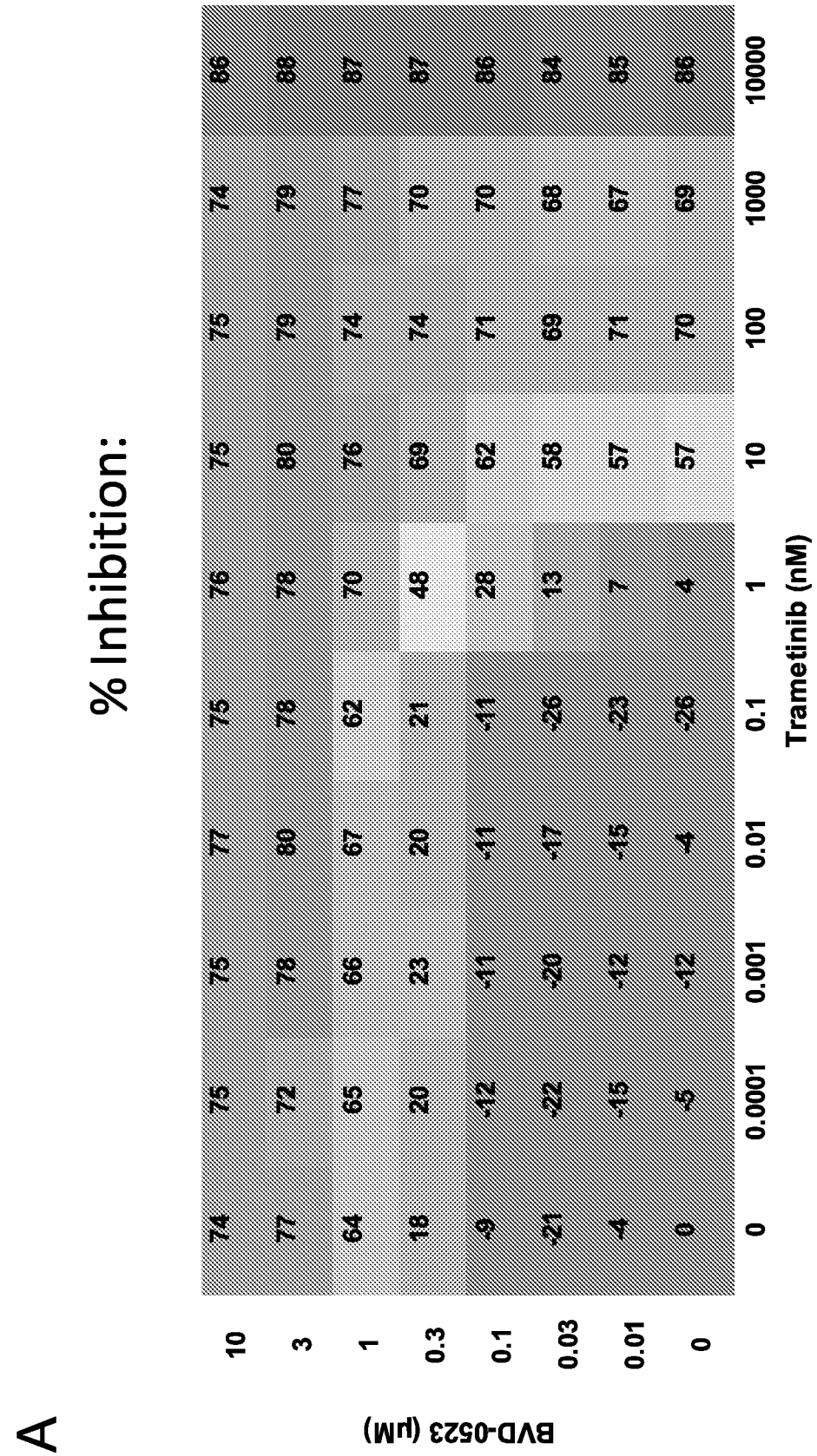

A
% Inhibition:
BVD-0523 (μM)
10
3
1
0.3
0.1
0.03
0.01
0
Trametinib (nM)
0
0.0001
0.001
0.01
0.1
1
10
100
1000
10000
74 75 75 77 75 76 75 75 74 86
77 72 78 80 78 78 80 79 79 88
64 65 66 67 62 70 76 74 77 87
18 20 23 20 21 48 69 74 70 87
-9 -12 -11 -11 -11 28 62 71 70 86
-21 -22 -20 -17 -26 13 58 69 68 84
-4 -15 -12 -15 -23 7 57 71 67 85
0 -5 -12 -4 -26 4 57 70 69 86

FIG. 15, Con't

B

Excess over Bliss:

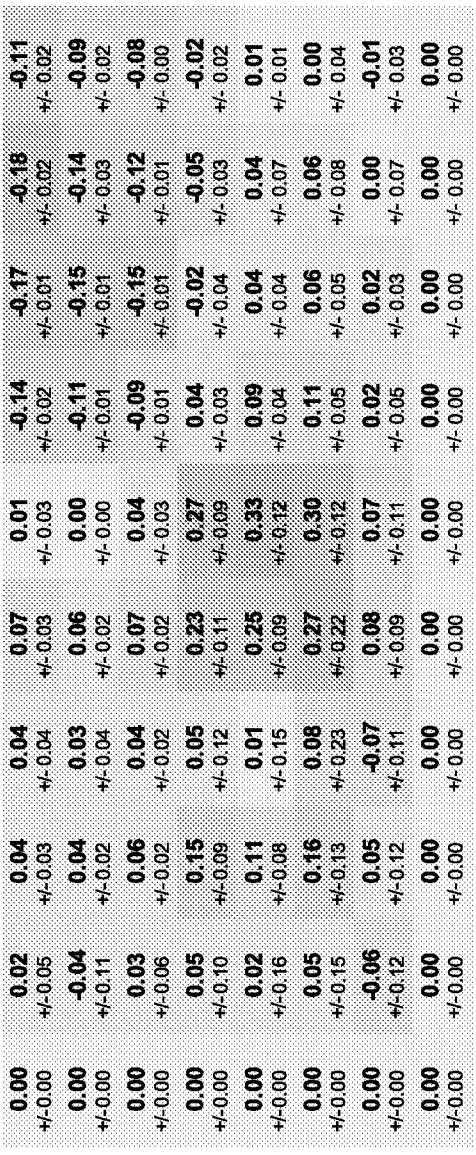


FIG. 15, Con't

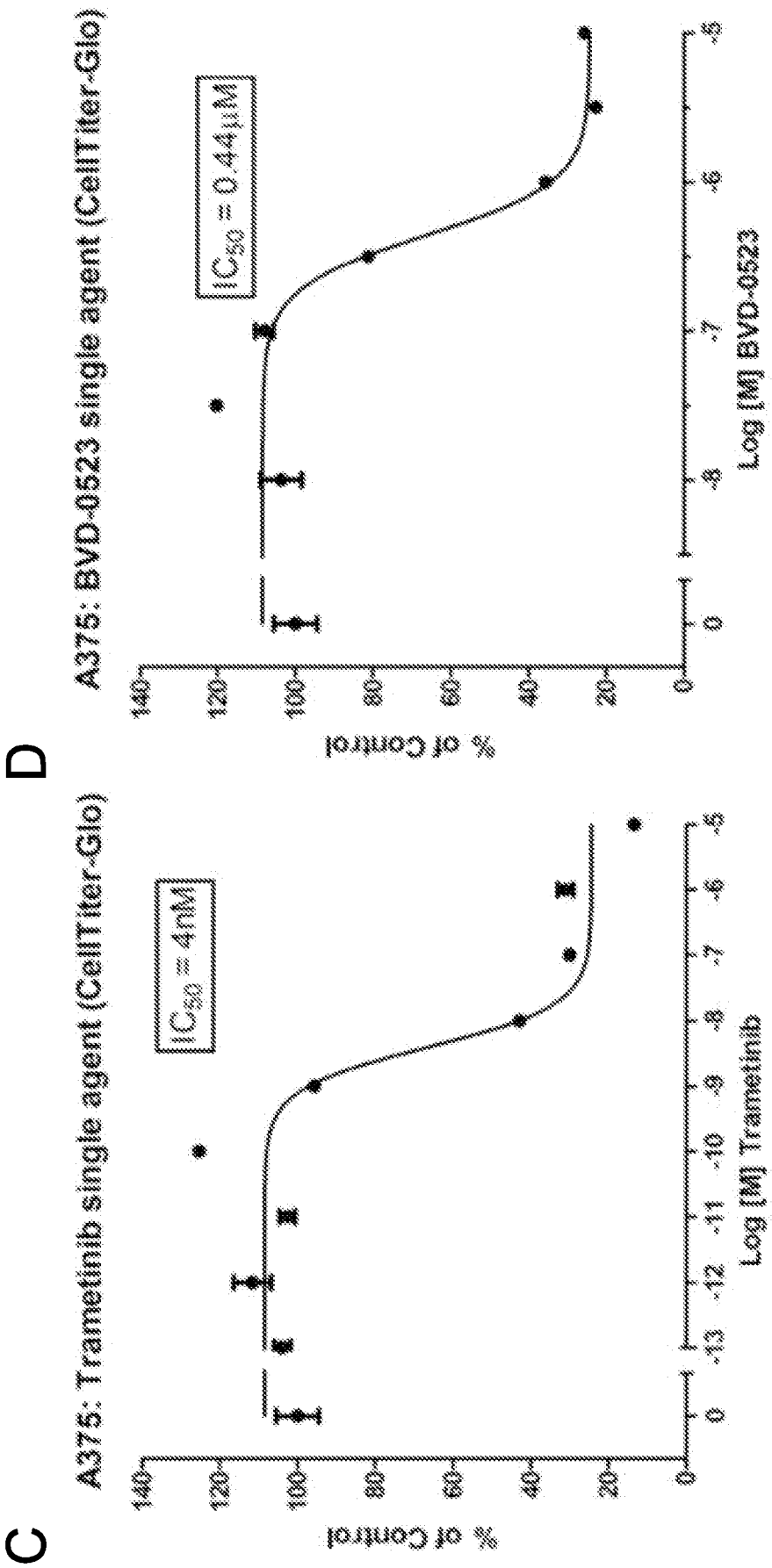

C
A375: Trametinib single agent (CellTiter-Glo)
IC50 = 4nM
% of Control
Log [M] Trametinib
D
A375: BVD-0523 single agent (CellTiter-Glo)
IC50 = 0.44μM
% of Control
Log [M] BVD-0523

FIG. 15, Con't

E

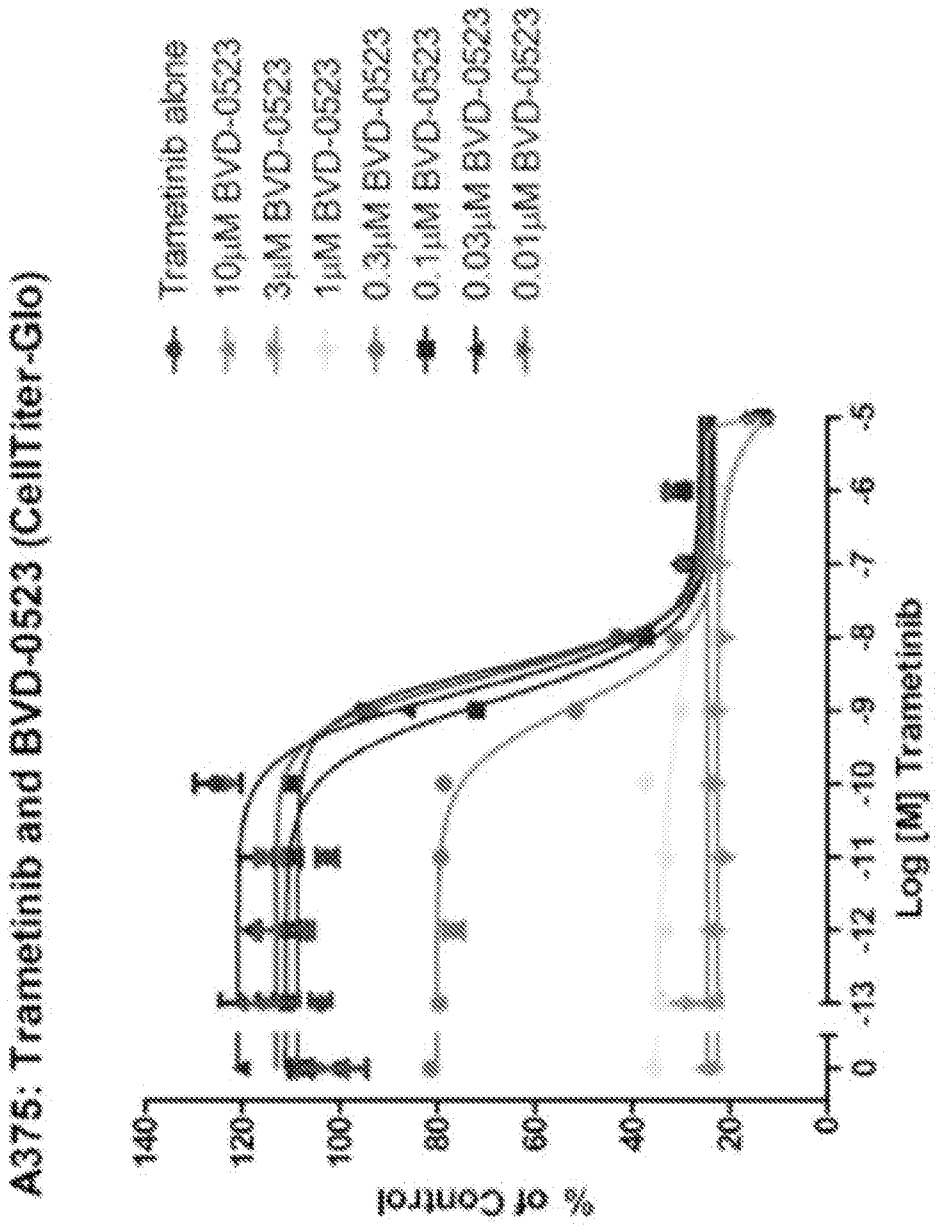

A375: Trametinib and BVD-0523 (CellTiter-Glo)
Trametinib alone
10μM BVD-0523
3μM BVD-0523
1μM BVD-0523
0.3μM BVD-0523
0.1μM BVD-0523
0.03μM BVD-0523
0.01μM BVD-0523
% of Control
140
120
100
80
60
40
20
0
0
-13
-12
-11
-10
-9
-8
-7
-6
-5
Log [M] Trametinib

FIG. 16

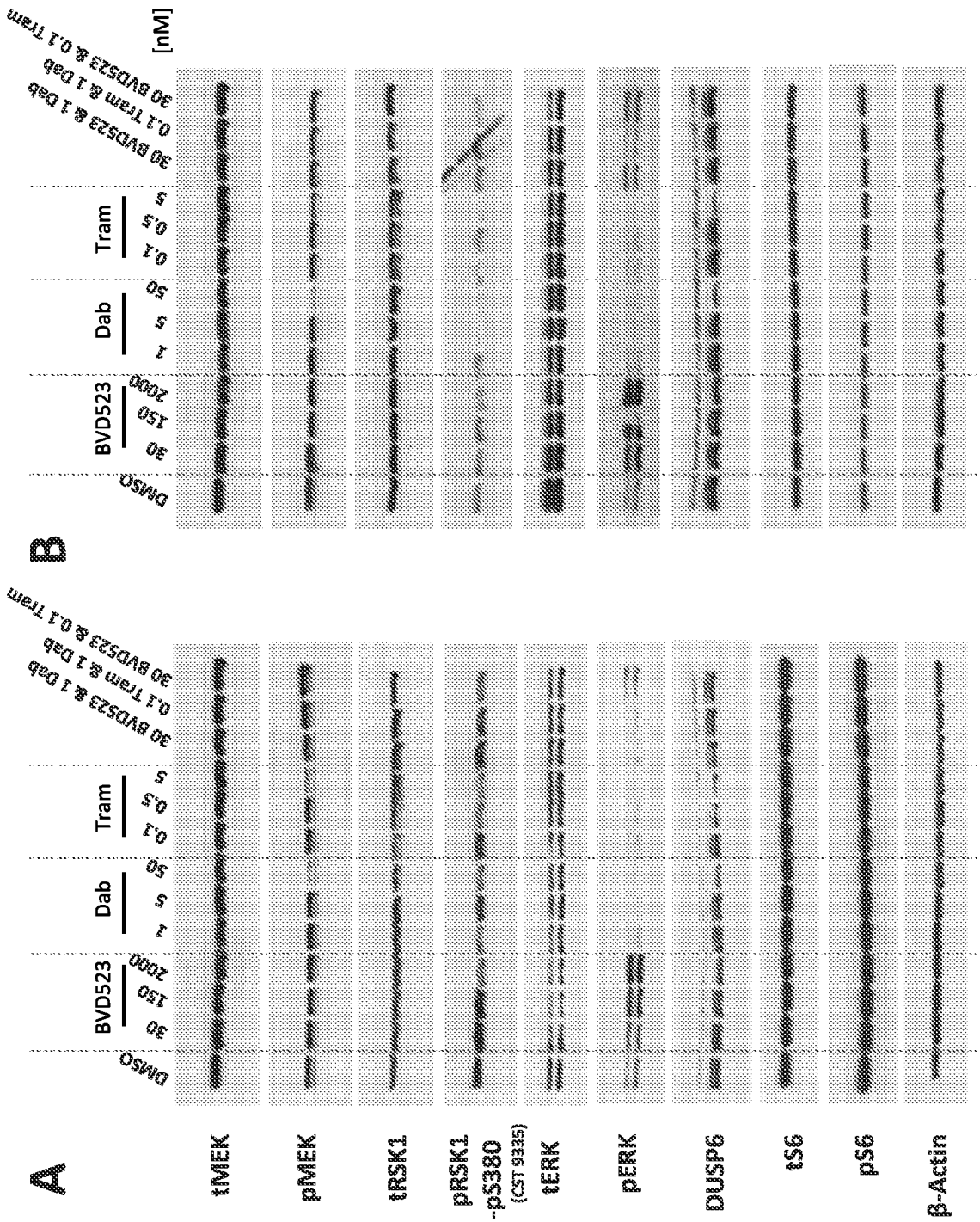

A
B
DMSO
BVD523
30
150
2000
Dab
1
5
50
Tram
0.1
0.5
5
30 BVD523 & 1 Dab
0.1 Tram & 1 Dab
30 BVD523 & 0.1 Tram
[nM]
tMEK
pMEK
tRSK1
pRSK1 -pS380 (CST 9335)
tERK
pERK
DUSP6
tS6
pS6
β-Actin

FIG. 16 Con't

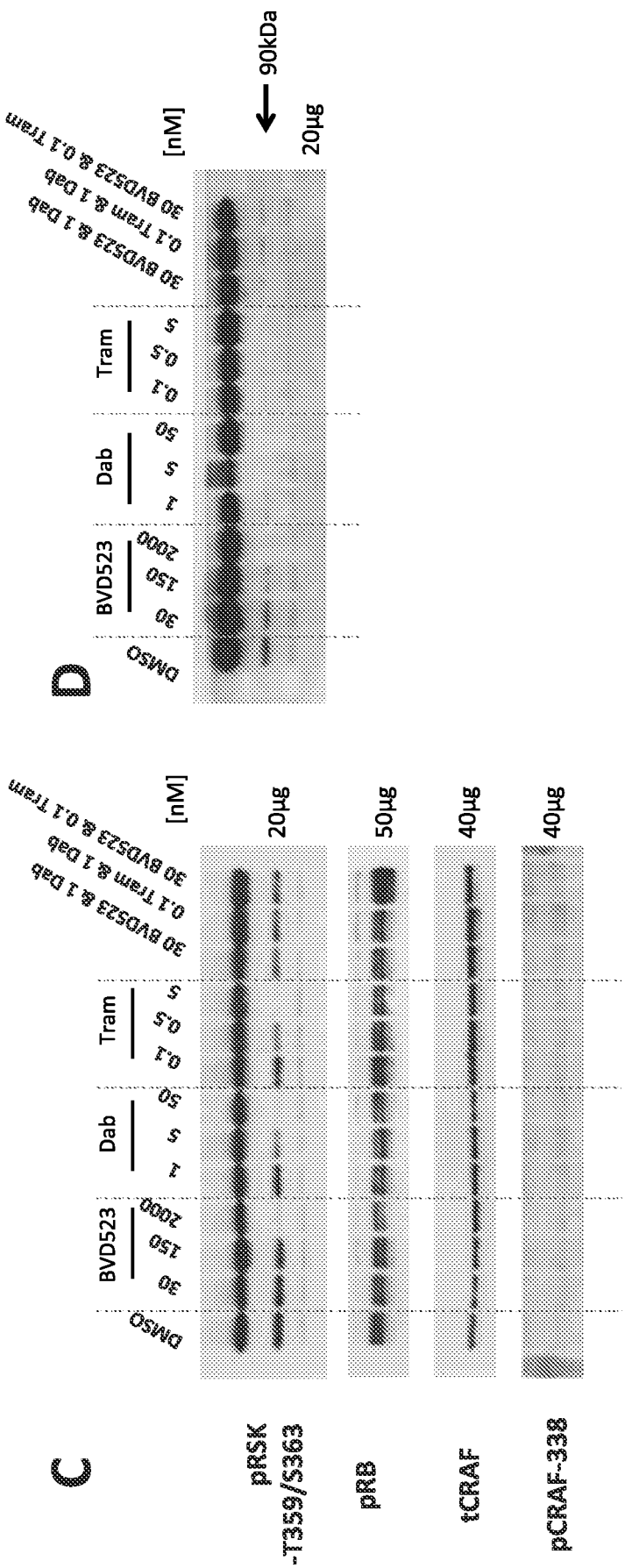

D
90kDa
20µg
[nM]
DMSO
BVD523
30
150
2000
Dab
1
5
50
Tram
0.1
0.5
5
30 BVD523 & 1 Dab
0.1 Tram & 1 Dab
30 BVD523 & 0.1 Tram
C
pRSK -T359/S363
pRB
tCRAF
pCRAF-338
20µg
50µg
40µg
40µg

FIG. 17

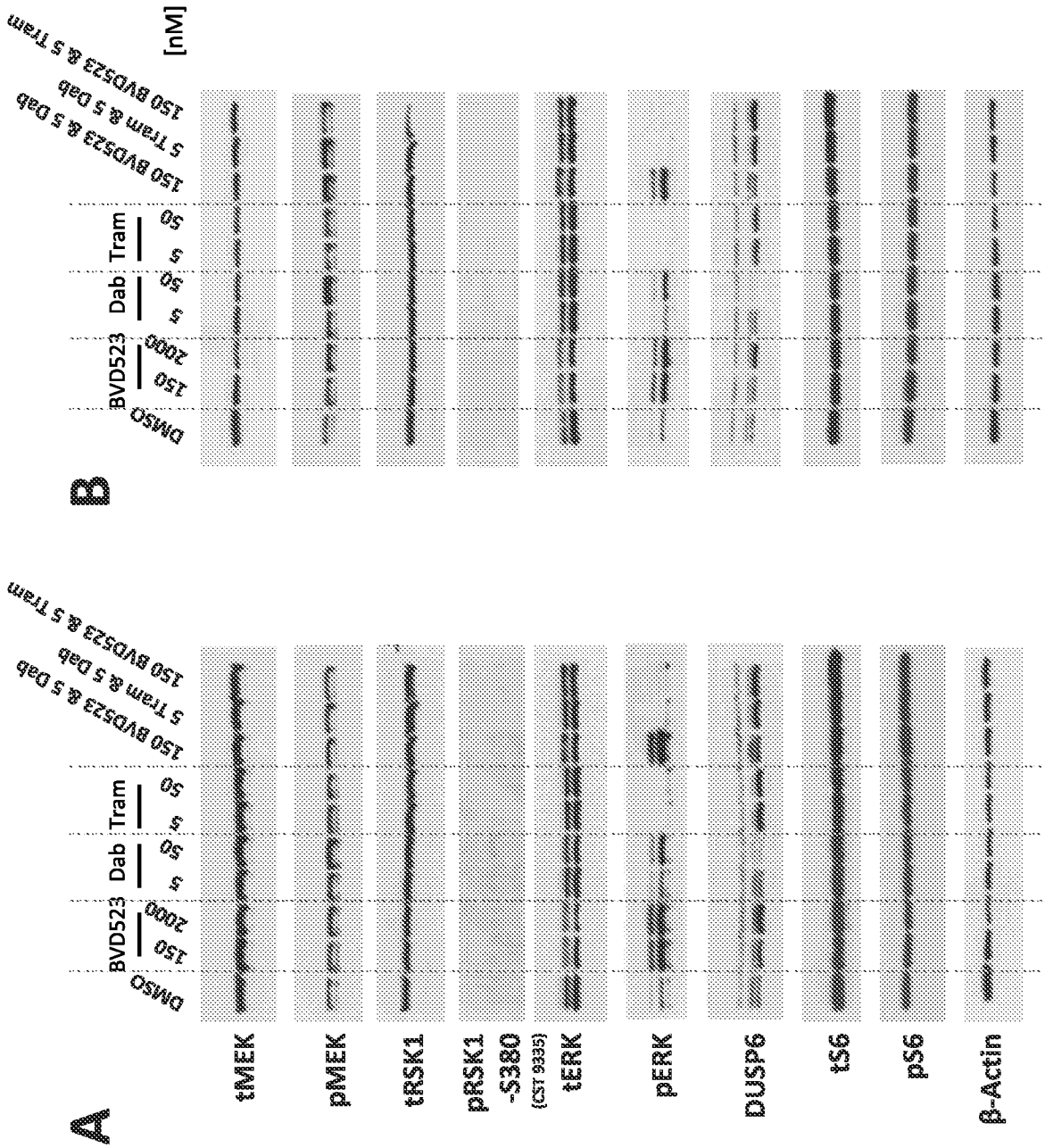

A
B
tMEK
pMEK
tRSK1
pRSK1 -S380 (CST 9335)
tERK
pERK
DUSP6
tS6
pS6
β-Actin
DMSO
BVD523
150
2000
Dab
5
50
Tram
5
50
150 BVD523 & 5 Dab
5 Tram & 5 Dab
150 BVD523 & 5 Tram
[nM]

FIG. 17 Con't

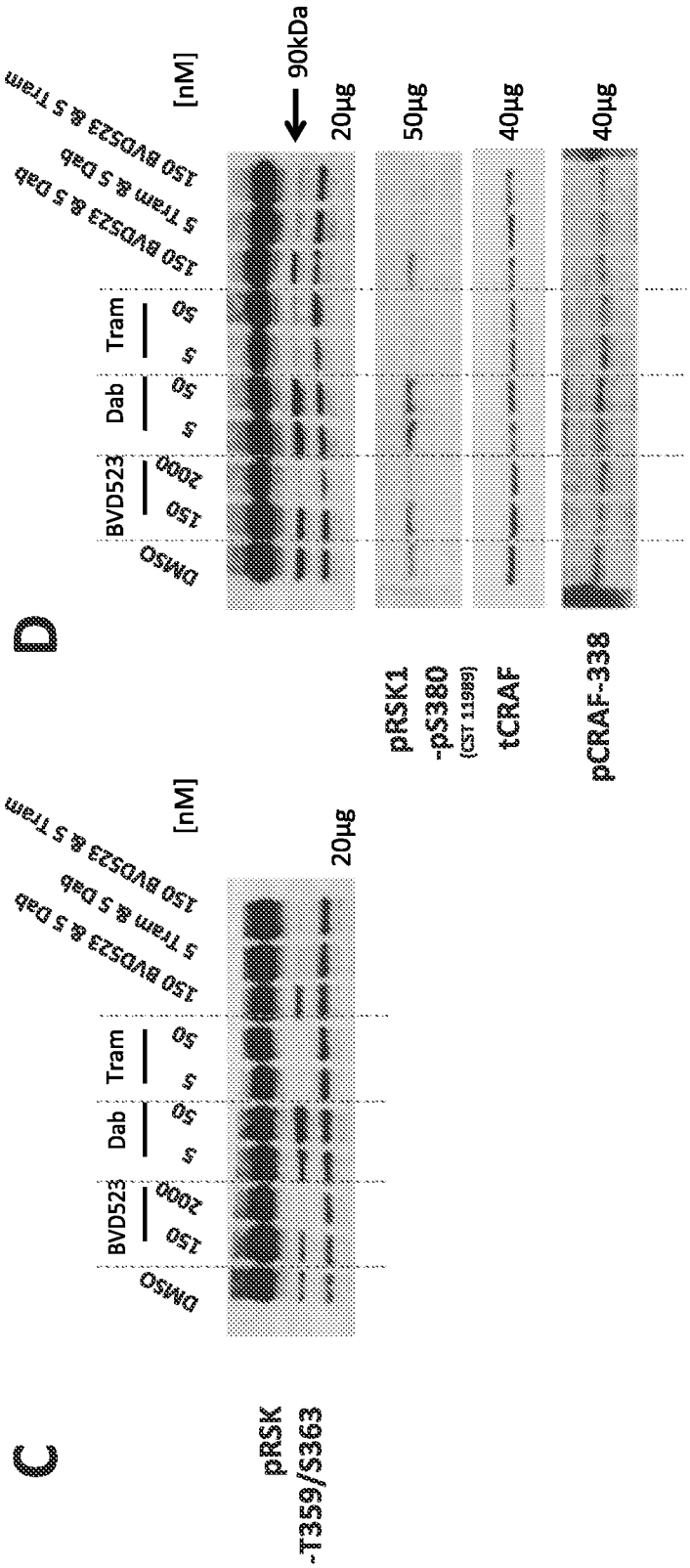

C
pRSK
-T359/S363
20µg
[nM]
DMSO
BVD523
150
2000
Dab
5
50
Tram
5
50
150 BVD523 & 5 Dab
5 Tram & 5 Dab
150 BVD523 & 5 Tram
D
90kDa
20µg
50µg
40µg
40µg
pRSK1
-pS380
(CST 11989)
tCRAF
pCRAF-338

FIG. 18

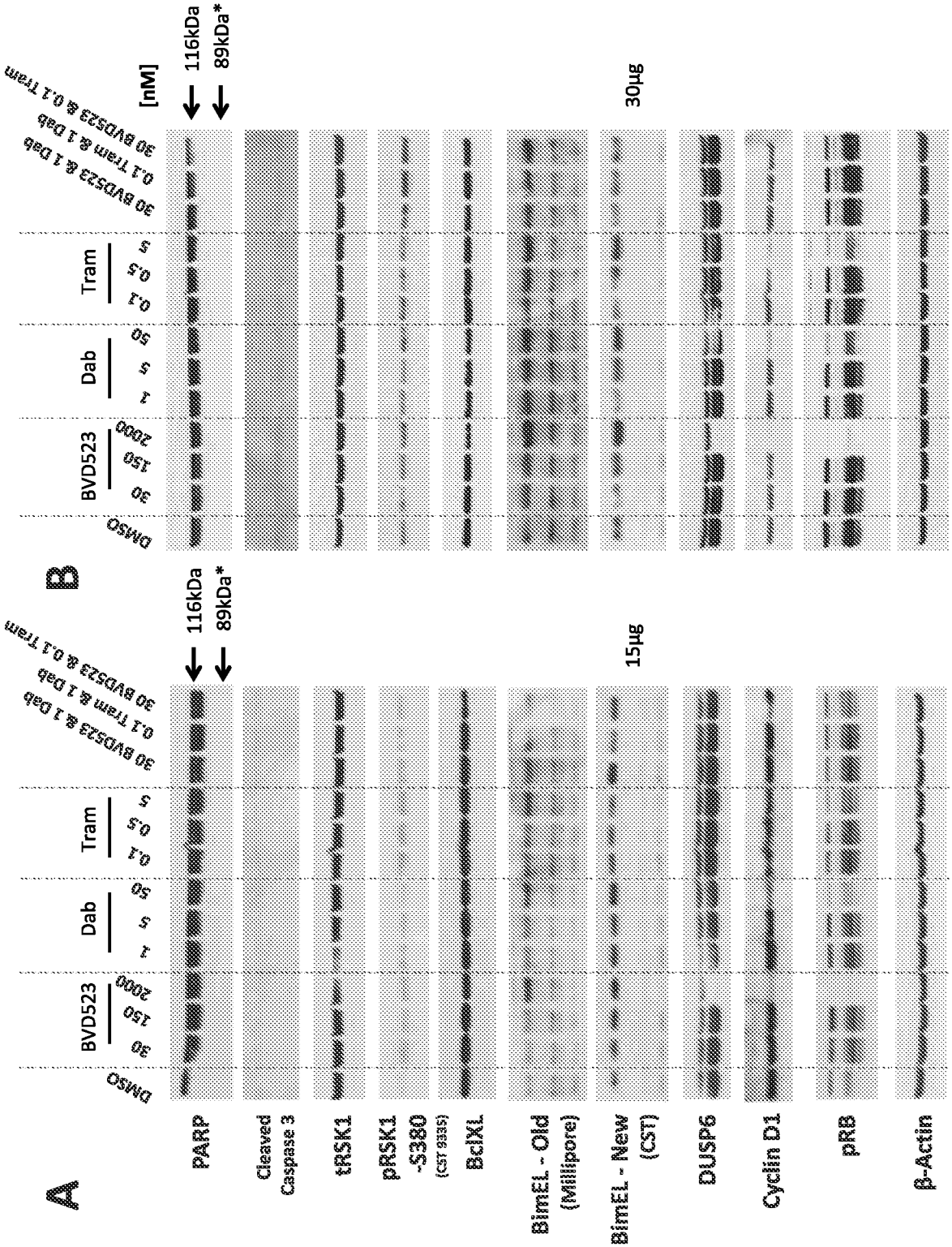

A
B
DMSO
BVD523
30
150
2000
Dab
1
5
50
Tram
0.1
0.5
5
30 BVD523 & 1 Dab
0.1 Tram & 1 Dab
30 BVD523 & 0.1 Tram
[nM]
116kDa
89kDa*
15µg
30µg
PARP
Cleaved Caspase 3
tRSK1
pRSK1 -S380 (CST 9335)
BclXL
BimEL - Old (Millipore)
BimEL - New (CST)
DUSP6
Cyclin D1
pRB
β-Actin

FIG. 18 Con't

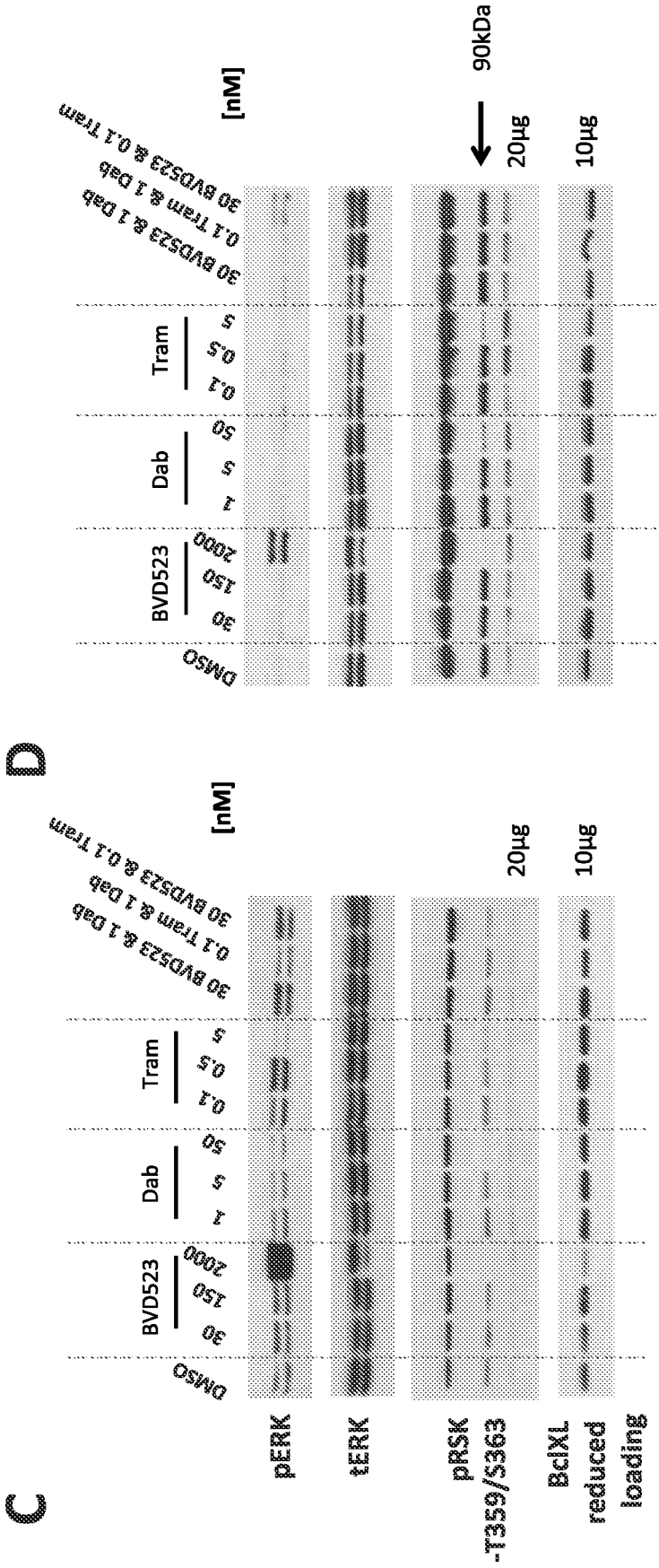

C
D
[nM]
DMSO
BVD523
30
150
2000
Dab
1
5
50
Tram
0.1
0.5
5
30 BVD523 & 1 Dab
0.1 Tram & 1 Dab
30 BVD523 & 0.1 Tram
20µg
10µg
90kDa
pERK
tERK
pRSK
-T359/S363
BclXL
reduced
loading

Figure 19

Resistance Condition	Starting Concentration (nM)	Maintenance Concentration (nM)		
		Month 1	Month 2	Month 3
Dabrafenib_R	5	25	400	3200
Trametinib_R	1	3	40	160
BVD-523_R	160	640	1800	1200
Dabrafenib/Trametinib_R	2.5/0.5	7.5/1.5	28/6	160/30
Dabrafenib/BVD-523_R	2.5/80	10/320	28/900	42/1500
Trametinib/BVD-523_R	0.5/80	2/320	2.5/400	4/600

Resistance Condition	Multiple Above Starting Concentration		
	Month 1	Month 2	Month 3
Dabrafenib_R	5X	80X	640X
Trametinib_R	3X	40X	160X
BVD-523_R	4X	11.25X	7.5X
Dabrafenib/Trametinib_R	3X	11.2X	64X
Dabrafenib/BVD-523_R	4X	11.2X	16.8X
Trametinib/BVD-523_R	4X	5X	8X

Resistance Condition	IC50 Fold Change			
	Dabrafenib	Trametinib	BVD-523	Paclitaxel
Parental	1.00	1.00	1.00	1.00
Trametinib_R	2.51	2.60	1.78	2.53
Dabrafenib_R	3.79	3.28	1.84	0.62
BVD-523_R	238.11	105.20	16.84	2.97
Trametinib + Dabrafenib_R	103.17	38.60	4.22	1.66
Dabrafenib + BVD-523_R	71.87	18.04	4.32	1.35
Trametinib + BVD-523_R	27.51	26.69	3.81	3.96

Resistance Condition	Percent Growth at ~10X Parental IC50			
	Dabrafenib	Trametinib	BVD-523	Paclitaxel
Parental	13.30%	6.80%	0.00%	1.95%
Trametinib_R	33.53%	30.15%	20.02%	13.52%
Dabrafenib_R	36.45%	25.96%	8.21%	9.36%
BVD-523_R	230.02%	172.91%	57.49%	34.02%
Trametinib + Dabrafenib_R	131.10%	125.01%	12.65%	15.44%
Dabrafenib + BVD-523_R	93.55%	71.37%	8.70%	11.41%
Trametinib + BVD-523_R	86.71%	64.03%	27.70%	30.71%
IC50, Parental (nM)	4.11	0.38	186.21	3.65
~10X Parental concentration	30.20	3.00	3019.95	36.48

FIG. 20

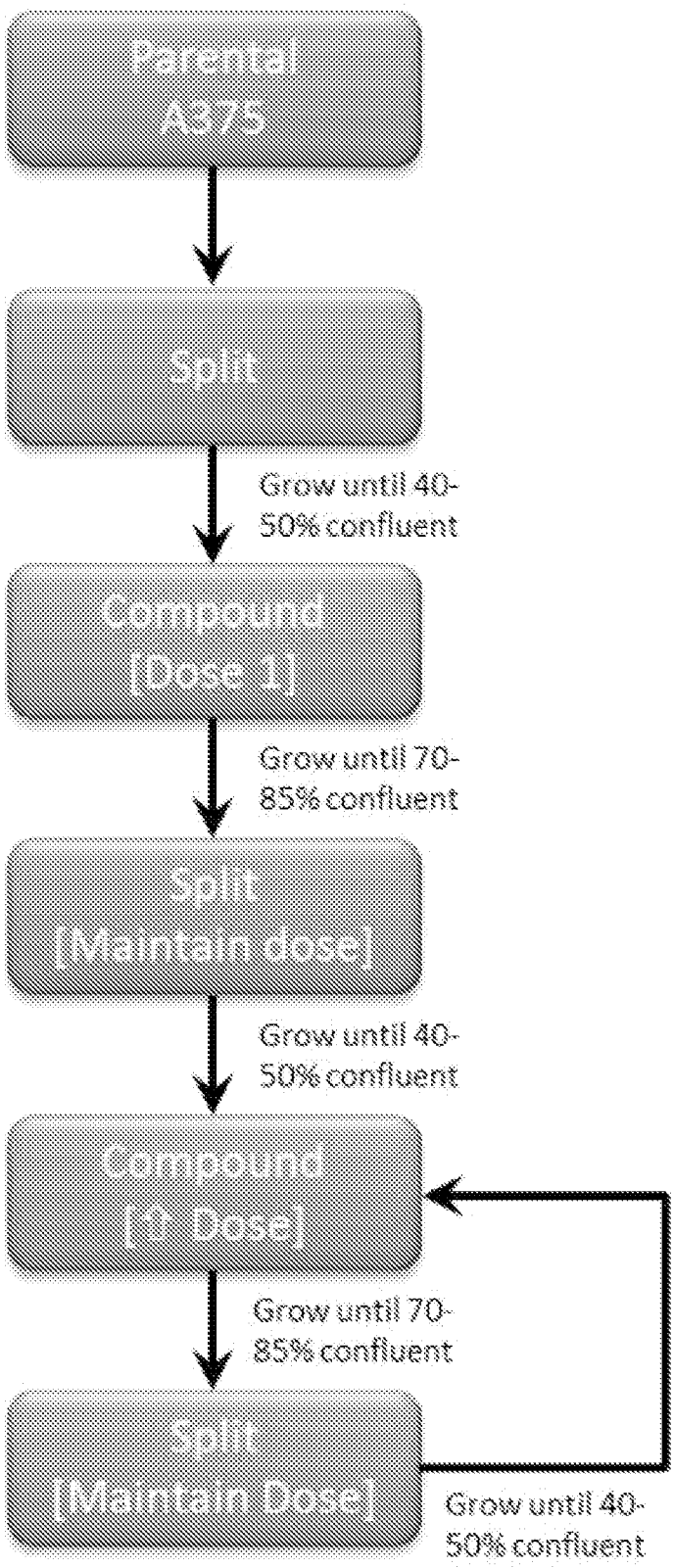

Parental
A375
Split
Grow until 40-
50% confluent
Compound
[Dose 1]
Grow until 70-
85% confluent
Split
[Maintain dose]
Grow until 40-
50% confluent
Compound
[↑ Dose]
Grow until 70-
85% confluent
Split
[Maintain Dose]
Grow until 40-
50% confluent

Fig. 21

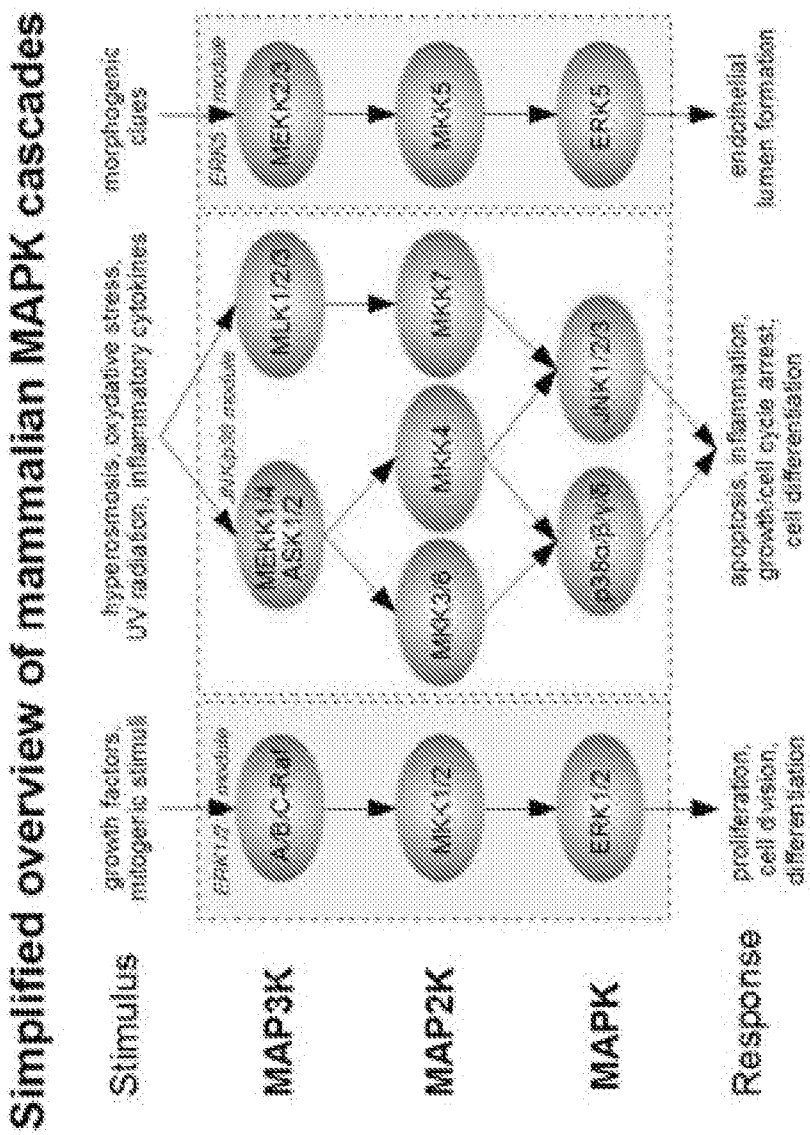

Simplified overview of mammalian MAPK cascades
Stimulus
growth factors, mitogenic stimuli
hyperosmosis, oxydative stress, UV radiation, inflammatory cytokines
morphogenic clues
MAP3K
ERK1/2 module
A-Raf, B-Raf, c-Raf
MEKK1/4 ASK1/2
MLK1/2/3
ERK5 module
MEKK2/3
MAP2K
MKK1/2
ERK1/2/5 module
ERK3/6
MKK4
MKK7
MKK5
MAPK
ERK1/2
p38α/β/γ/δ
JNK1/2/3
ERK5
Response
proliferation, cell division, differentiation
apoptosis, inflammation, growth/cell cycle arrest, cell differentiation
endothelial lumen formation

FIG. 22

A

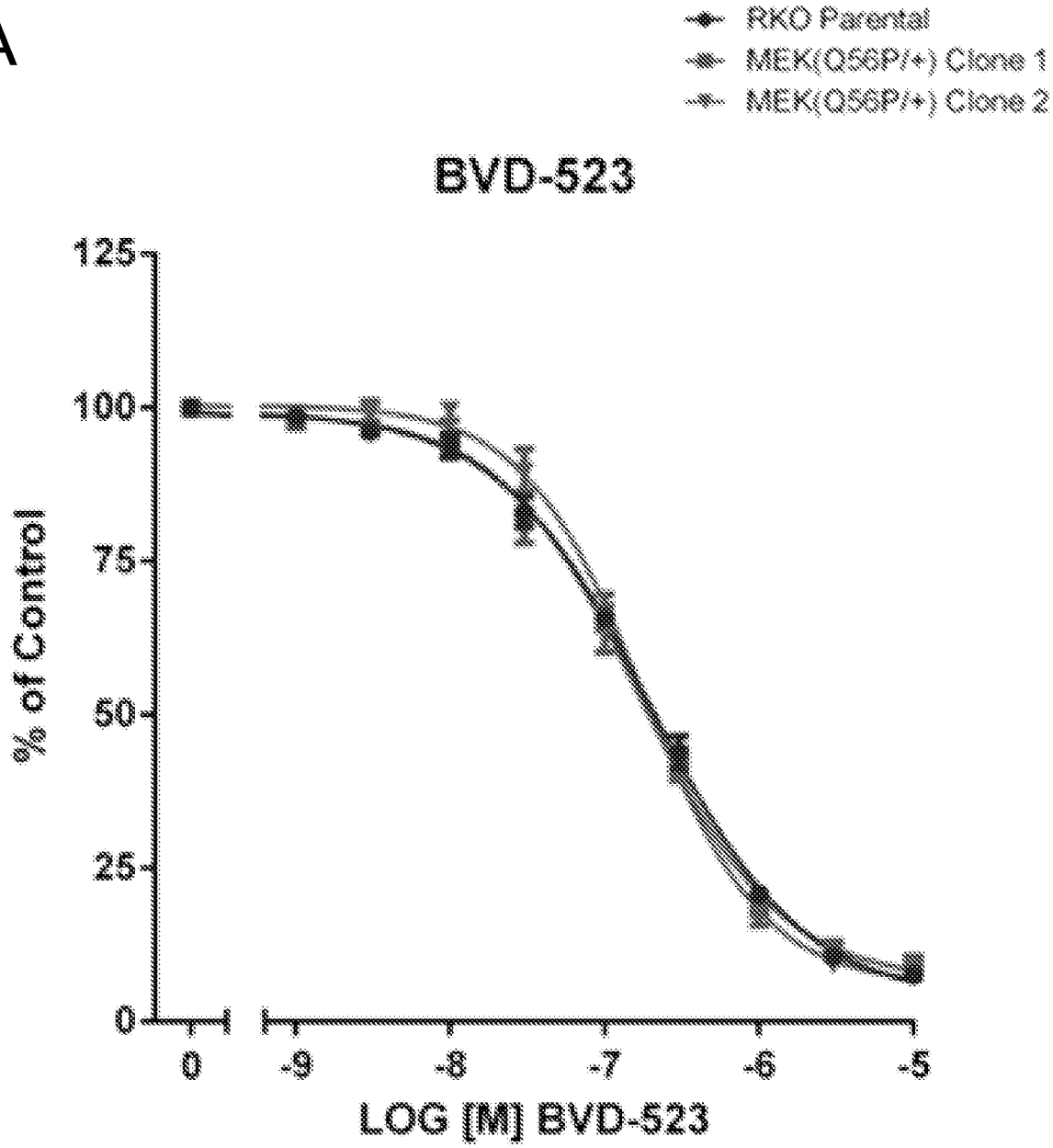

RKO Parental
MEK(Q56P/+) Clone 1
MEK(Q56P/+) Clone 2
BVD-523
% of Control
125
100
75
50
25
0
0
-9
-8
-7
-6
-5
LOG [M] BVD-523

FIG. 22, Con't

B

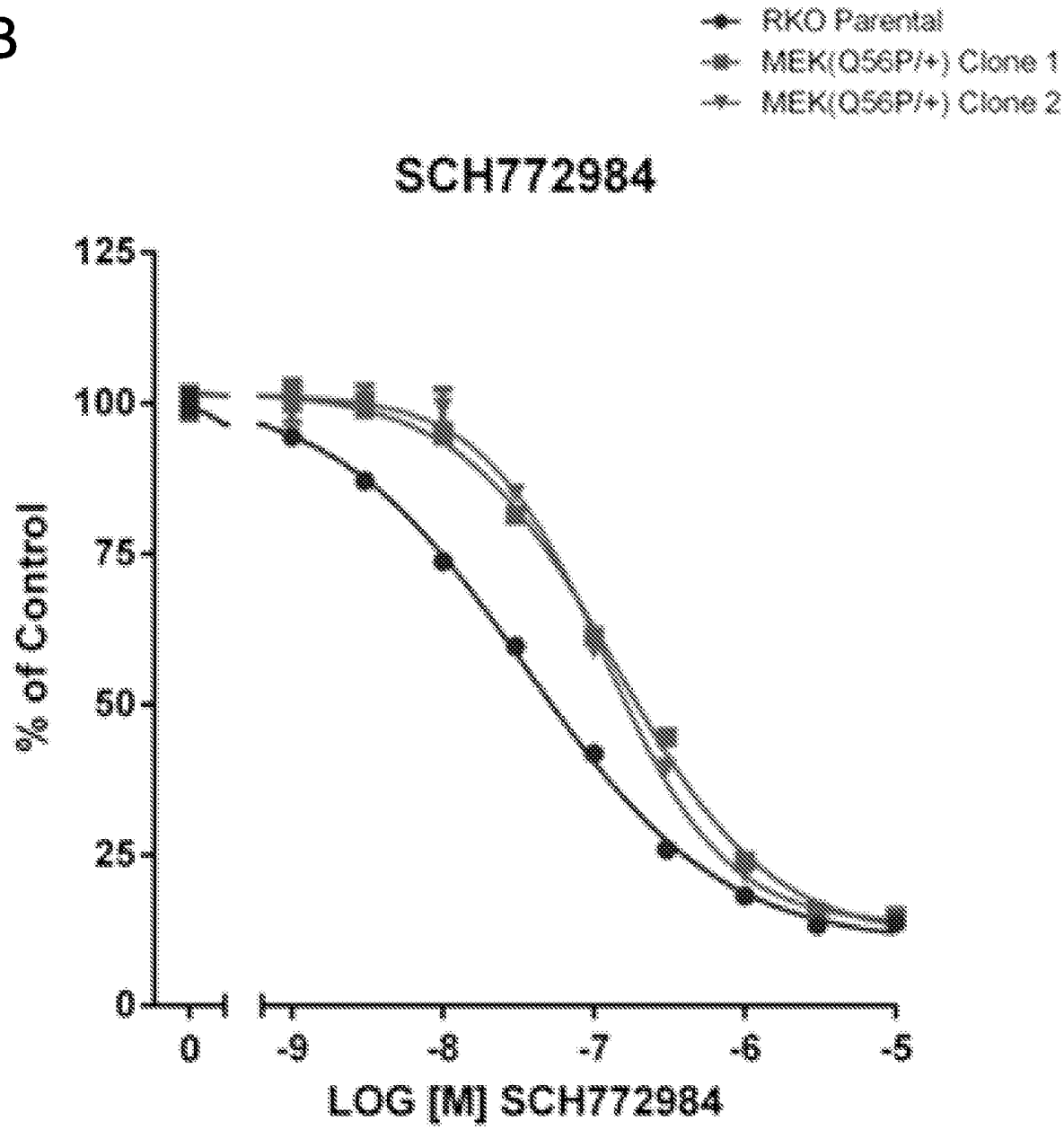

RKO Parental
MEK(Q56P/+) Clone 1
MEK(Q56P/+) Clone 2
SCH772984
% of Control
125
100
75
50
25
0
0
-9
-8
-7
-6
-5
LOG [M] SCH772984

FIG. 22 Con't

C

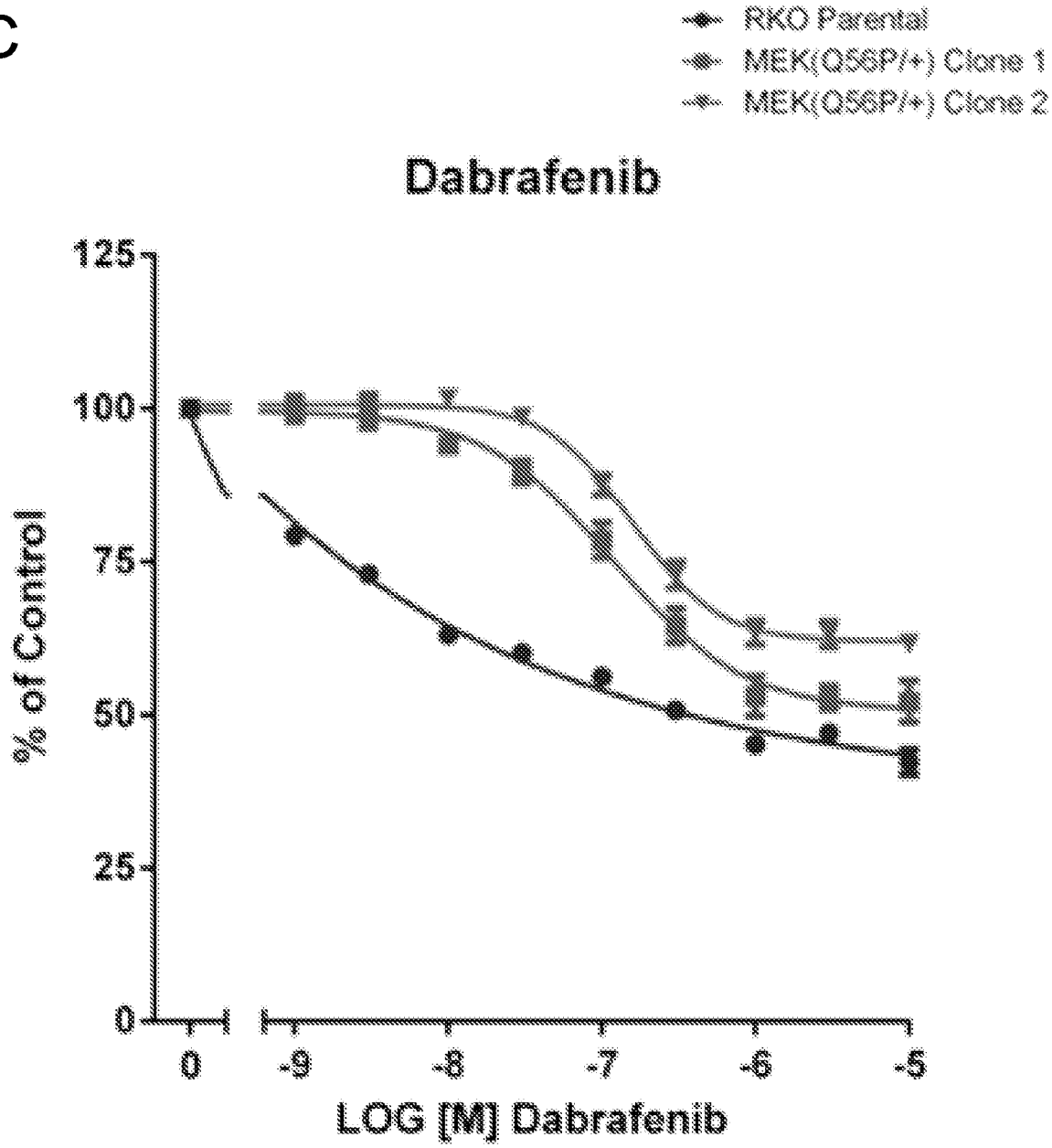

RKO Parental
MEK(Q56P/+) Clone 1
MEK(Q56P/+) Clone 2
Dabrafenib
% of Control
125
100
75
50
25
0
0
-9
-8
-7
-6
-5
LOG [M] Dabrafenib

FIG. 22 Con't

D

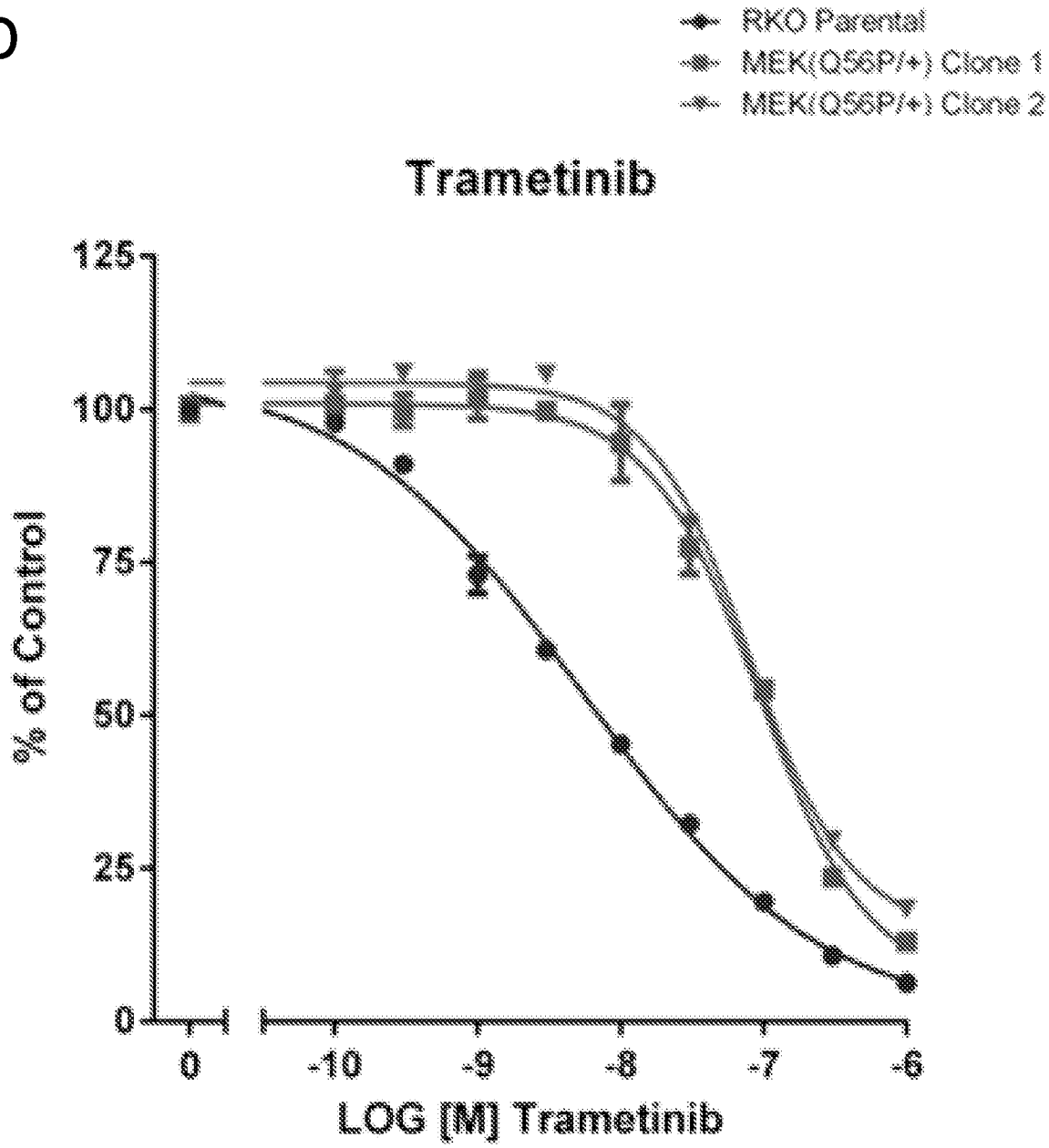

RKO Parental
MEK(Q56P/+) Clone 1
MEK(Q56P/+) Clone 2
Trametinib
% of Control
125
100
75
50
25
0
0
-10
-9
-8
-7
-6
LOG [M] Trametinib

FIG. 22, Con't

E

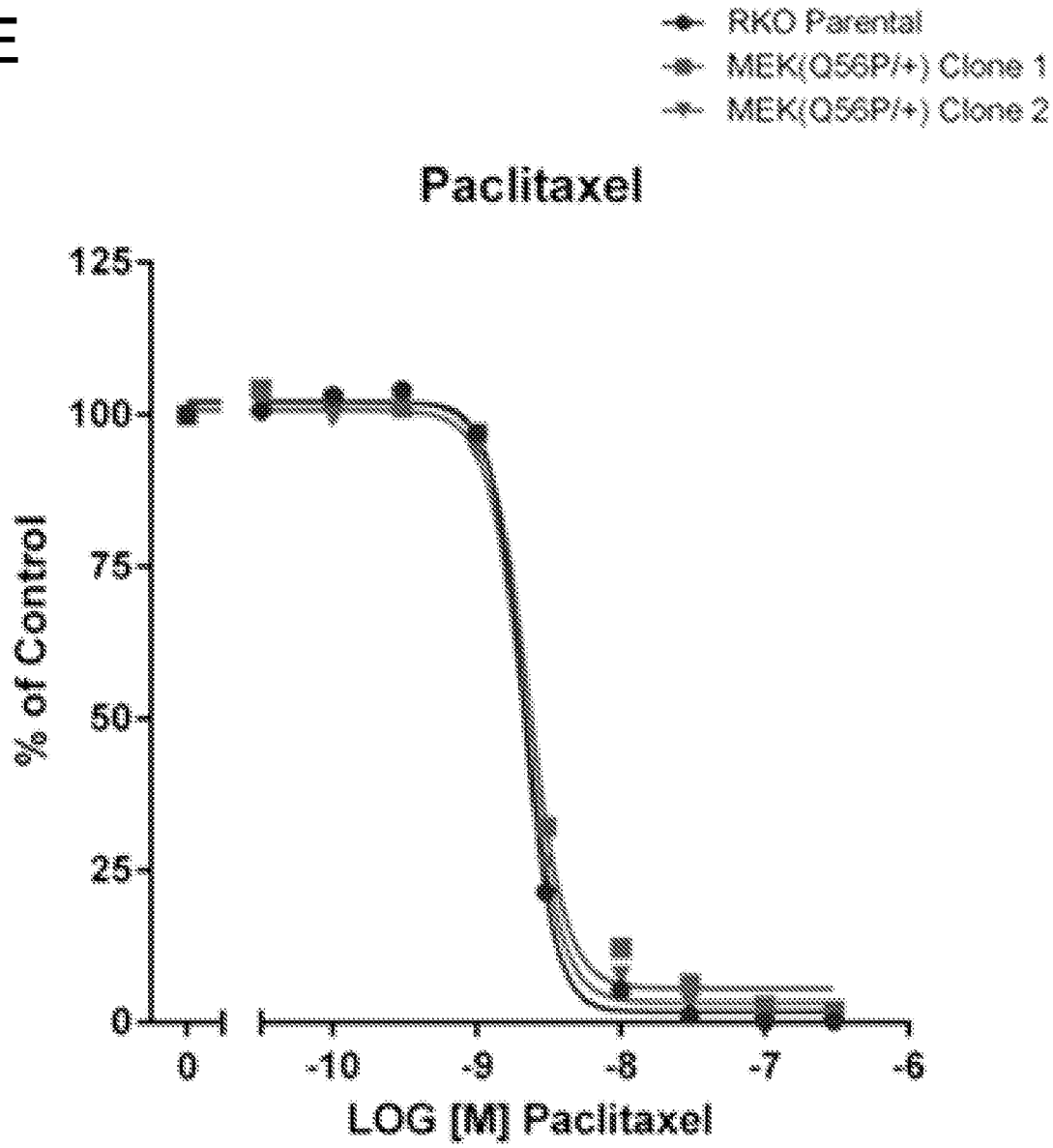

RKO Parental
MEK(Q56P/+) Clone 1
MEK(Q56P/+) Clone 2
Paclitaxel
% of Control
125
100
75
50
25
0
0
-10
-9
-8
-7
-6
LOG [M] Paclitaxel

FIG. 23

A

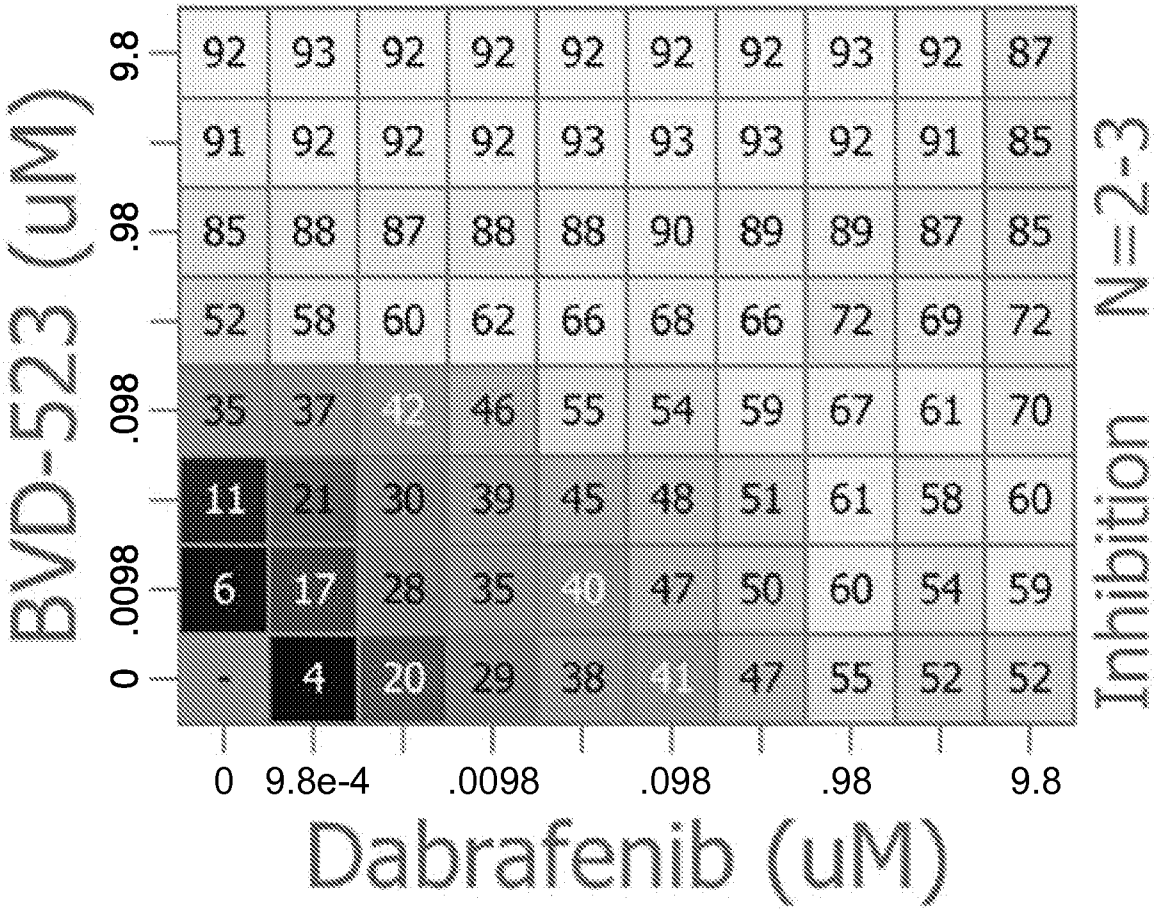
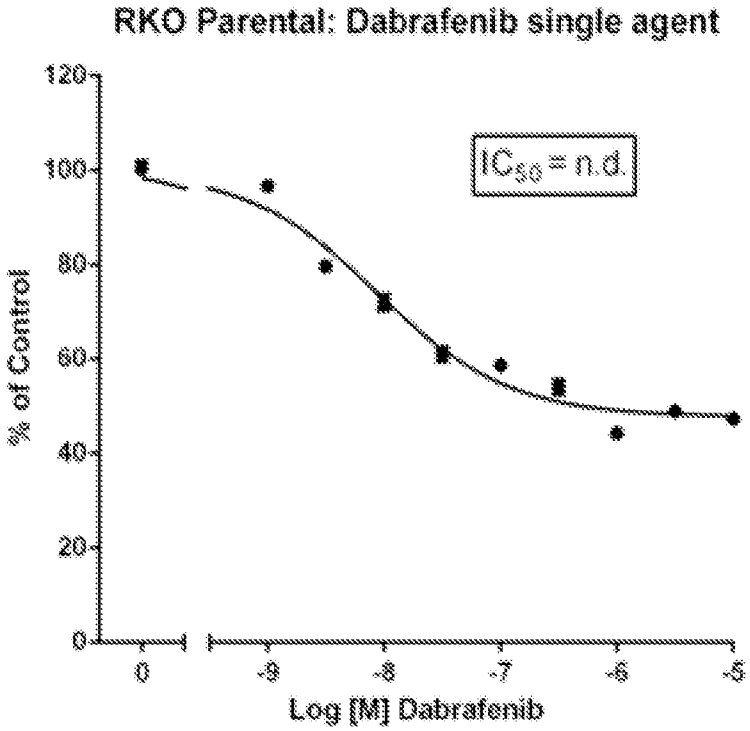

BVD-523 (uM)
9.8
.98
.098
.0098
0
92 93 92 92 92 92 92 93 92 87
91 92 92 92 93 93 93 92 91 85
85 88 87 88 88 90 89 89 87 85
52 58 60 62 66 68 66 72 69 72
35 37 42 46 55 54 59 67 61 70
11 21 30 39 45 48 51 61 58 60
6 17 28 35 40 47 50 60 54 59
- 4 20 29 38 41 47 55 52 52
0 9.8e-4 .0098 .098 .98 9.8
Dabrafenib (uM)
Inhibition N=2-3

FIG. 23 Con't

B



C

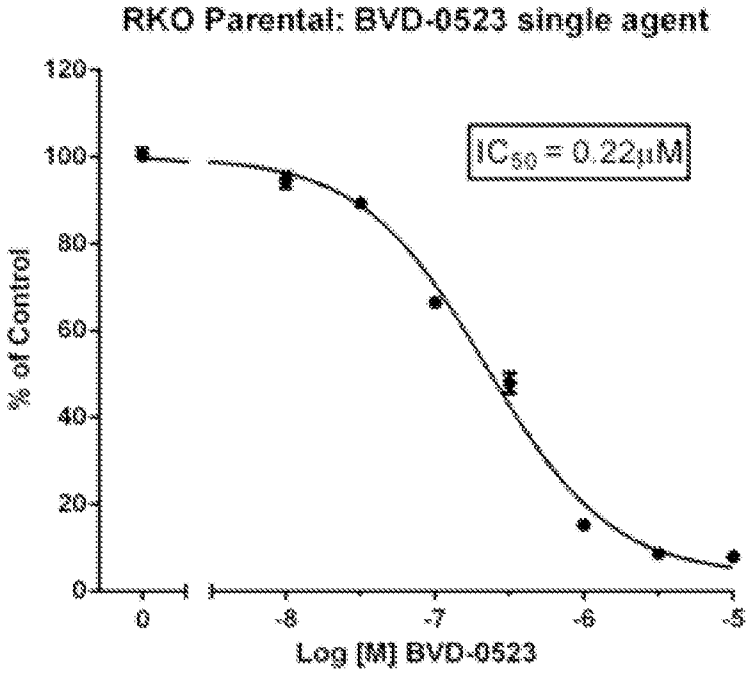


FIG. 23, Con't

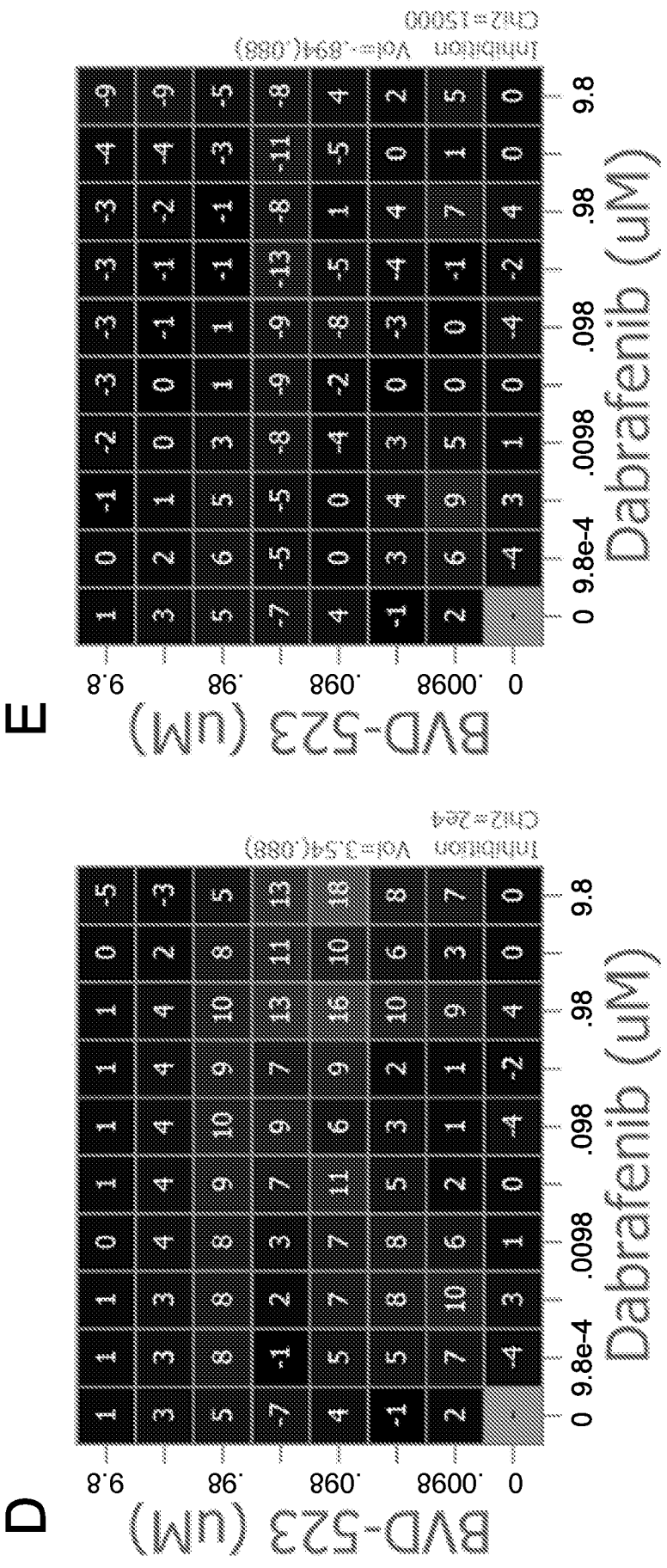

E
BVD-523 (uM)
Dabrafenib (uM)
Inhibition Val=.894(.088)
CN2=15000
D
BVD-523 (uM)
Dabrafenib (uM)
Inhibition Val=3.54(.088)
CN2=264

FIG. 23, Con't

F

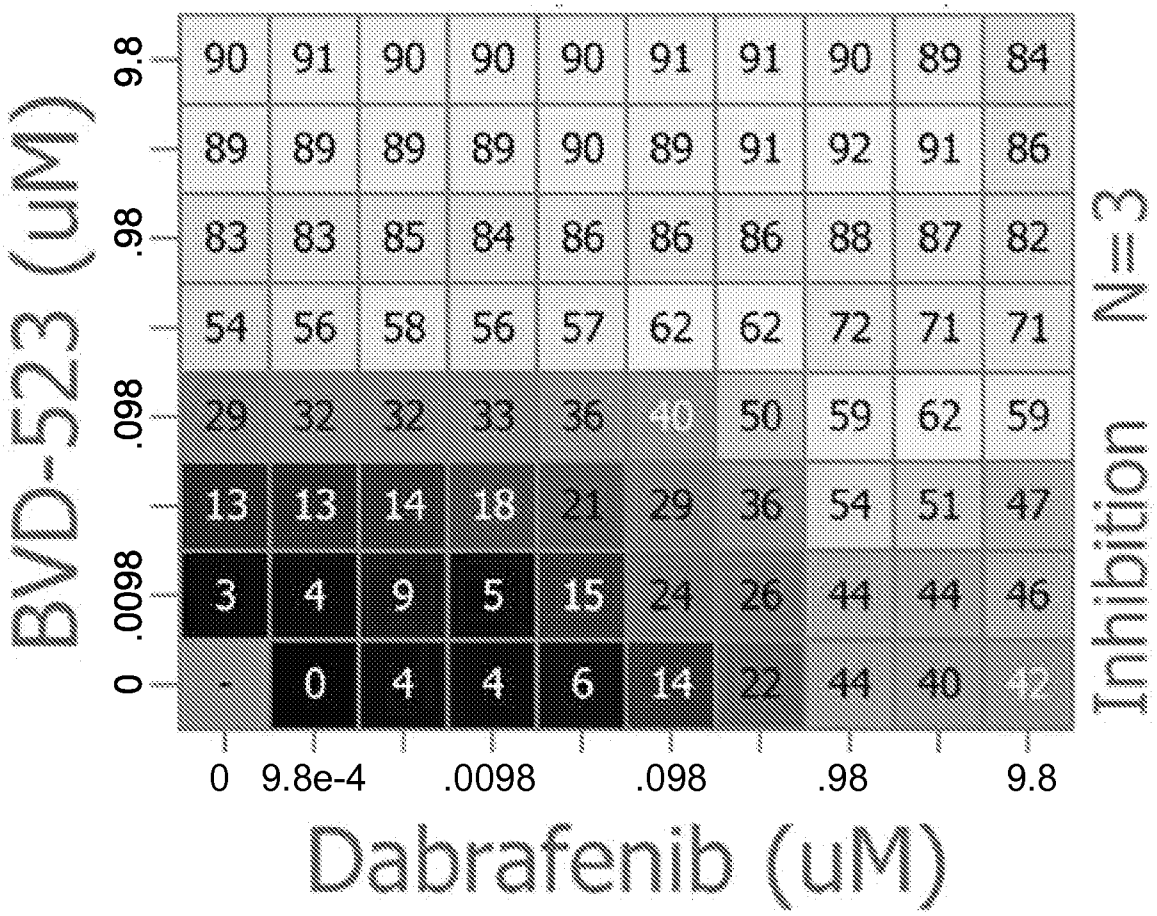
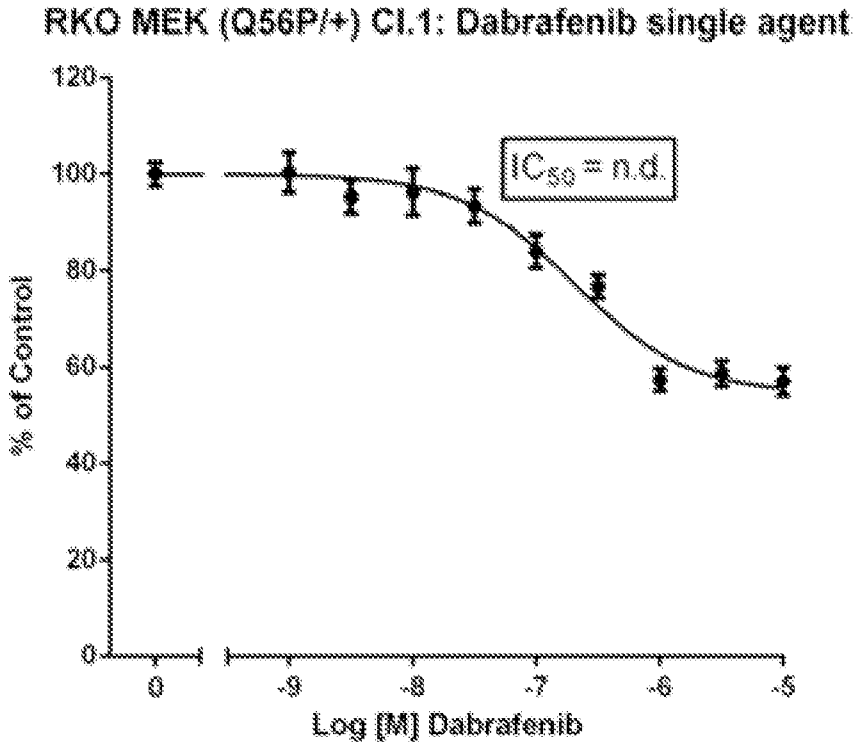

BVD-523 (uM)
9.8
.98
.098
.0098
0
90 91 90 90 90 91 91 90 89 84
89 89 89 89 90 89 91 92 91 86
83 83 85 84 86 86 86 88 87 82
54 56 58 56 57 62 62 72 71 71
29 32 32 33 36 40 50 59 62 59
13 13 14 18 21 29 36 54 51 47
3 4 9 5 15 24 26 44 44 46
- 0 4 4 6 14 22 44 40 42
0 9.8e-4 .0098 .098 .98 9.8
Dabrafenib (uM)
Inhibition N=3

FIG. 23, Con't

G



H

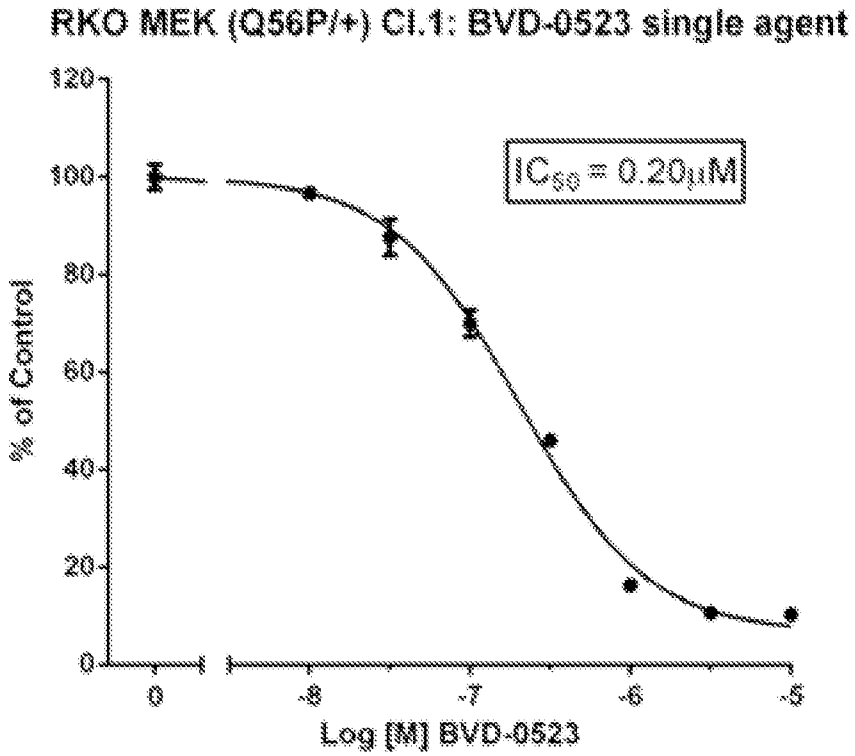


FIG. 23, Con't

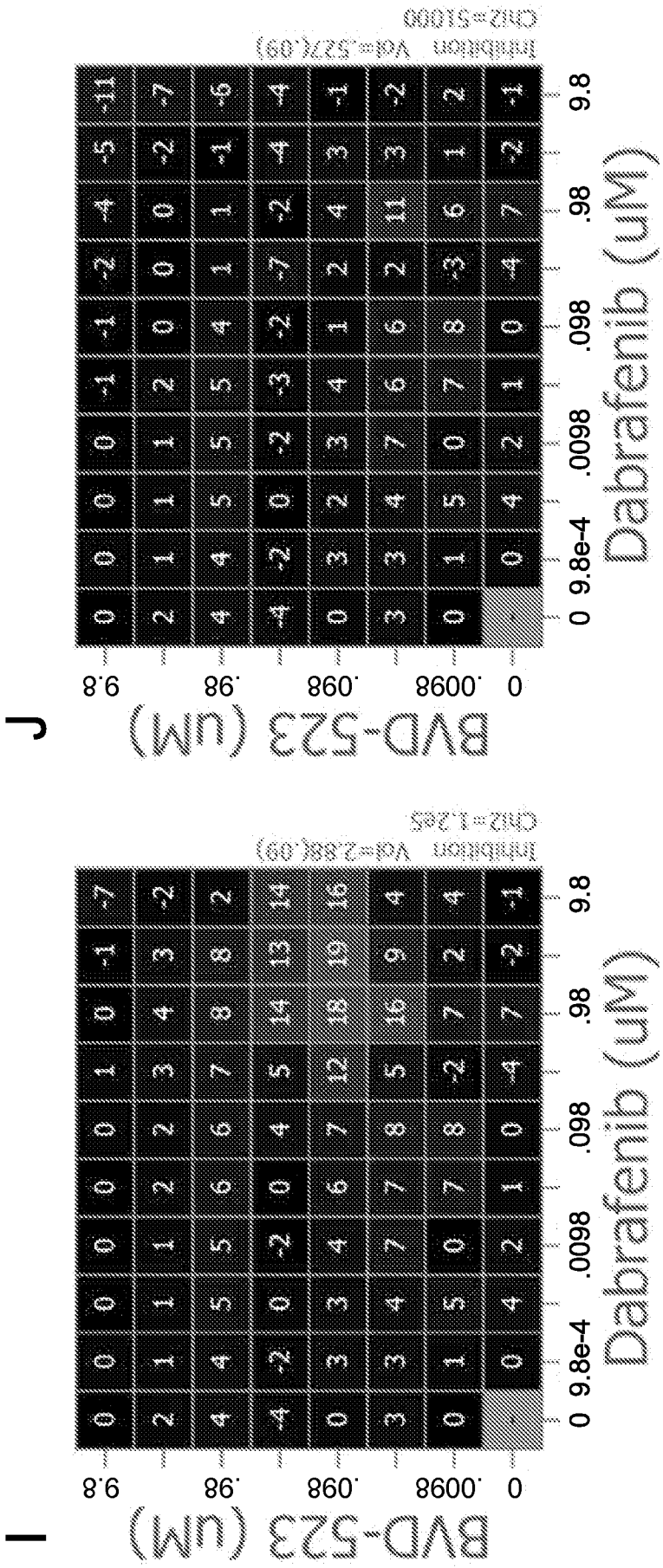

J
BVD-523 (uM)
Dabrafenib (uM)
Inhibition Vol=.527(.09)
CH2=5109
I
BVD-523 (uM)
Dabrafenib (uM)
Inhibition Vol=2.86(.09)
CH2=1.2e5

FIG. 23, Con't

K

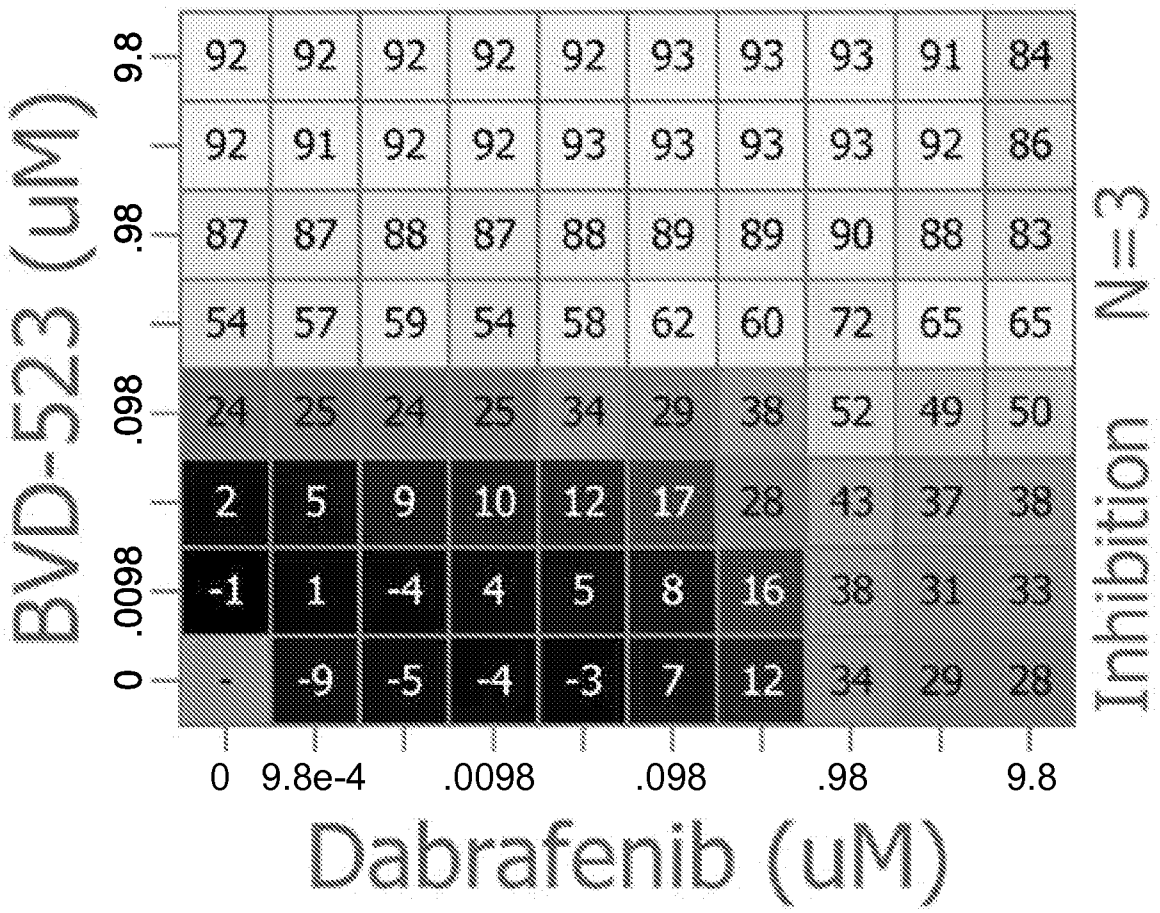
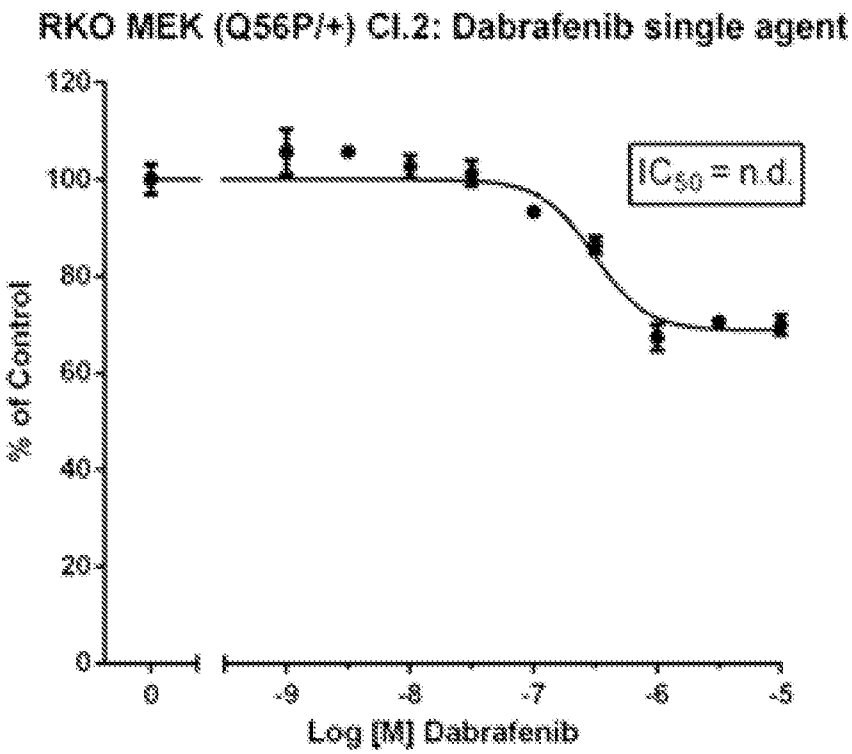

BVD-523 (uM)
9.8
.98
.098
.0098
0
92 92 92 92 92 93 93 93 91 84
92 91 92 92 93 93 93 93 92 86
87 87 88 87 88 89 89 90 88 83
54 57 59 54 58 62 60 72 65 65
24 25 24 25 34 29 38 52 49 50
2 5 9 10 12 17 28 43 37 38
-1 1 -4 4 5 8 16 38 31 33
- -9 -5 -4 -3 7 12 34 29 28
0 9.8e-4 .0098 .098 .98 9.8
Dabrafenib (uM)
Inhibition N=3

FIG. 23, Con't

L



M

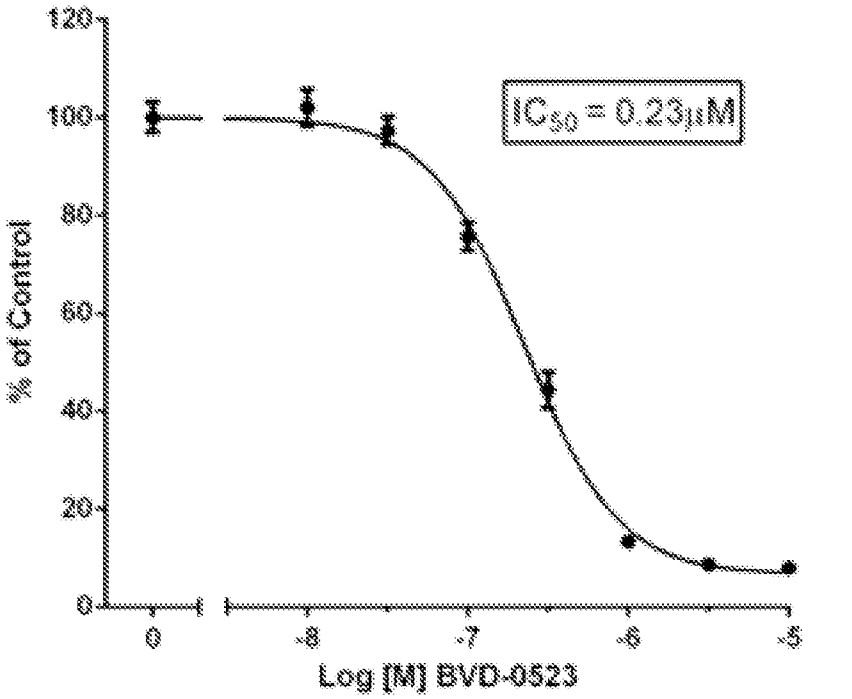


FIG. 23, Con't

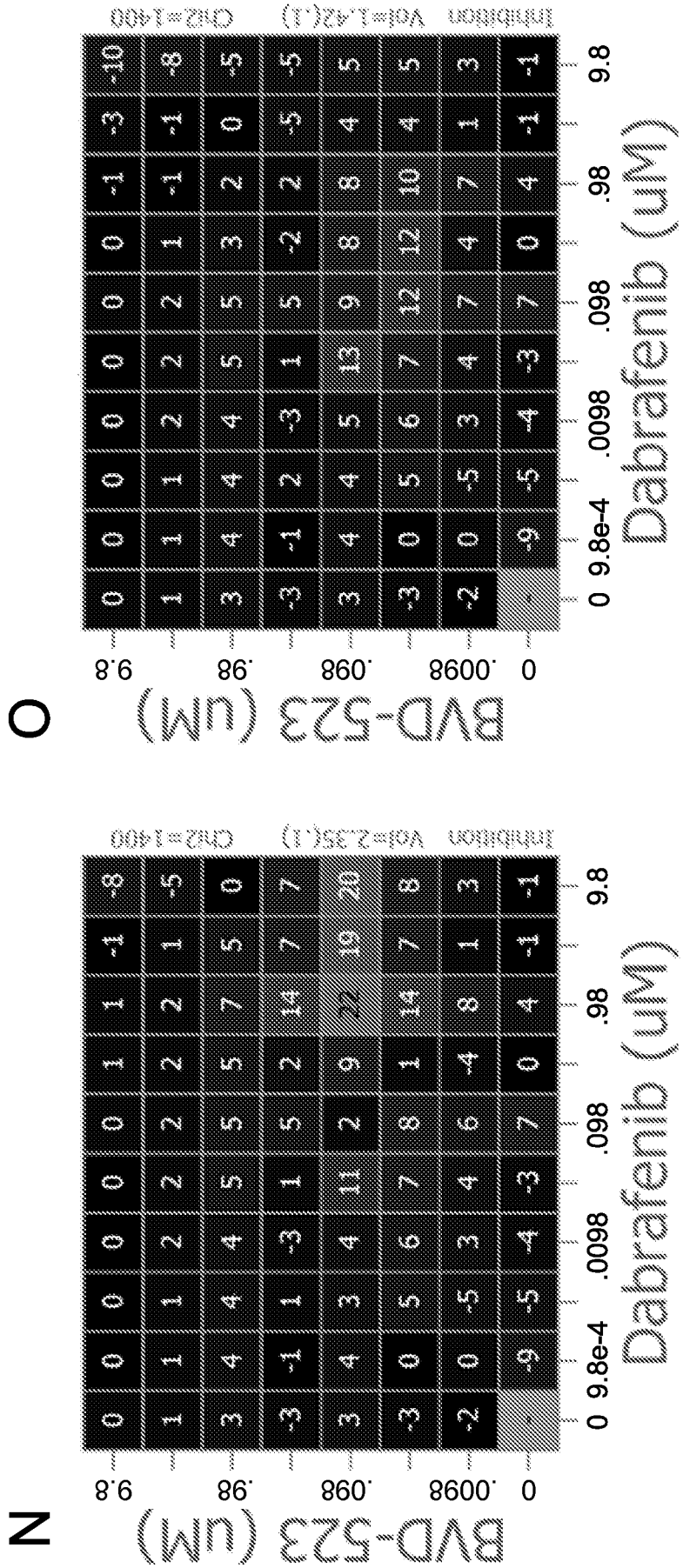

O
Inhibition Vol=1.42(.1) CN2=1400
BVD-523 (uM)
0 .0098 .098 .98 9.8
Dabrafenib (uM)
0 9.8e-4 .0098 .098 .98 9.8
N
Inhibition Vol=2.35(.1) CN2=1400
BVD-523 (uM)
0 .0098 .098 .98 9.8
Dabrafenib (uM)
0 9.8e-4 .0098 .098 .98 9.8

FIG. 24

A

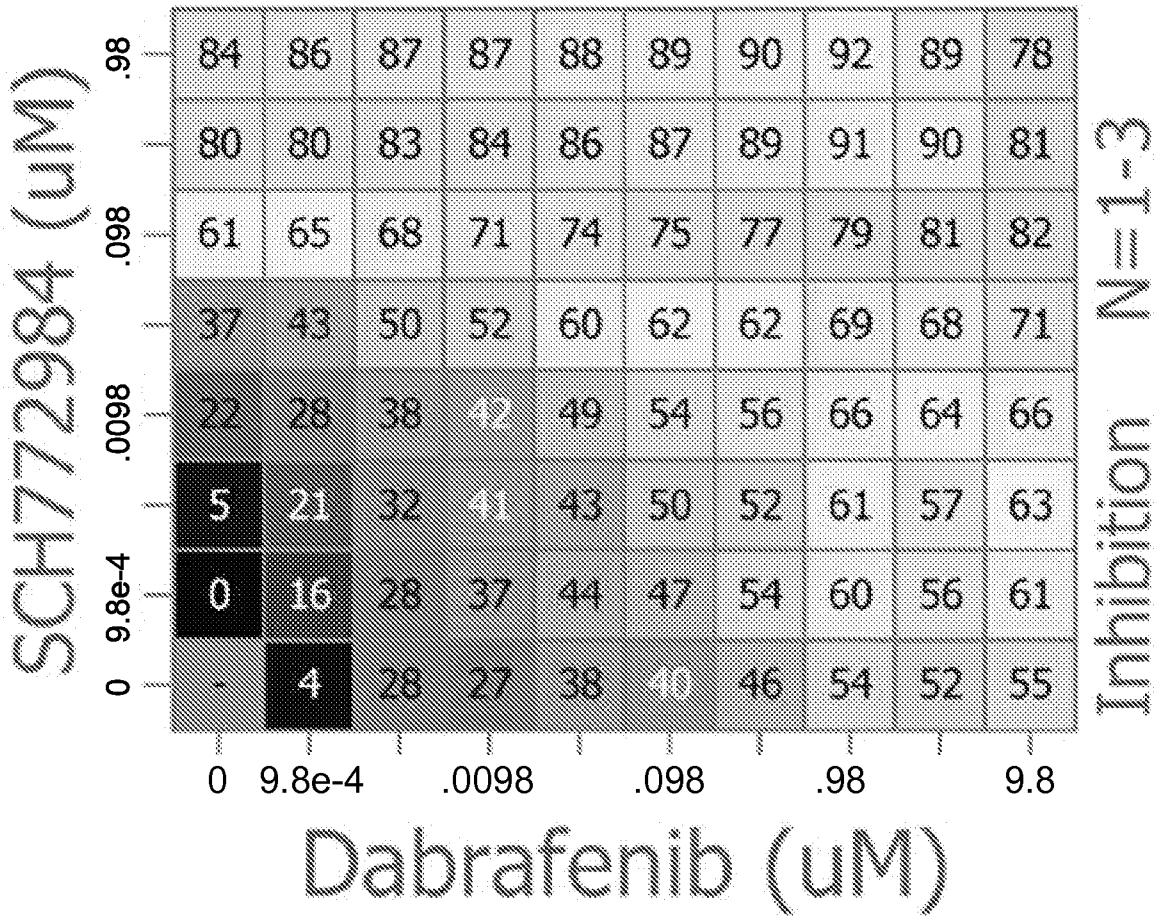
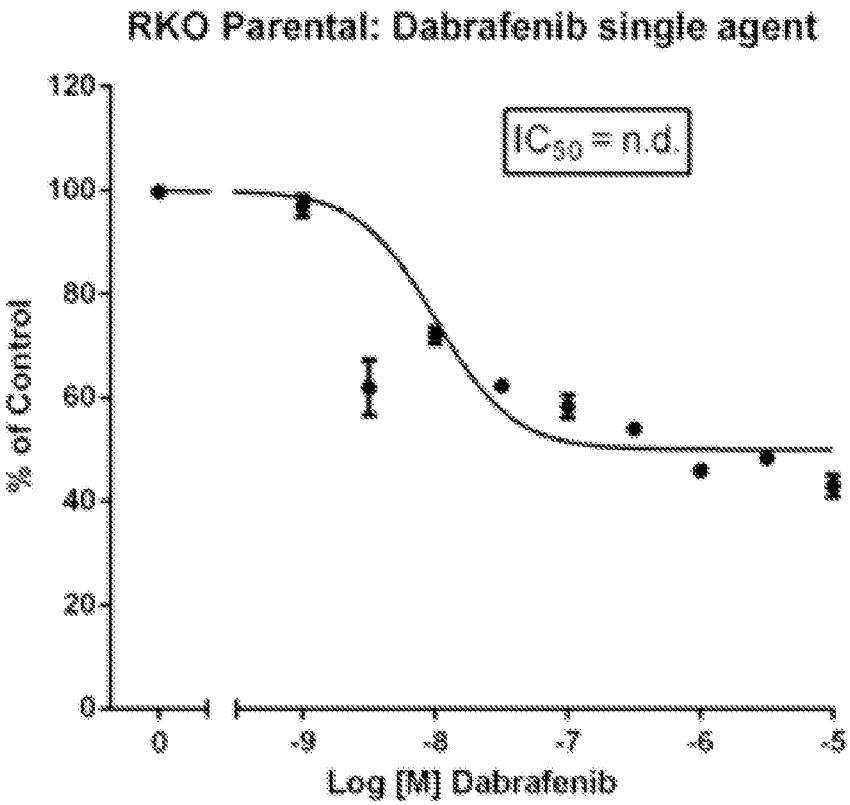

SCH772984 (uM)
.98
.098
.0098
9.8e-4
0
84 86 87 87 88 89 90 92 89 78
80 80 83 84 86 87 89 91 90 81
61 65 68 71 74 75 77 79 81 82
37 43 50 52 60 62 62 69 68 71
22 28 38 42 49 54 56 66 64 66
5 21 32 41 43 50 52 61 57 63
0 16 28 37 44 47 54 60 56 61
- 4 28 27 38 40 46 54 52 55
0 9.8e-4 .0098 .098 .98 9.8
Dabrafenib (uM)
Inhibition N=1-3

FIG. 24, Con't

B



C

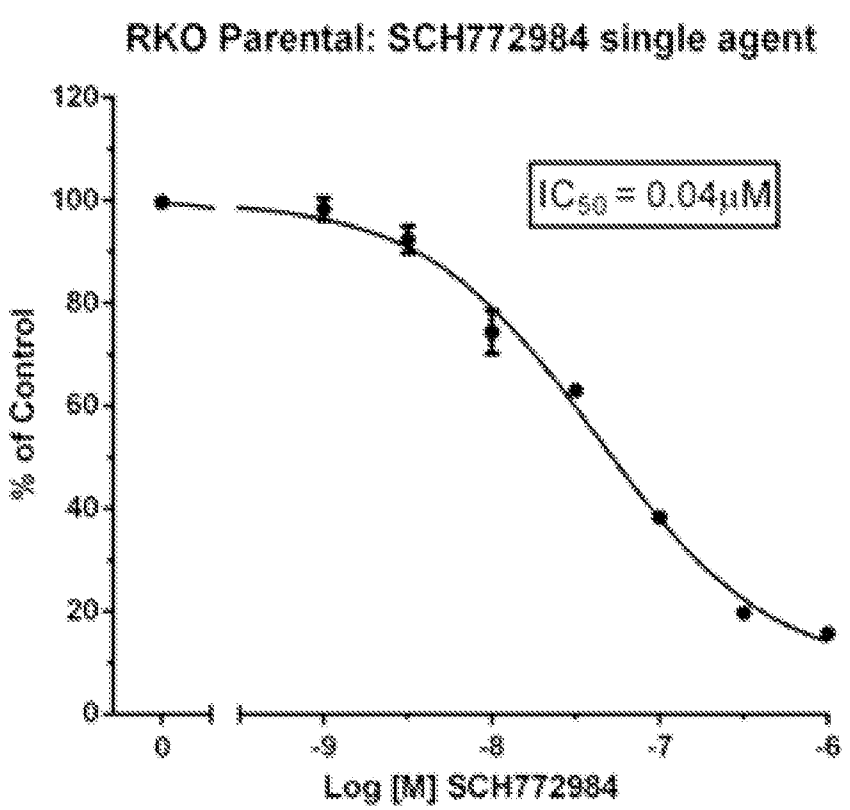


FIG. 24, Con't

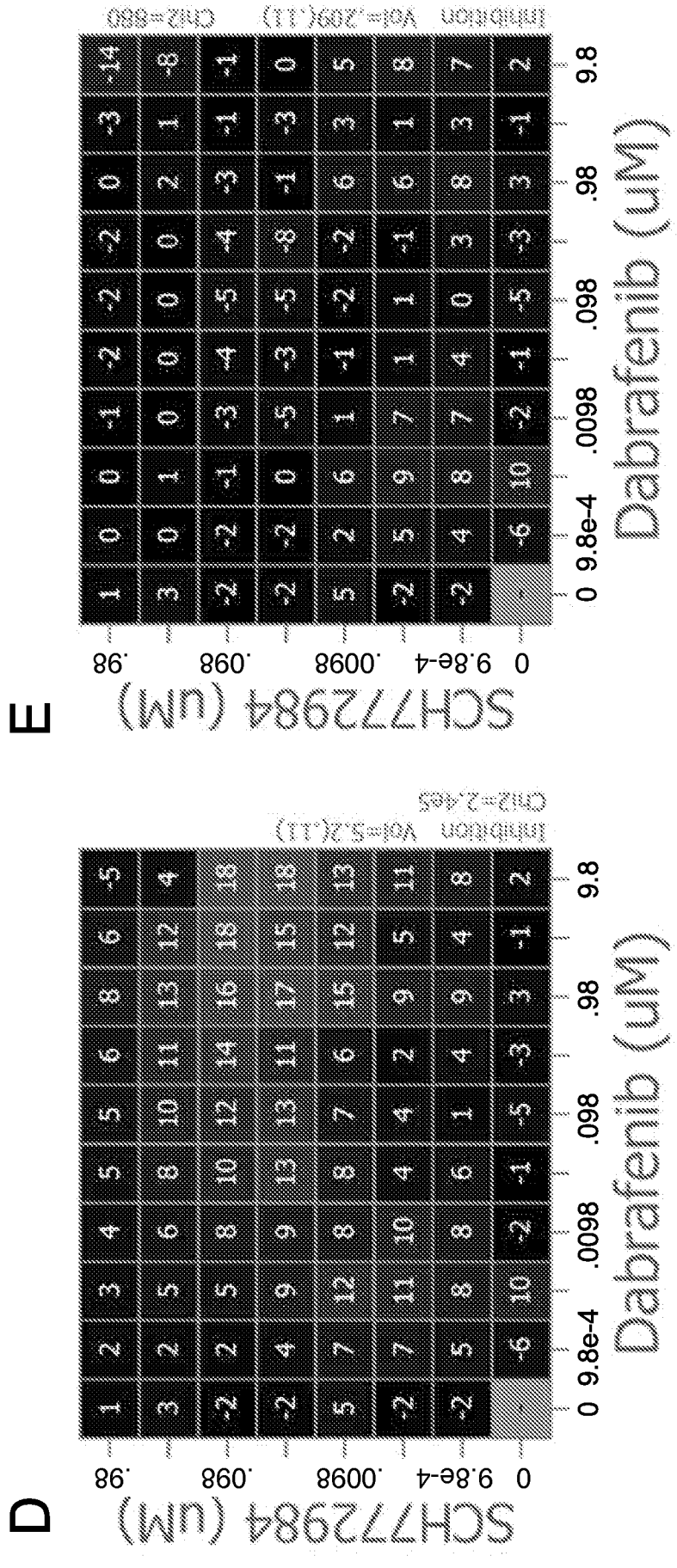

E
SCH772984 (uM)
0 9.8e-4 .0098 .098 .98
Dabrafenib (uM)
0 9.8e-4 .0098 .098 .98 9.8
D
SCH772984 (uM)
0 9.8e-4 .0098 .098 .98
Dabrafenib (uM)
0 9.8e-4 .0098 .098 .98 9.8

FIG. 24, Con't

F

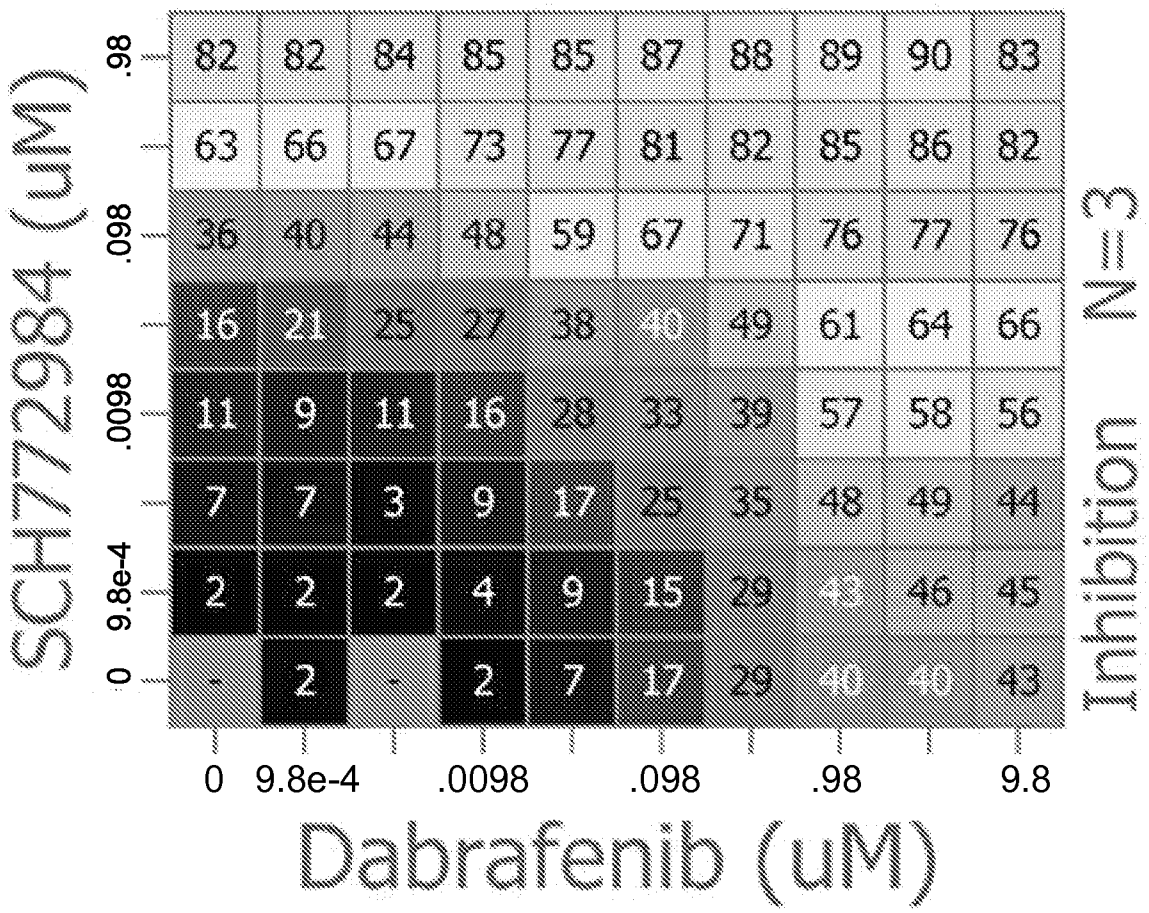
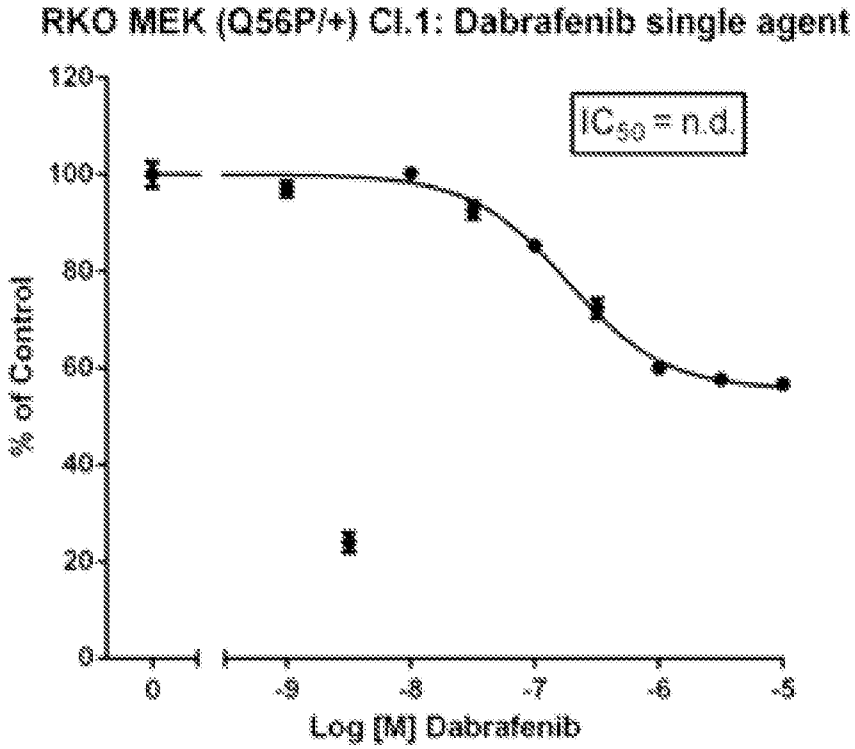

SCH772984 (uM)
.98
.098
.0098
9.8e-4
0
82 82 84 85 85 87 88 89 90 83
63 66 67 73 77 81 82 85 86 82
36 40 44 48 59 67 71 76 77 76
16 21 25 27 38 40 49 61 64 66
11 9 11 16 28 33 39 57 58 56
7 7 3 9 17 25 35 48 49 44
2 2 2 4 9 15 29 43 46 45
- 2 - 2 7 17 29 40 40 43
Inhibition N=3
0 9.8e-4 .0098 .098 .98 9.8
Dabrafenib (uM)

FIG. 24, Con't

G



H

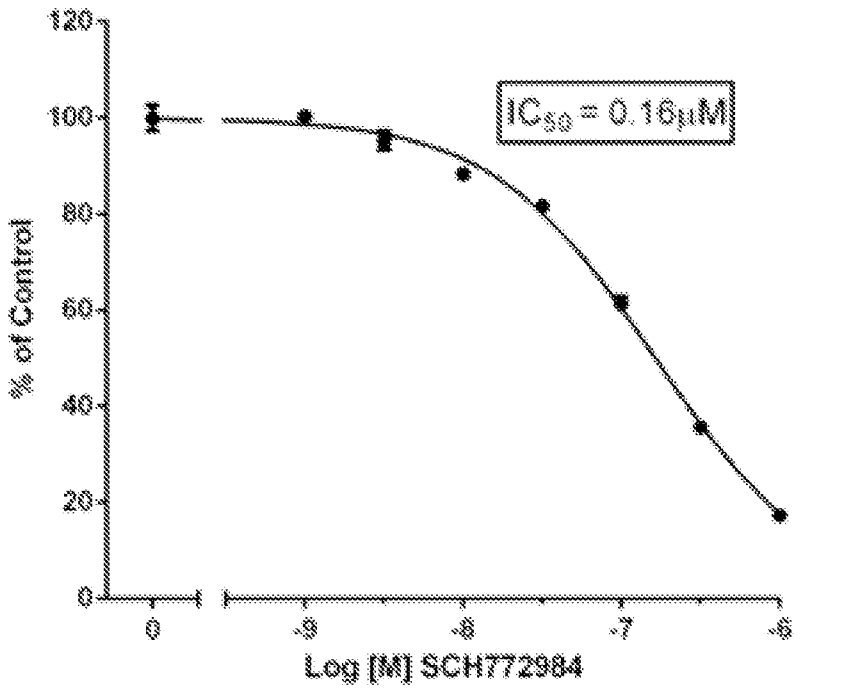


FIG. 24, Con't

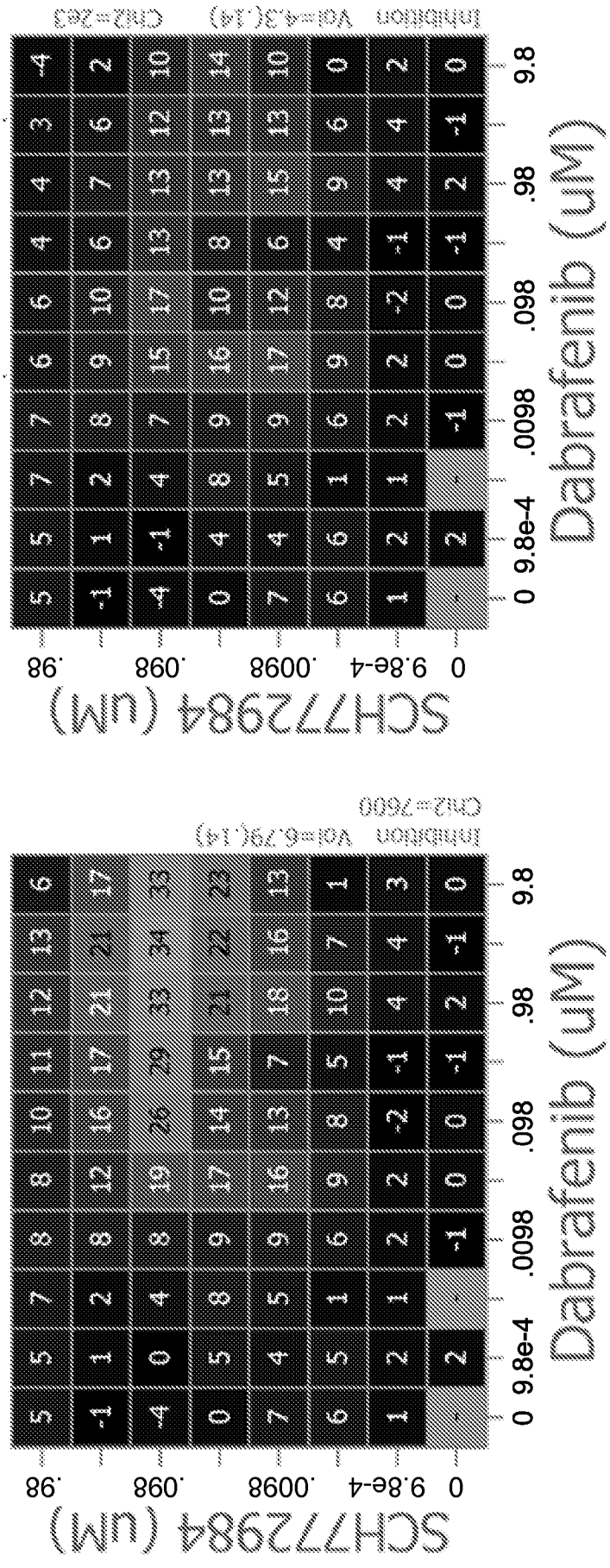

J
SCH772984 (uM)
0
9.8e-4
.0098
.098
.98
Dabrafenib (uM)
0
9.8e-4
.0098
.098
.98
9.8
Inhibition Vol=4.3(.14) Chi2=263
I
SCH772984 (uM)
0
9.8e-4
.0098
.098
.98
Dabrafenib (uM)
0
9.8e-4
.0098
.098
.98
9.8
Inhibition Vol=6.79(.14)
Chi2=7600

FIG. 24, Con't

K

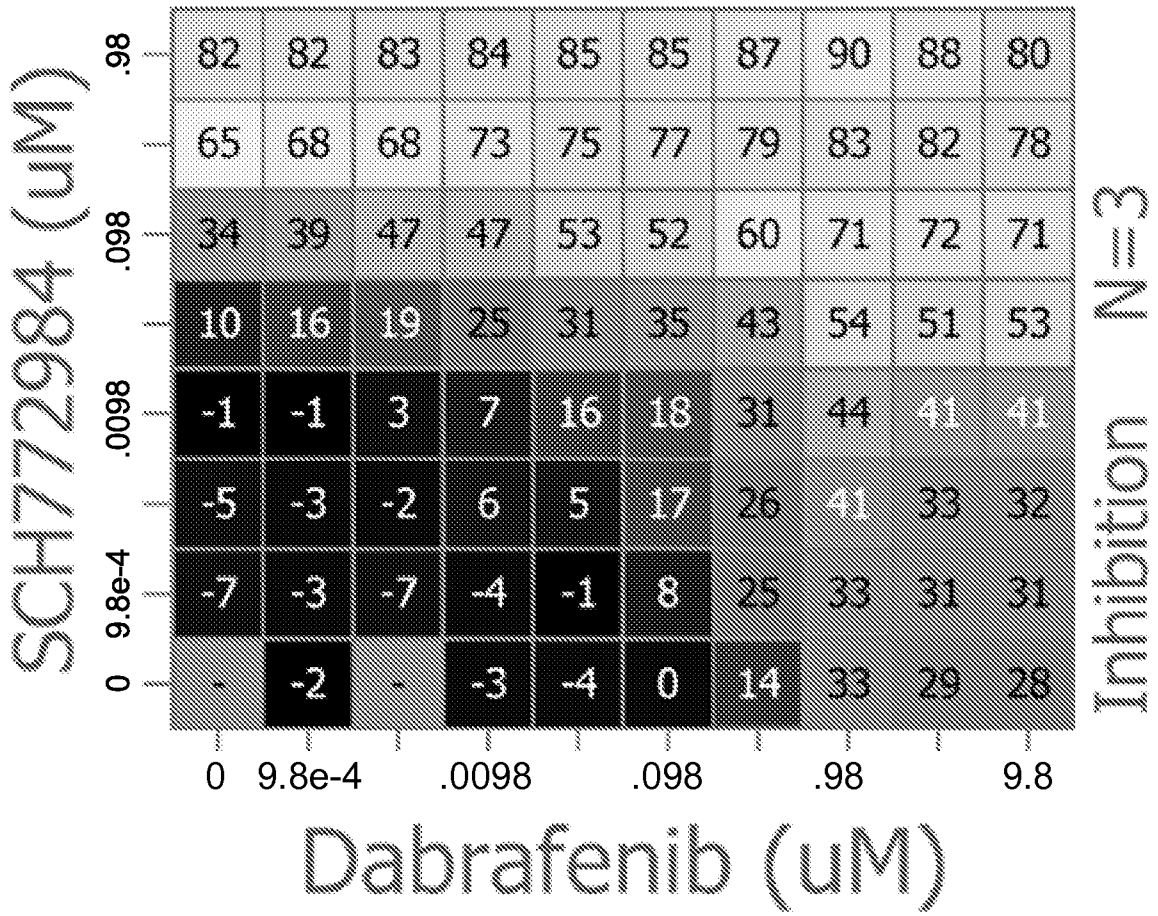
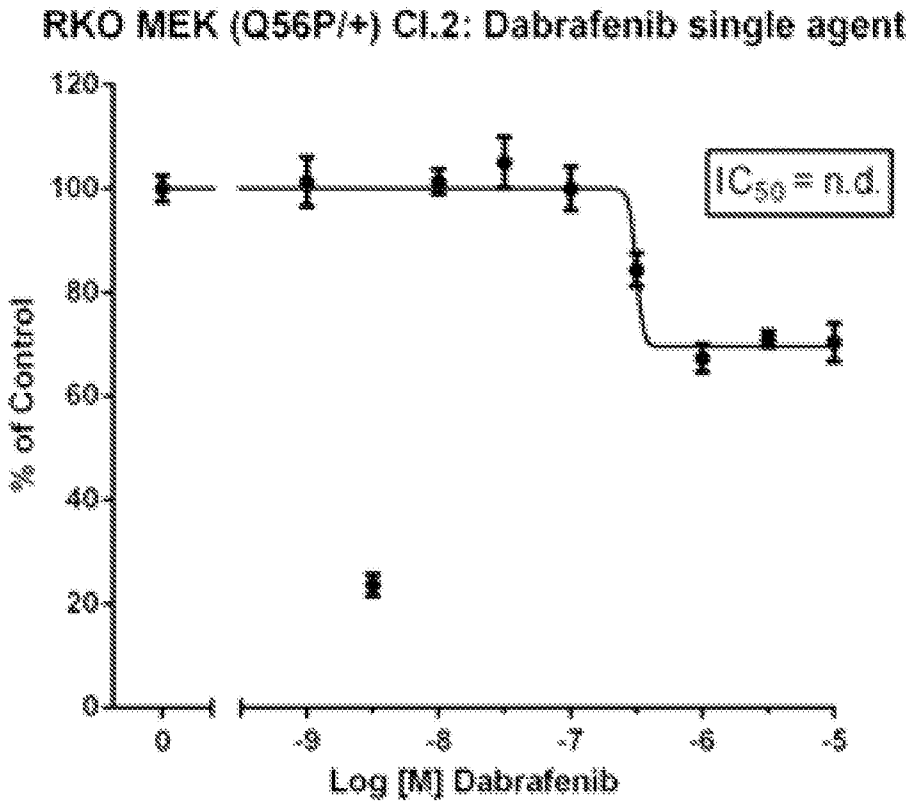

SCH772984 (uM)
.98
.098
.0098
9.8e-4
0
82 82 83 84 85 85 87 90 88 80
65 68 68 73 75 77 79 83 82 78
34 39 47 47 53 52 60 71 72 71
10 16 19 25 31 35 43 54 51 53
-1 -1 3 7 16 18 31 44 41 41
-5 -3 -2 6 5 17 26 41 33 32
-7 -3 -7 -4 -1 8 25 33 31 31
- -2 - -3 -4 0 14 33 29 28
0 9.8e-4 .0098 .098 .98 9.8
Dabrafenib (uM)
Inhibition N=3

FIG. 24, Con't

L



M

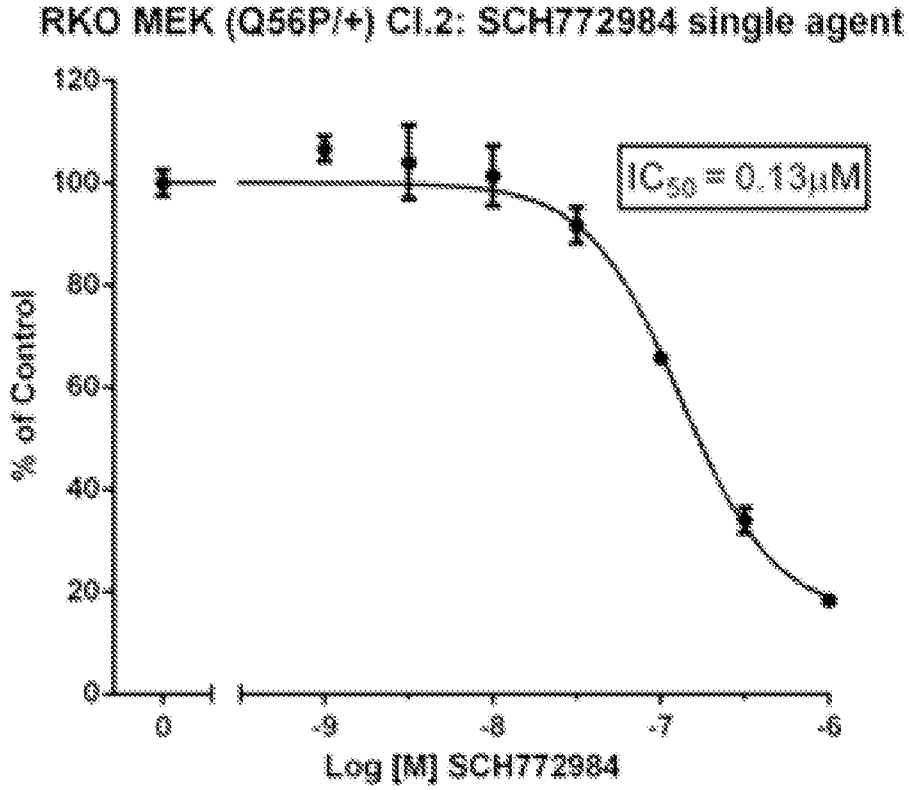


FIG. 24, Con't

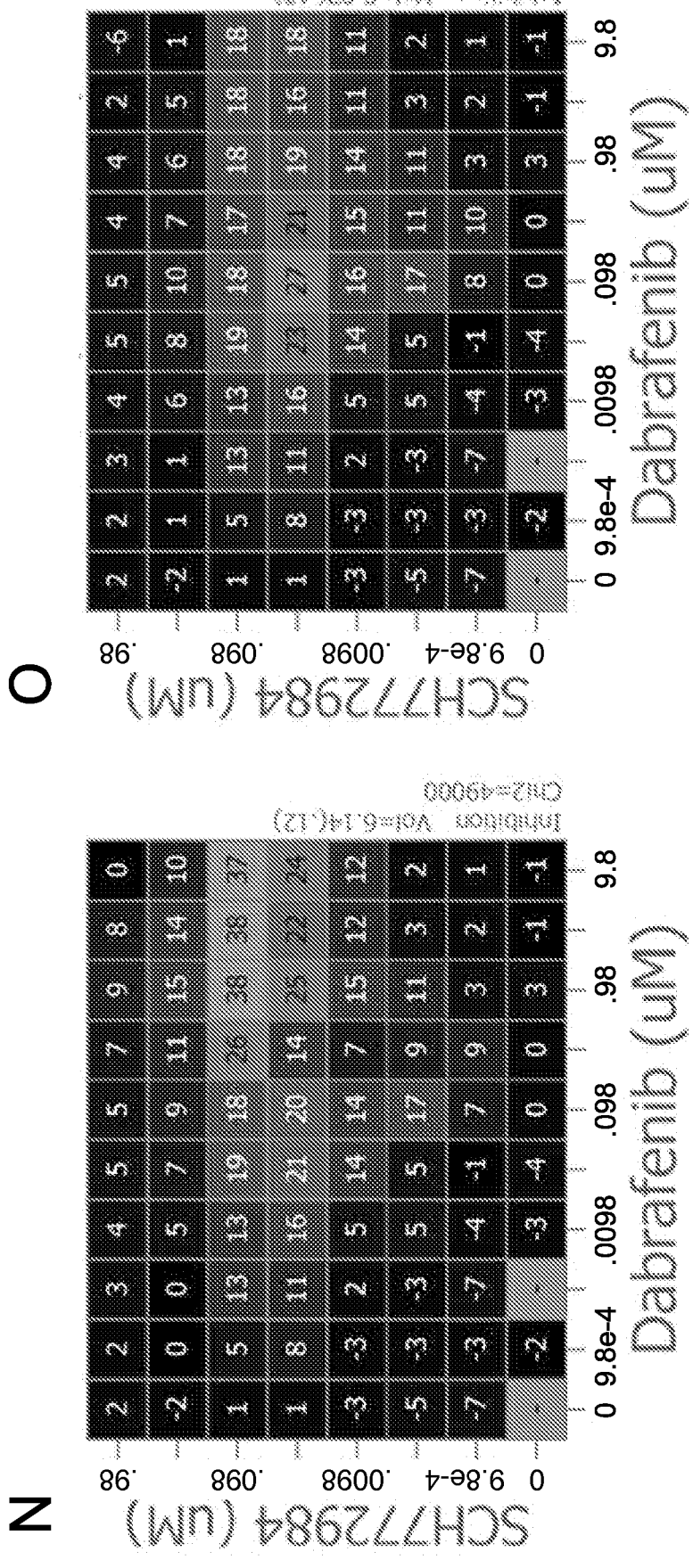

O
SCH772984 (uM)
0 9.8e-4 .0098 .098 .98
Dabrafenib (uM)
0 9.8e-4 .0098 .098 .98 9.8
Inhibition Vol=5.07(.12)
CN2=22000
N
SCH772984 (uM)
0 9.8e-4 .0098 .098 .98
Dabrafenib (uM)
0 9.8e-4 .0098 .098 .98 9.8
Inhibition Vol=6.14(.12)
CN2=49000

FIG. 25

A

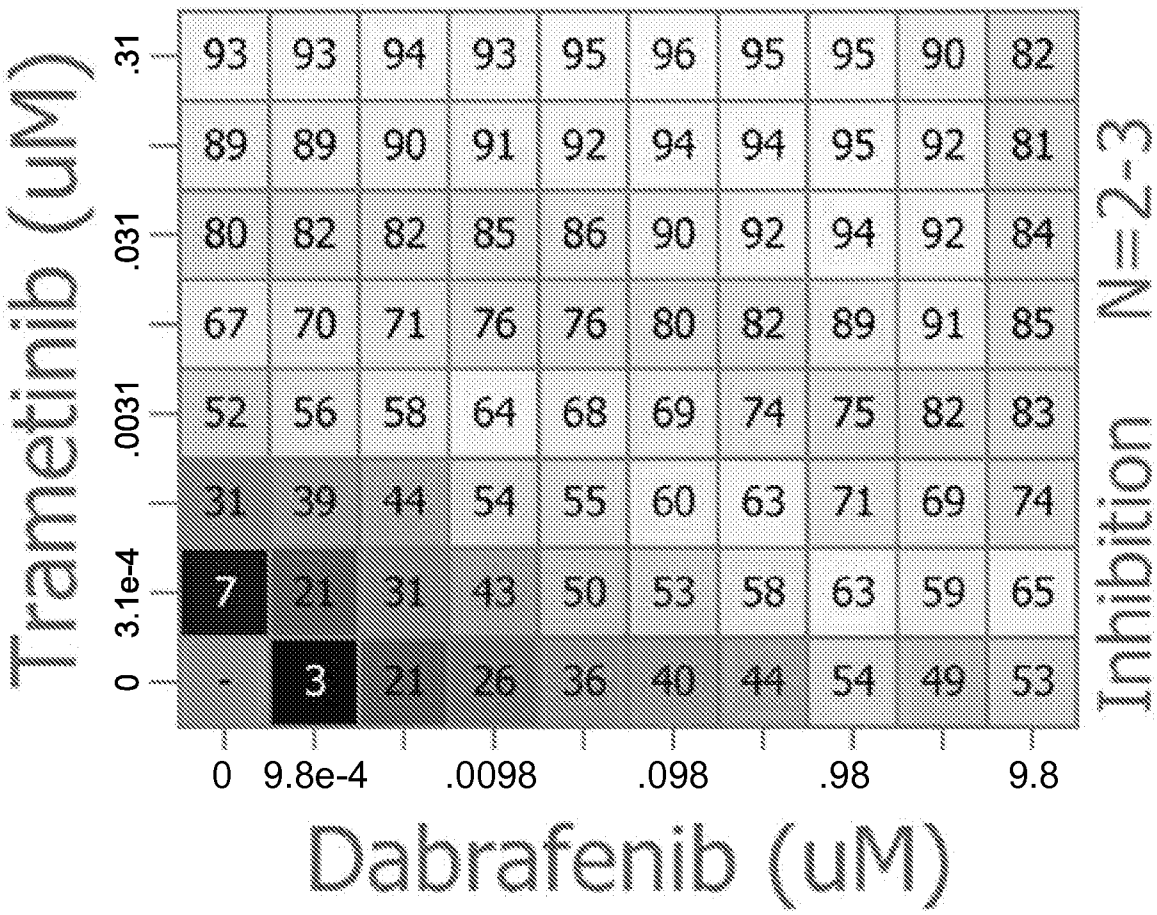
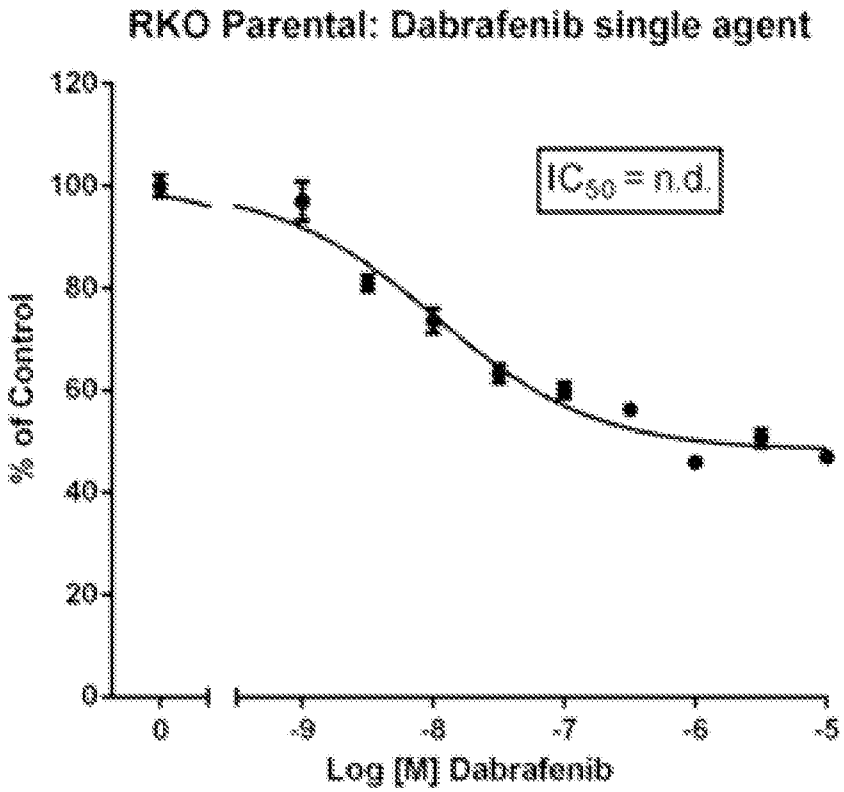

Trametinib (uM)
.31
.031
.0031
3.1e-4
0
93 93 94 93 95 96 95 95 90 82
89 89 90 91 92 94 94 95 92 81
80 82 82 85 86 90 92 94 92 84
67 70 71 76 76 80 82 89 91 85
52 56 58 64 68 69 74 75 82 83
31 39 44 54 55 60 63 71 69 74
7 21 31 43 50 53 58 63 59 65
- 3 21 26 36 40 44 54 49 53
0 9.8e-4 .0098 .098 .98 9.8
Dabrafenib (uM)
Inhibition N=2-3

FIG. 25 Con't

B



C

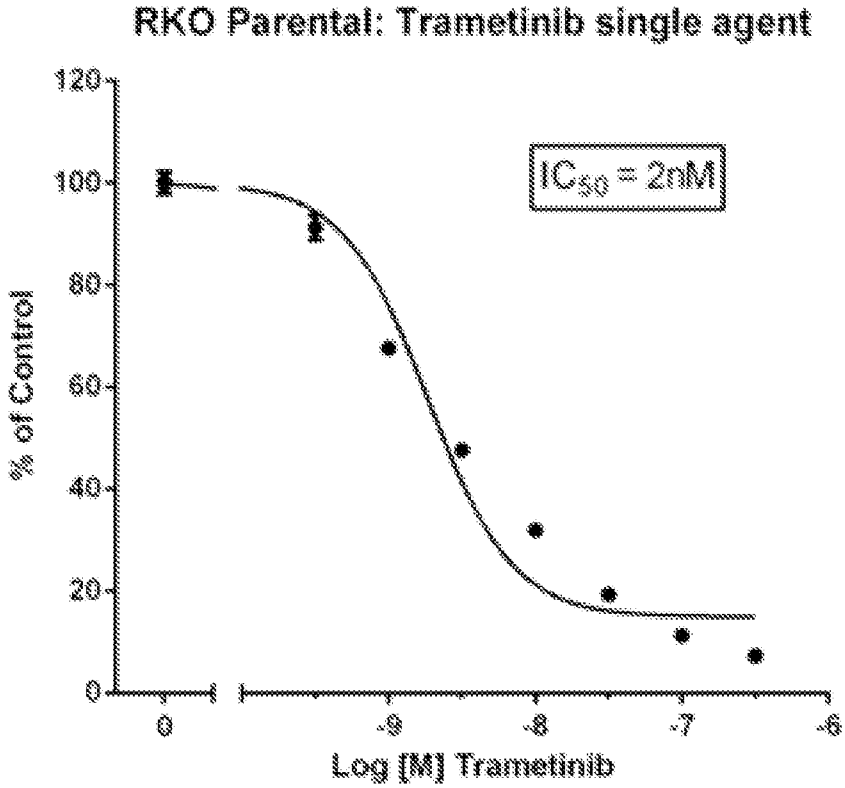


FIG. 25, Con't

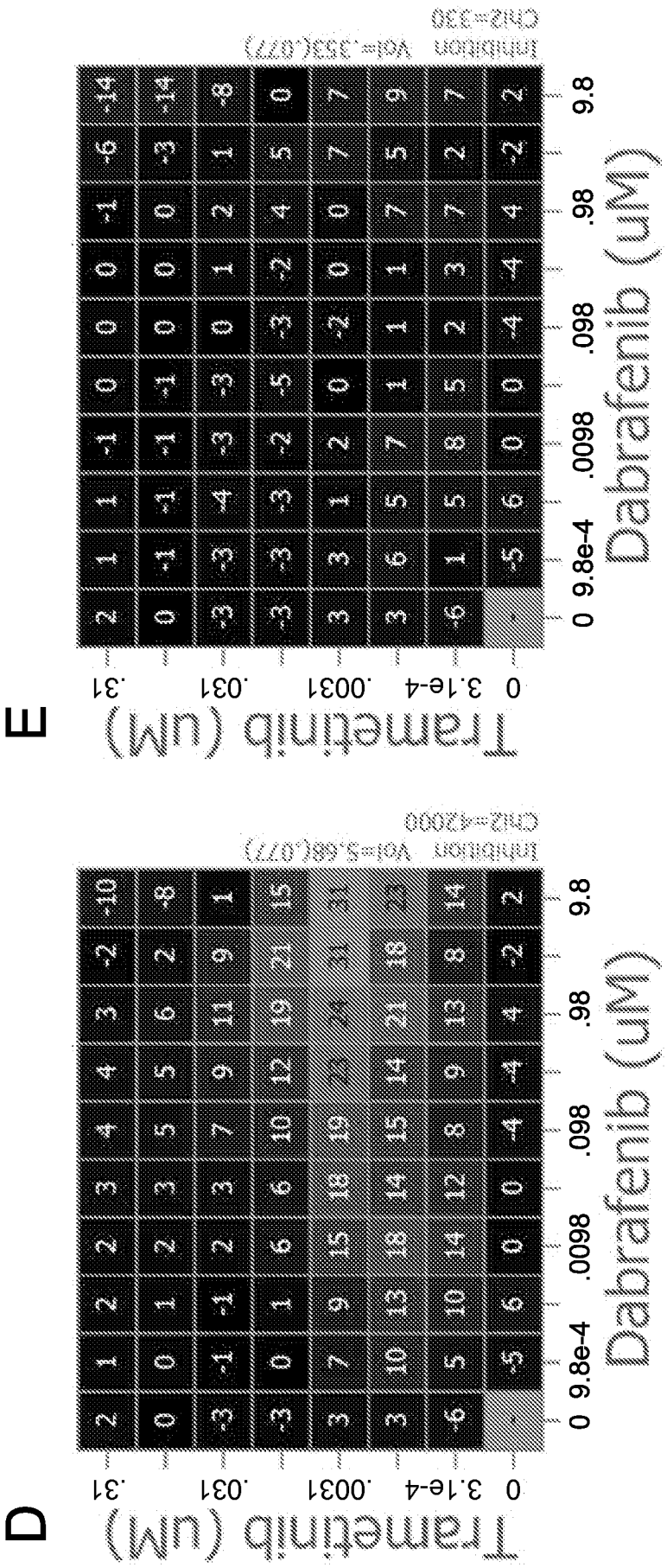

D
E
Trametinib (uM)
Dabrafenib (uM)
0
3.1e-4
.0031
.031
.31
0
9.8e-4
.0098
.098
.98
9.8
Inhibition Vol=5.68(.077)
Chi2=4200
Inhibition Vol=.353(.077)
Chi2=330

FIG. 25, Con't

F

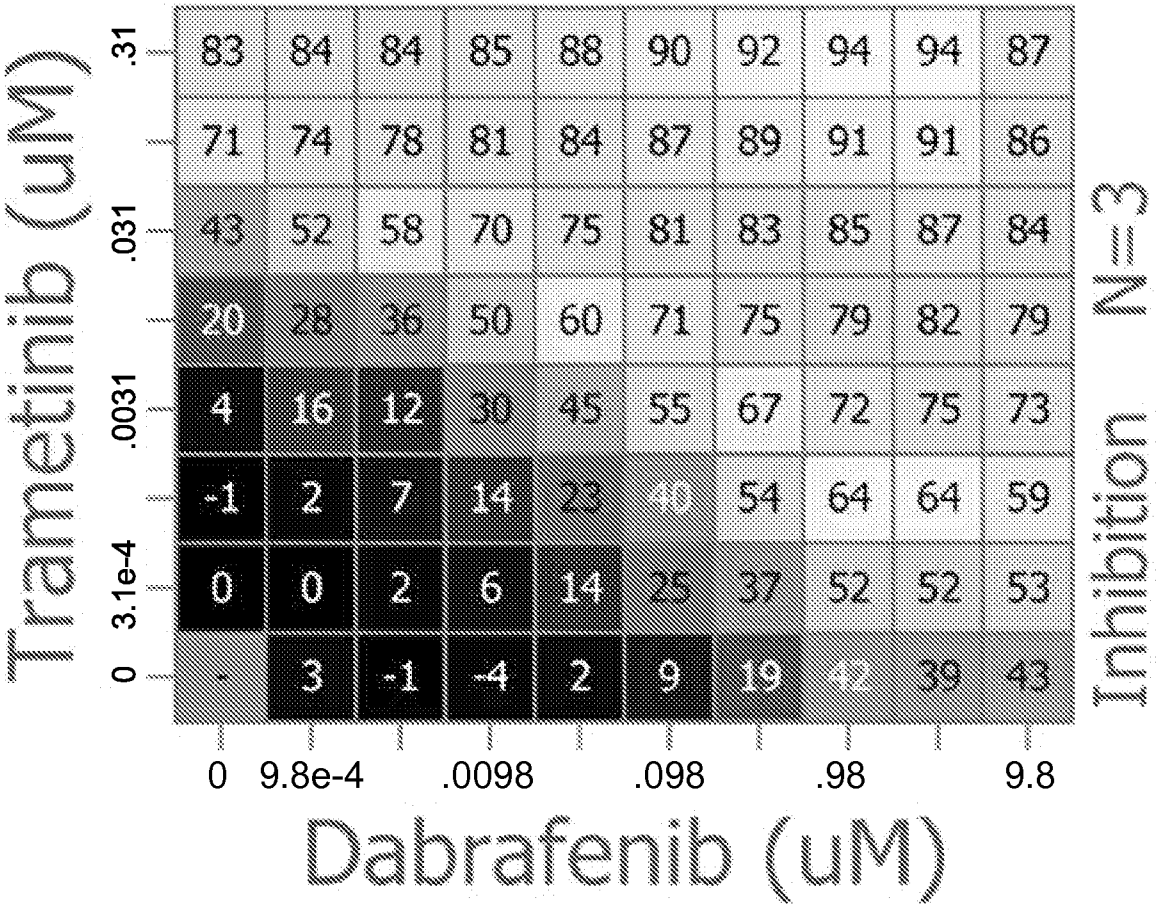
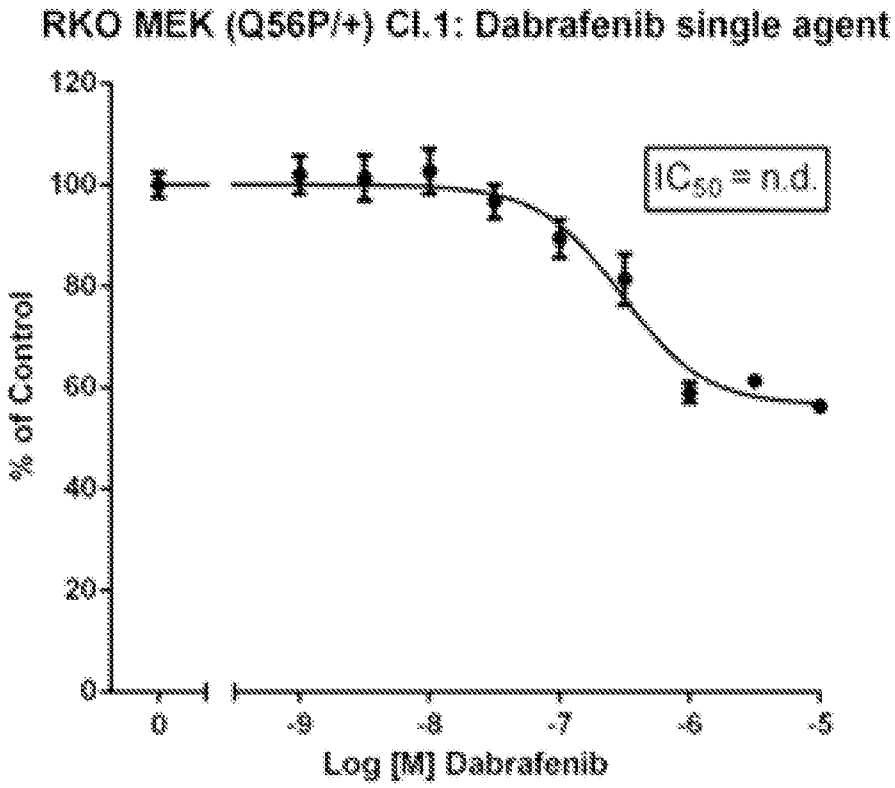

Trametinib (uM)
.31
.031
.0031
3.1e-4
0
83 84 84 85 88 90 92 94 94 87
71 74 78 81 84 87 89 91 91 86
43 52 58 70 75 81 83 85 87 84
20 28 36 50 60 71 75 79 82 79
4 16 12 30 45 55 67 72 75 73
-1 2 7 14 23 40 54 64 64 59
0 0 2 6 14 25 37 52 52 53
- 3 -1 -4 2 9 19 42 39 43
0 9.8e-4 .0098 .098 .98 9.8
Dabrafenib (uM)
Inhibition N=3

FIG. 25, Con't

G



H

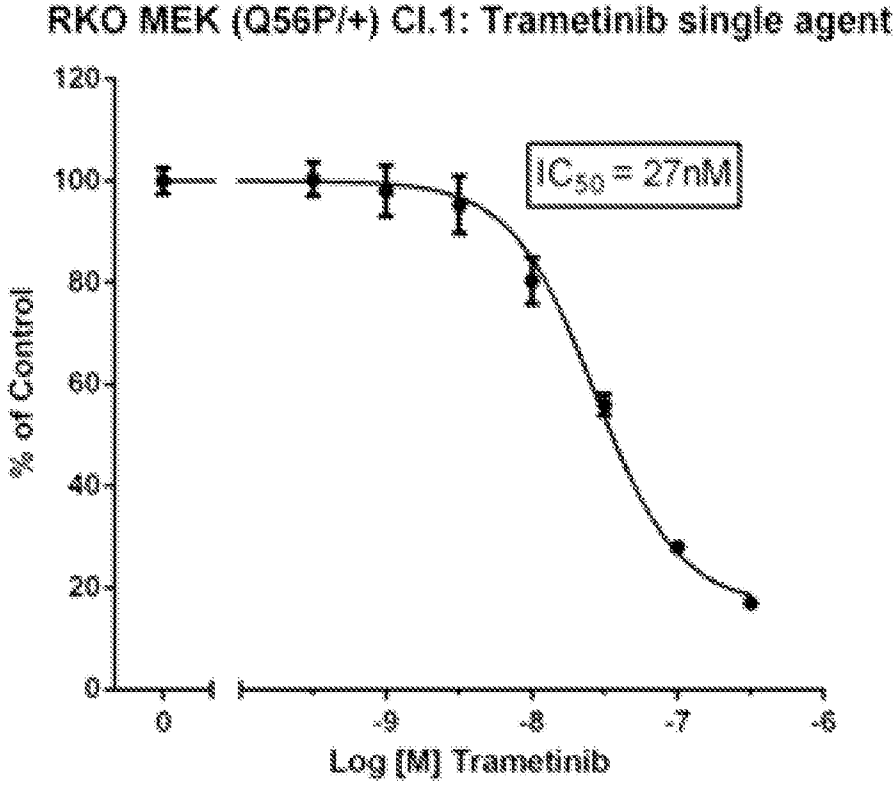


FIG. 25, Con't

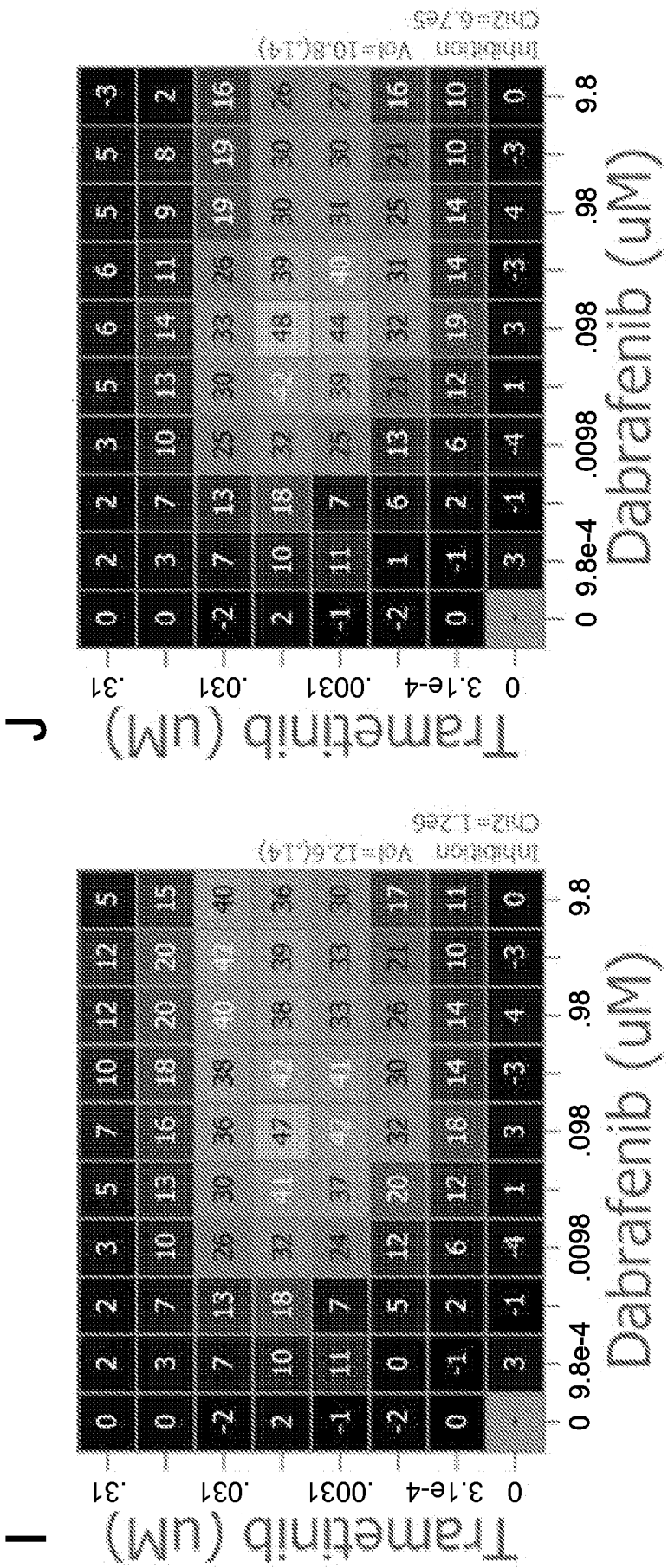

I
J
Trametinib (uM)
Dabrafenib (uM)
0
3.1e-4
.0031
.031
.31
0
9.8e-4
.0098
.098
.98
9.8
Inhibition Vol=12.6(.14)
Cn2=1.2e6
Inhibition Vol=10.8(.14)
Cn2=6.7e5

FIG. 25, Con't

K

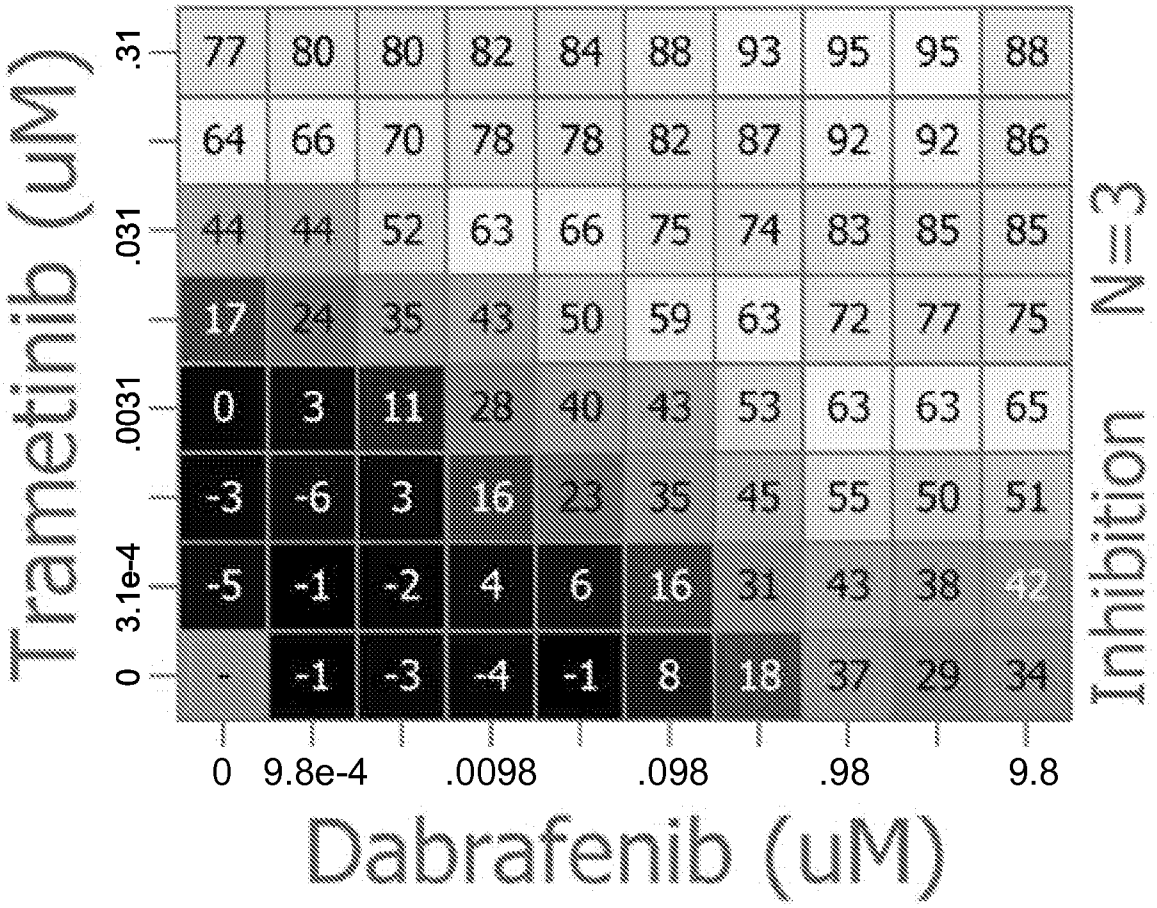
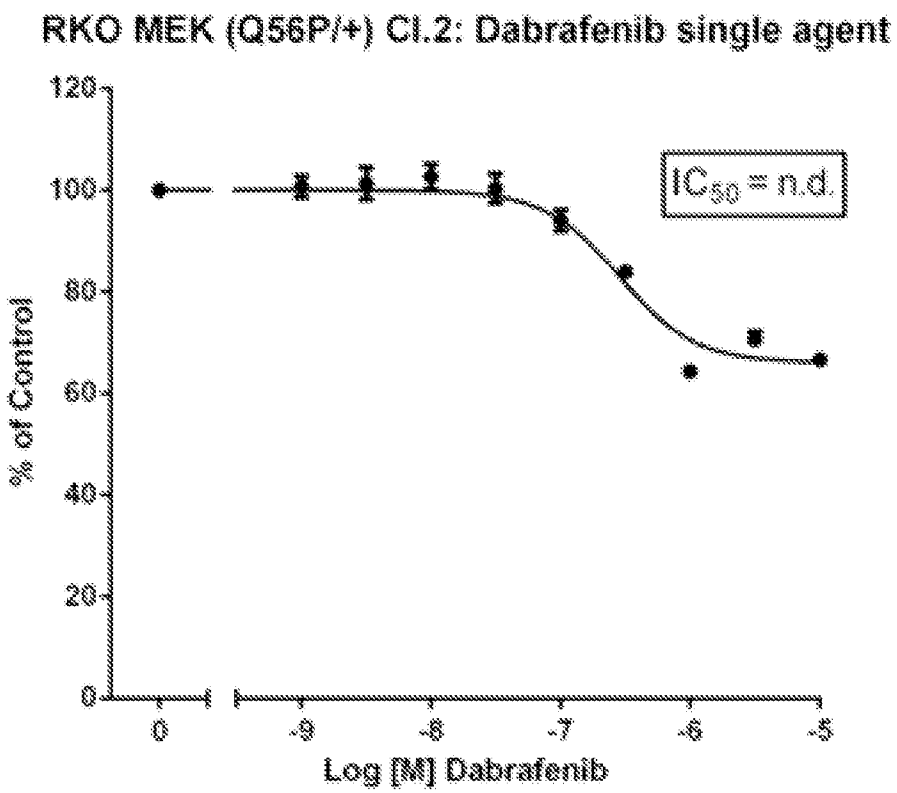

Trametinib (uM)
.31
.031
.0031
3.1e-4
0
77 80 80 82 84 88 93 95 95 88
64 66 70 78 78 82 87 92 92 86
44 44 52 63 66 75 74 83 85 85
17 24 35 43 50 59 63 72 77 75
0 3 11 28 40 43 53 63 63 65
-3 -6 3 16 23 35 45 55 50 51
-5 -1 -2 4 6 16 31 43 38 42
- -1 -3 -4 -1 8 18 37 29 34
0 9.8e-4 .0098 .098 .98 9.8
Dabrafenib (uM)
Inhibition N=3

FIG. 25, Con't

L



M

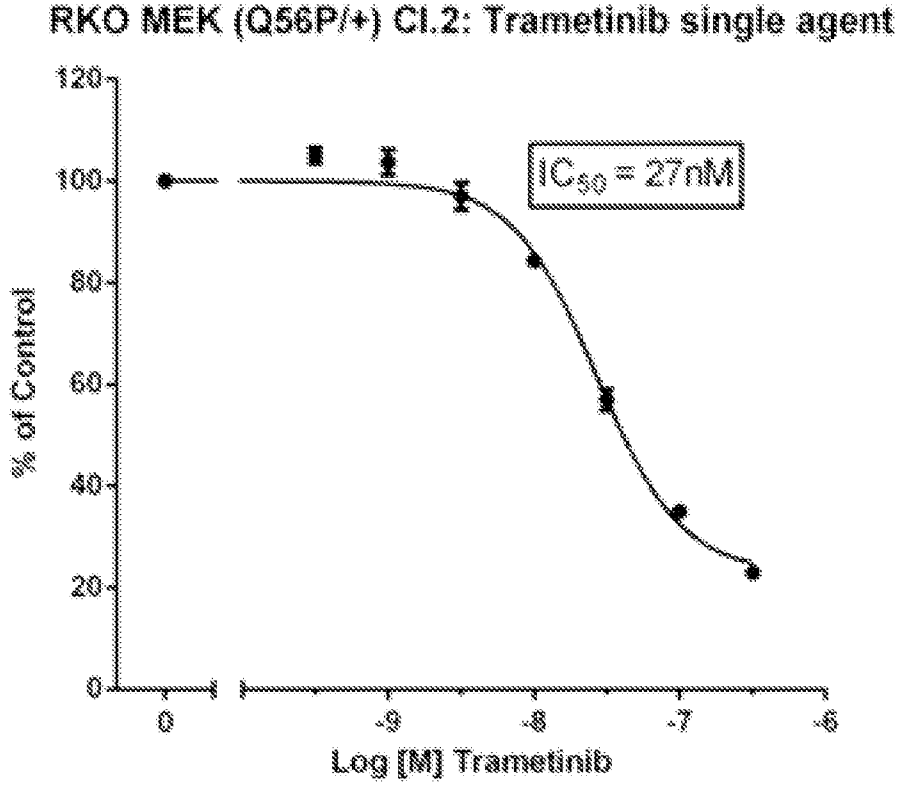


FIG. 25, Con't

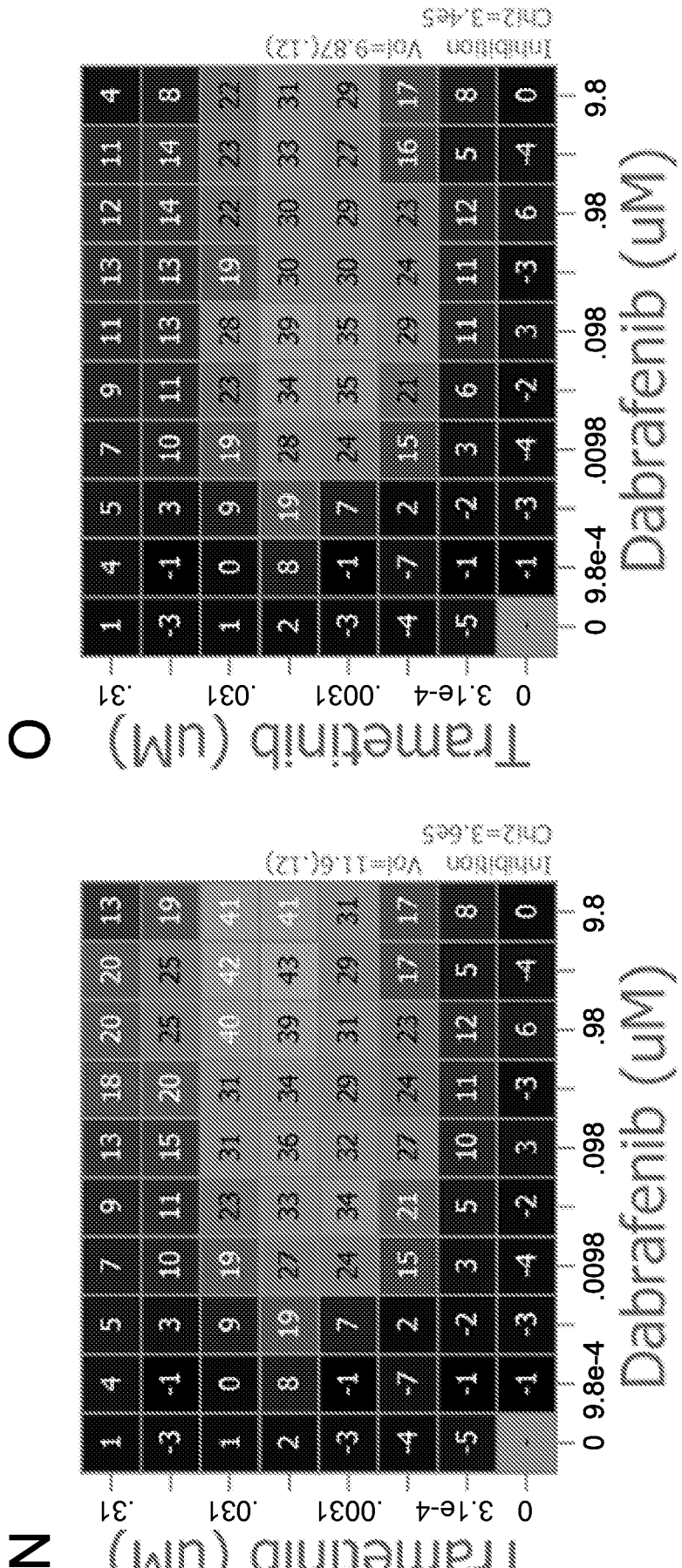

O
Trametinib (uM)
0 3.1e-4 .0031 .031 .31
Dabrafenib (uM)
0 9.8e-4 .0098 .098 .98 9.8
Inhibition Vol=9.87(.12)
CHI2=3.4e5
N
Trametinib (uM)
0 3.1e-4 .0031 .031 .31
Dabrafenib (uM)
0 9.8e-4 .0098 .098 .98 9.8
Inhibition Vol=11.6(.12)
CHI2=3.6e5

FIG. 26

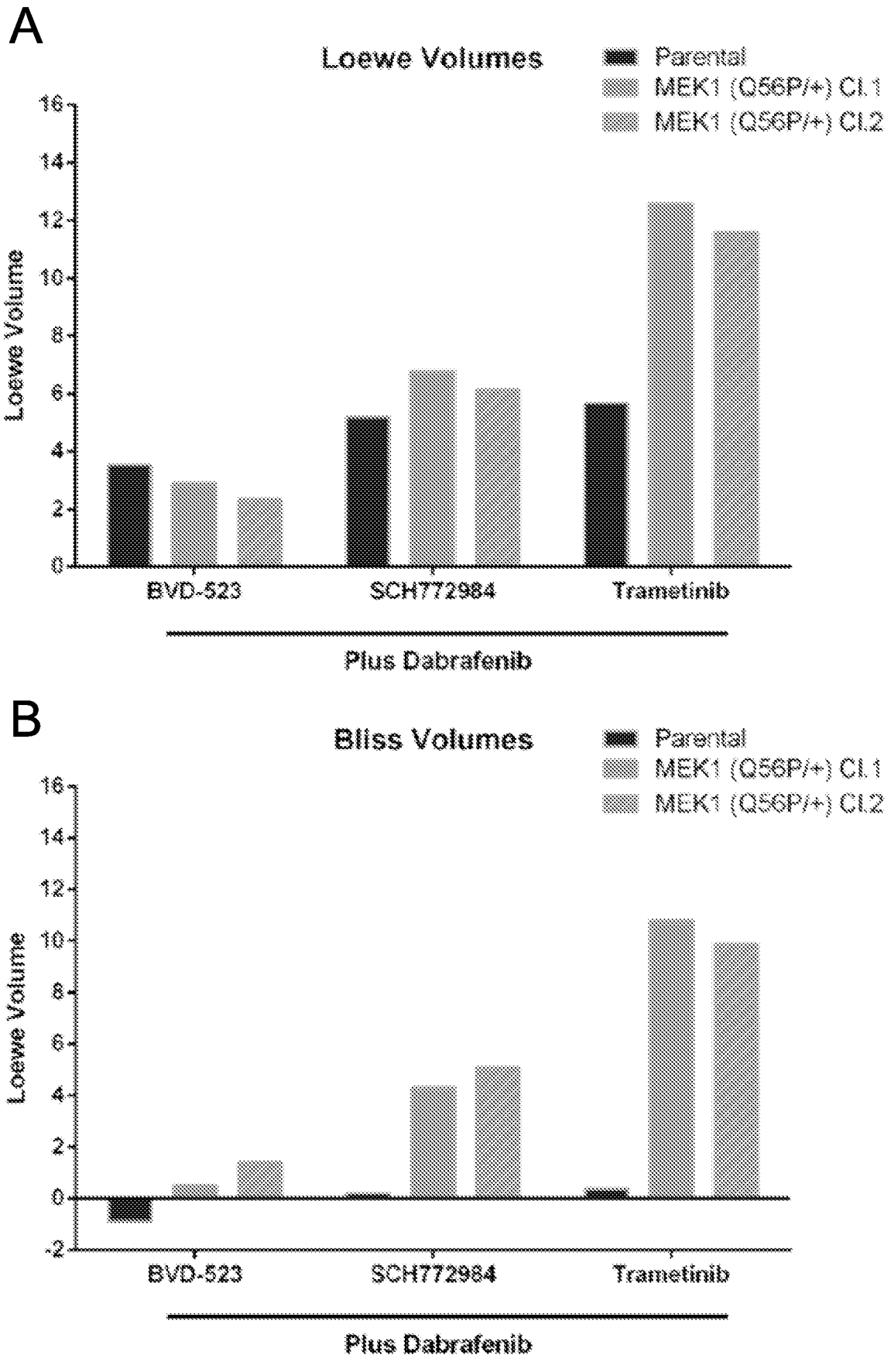

A
Loewe Volumes
Parental
MEK1 (Q56P/+) Cl.1
MEK1 (Q56P/+) Cl.2
Loewe Volume
16
14
12
10
8
6
4
2
0
BVD-523
SCH772984
Trametinib
Plus Dabrafenib
B
Bliss Volumes
Parental
MEK1 (Q56P/+) Cl.1
MEK1 (Q56P/+) Cl.2
Loewe Volume
16
14
12
10
8
6
4
2
0
-2
BVD-523
SCH772984
Trametinib
Plus Dabrafenib

FIG. 26, Con't

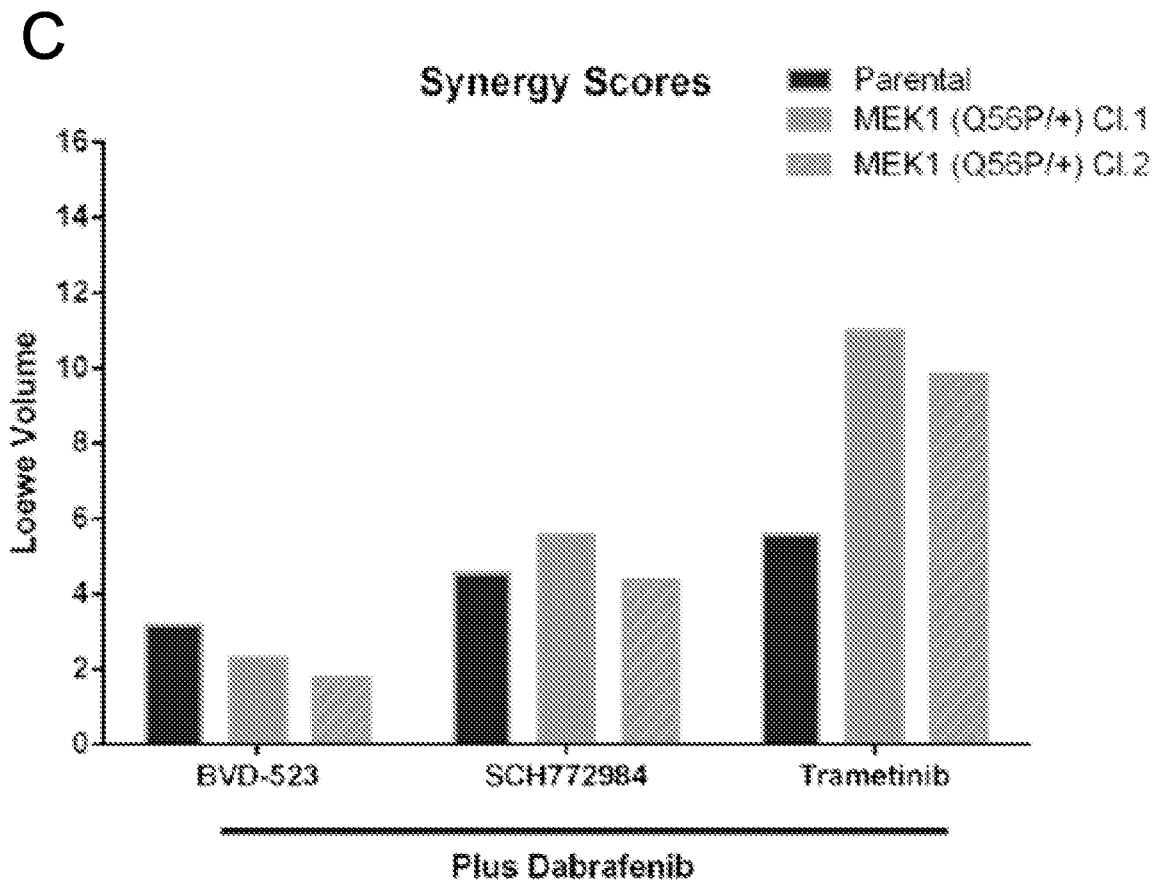

C
Synergy Scores
Parental
MEK1 (Q56P/+) Cl.1
MEK1 (Q56P/+) Cl.2
Loewe Volume
0
2
4
6
8
10
12
14
16
BVD-523
SCH772984
Trametinib
Plus Dabrafenib

FIG. 27

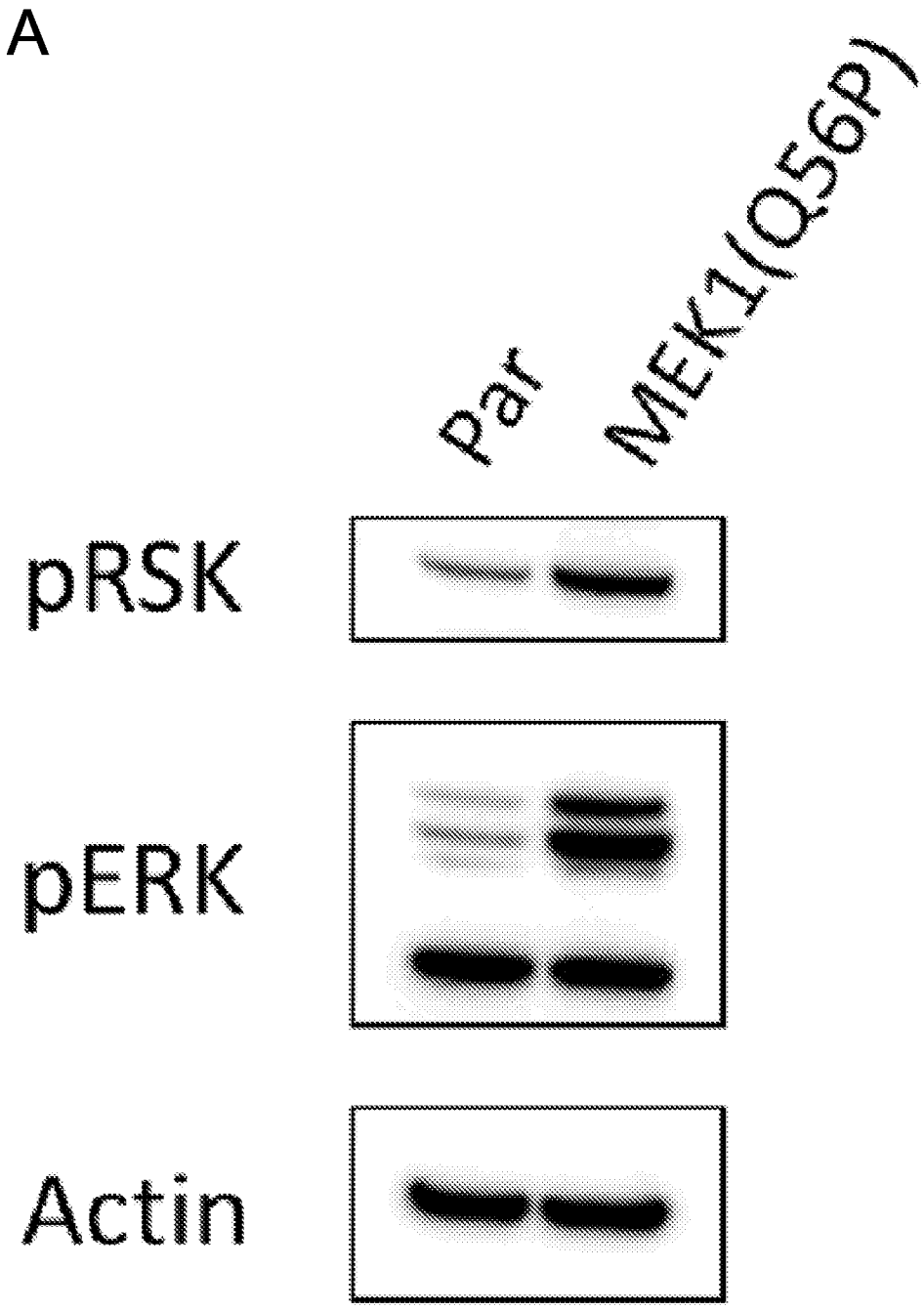


FIG. 27, Con't

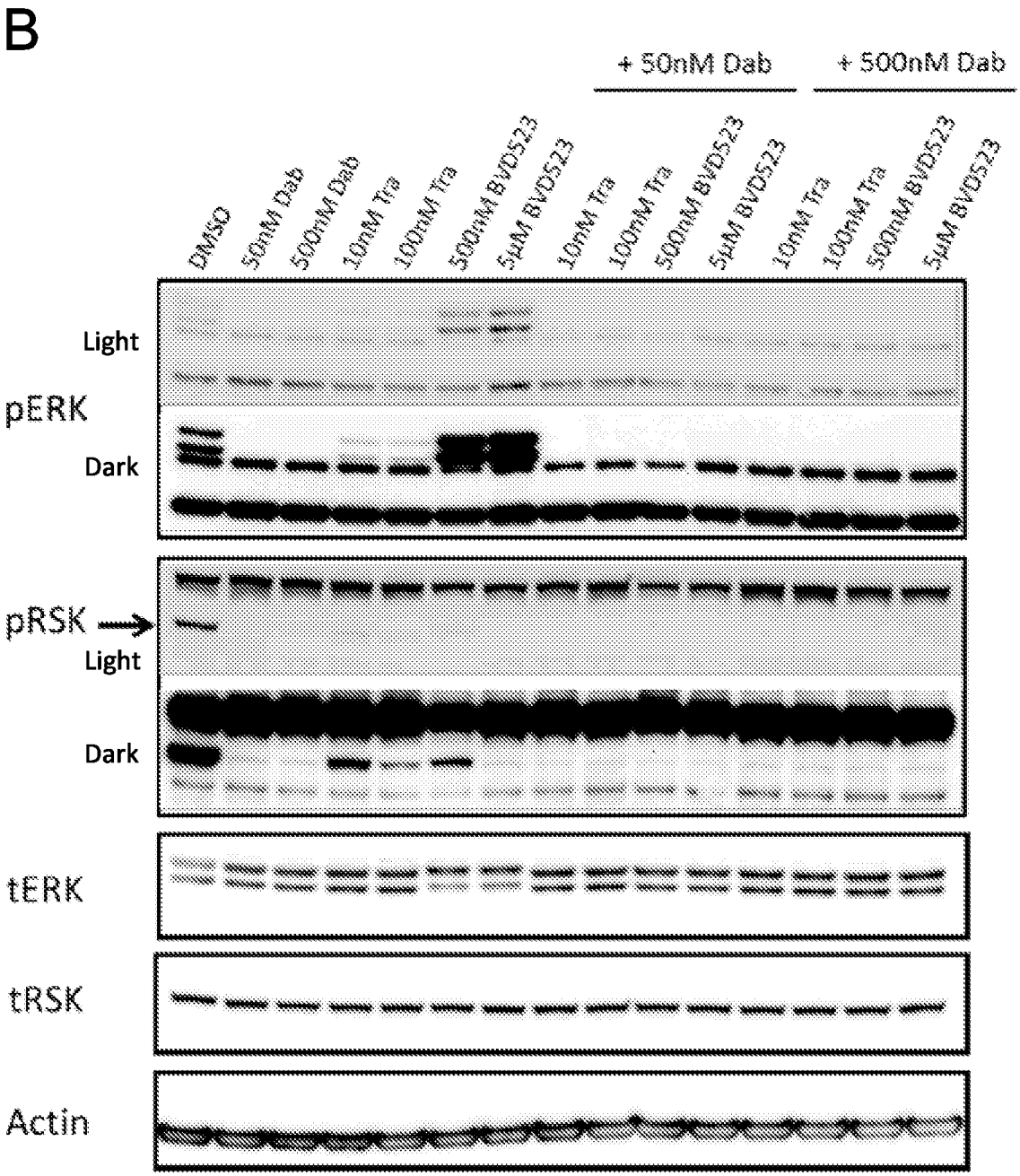

B
+ 50nM Dab
+ 500nM Dab
DMSO
50nM Dab
500nM Dab
10nM Tra
100nM Tra
500nM BVD523
5μM BVD523
10nM Tra
100nM Tra
500nM BVD523
5μM BVD523
10nM Tra
100nM Tra
500nM BVD523
5μM BVD523
Light
pERK
Dark
pRSK
Light
Dark
tERK
tRSK
Actin

FIG. 27, Con't

C

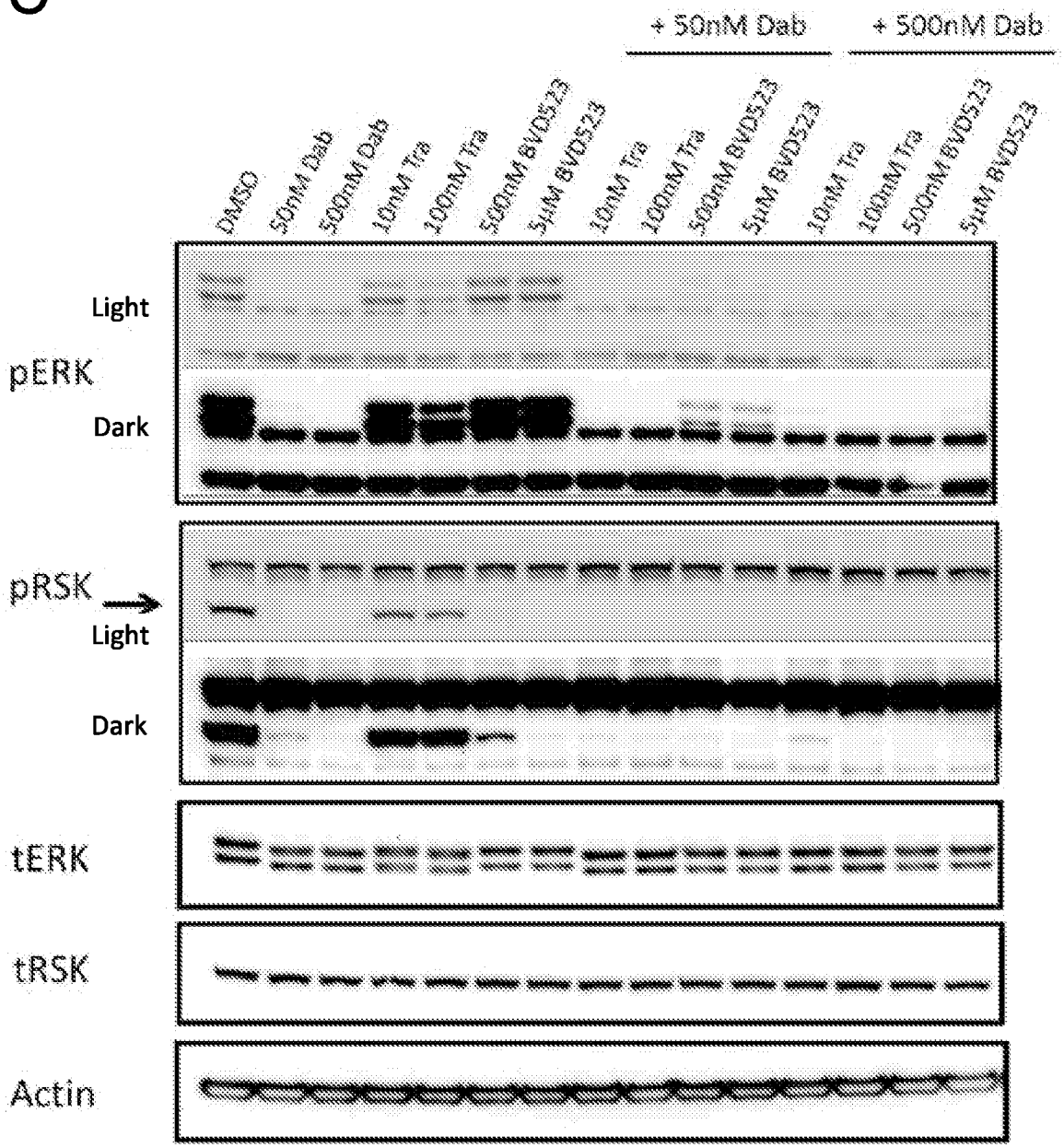

+ 50nM Dab
+ 500nM Dab
DMSO
50nM Dab
500nM Dab
10nM Tra
100nM Tra
500nM BVD523
5μM BVD523
10nM Tra
100nM Tra
500nM BVD523
5μM BVD523
10nM Tra
100nM Tra
500nM BVD523
5μM BVD523
Light
pERK
Dark
pRSK
Light
Dark
tERK
tRSK
Actin

FIG. 27, Con't

D

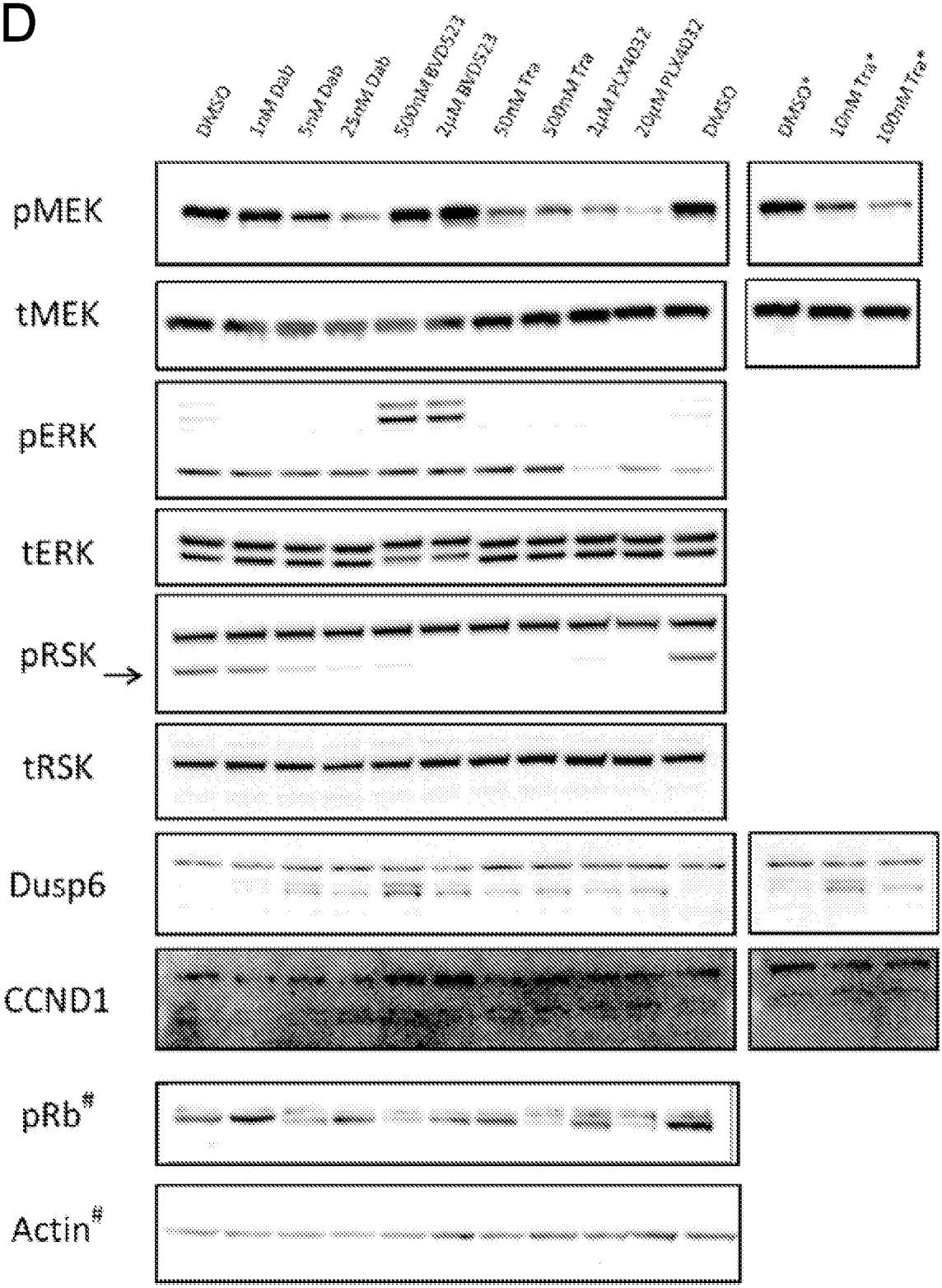

DMSO
1nM Dab
5nM Dab
25nM Dab
500nM BVD523
2μM BVD523
50nM Tra
500nM Tra
2μM PLX4032
20μM PLX4032
DMSO
DMSO*
10nM Tra*
100nM Tra*
pMEK
tMEK
pERK
tERK
pRSK
tRSK
Dusp6
CCND1
pRb#
Actin#

FIG. 27, Con't

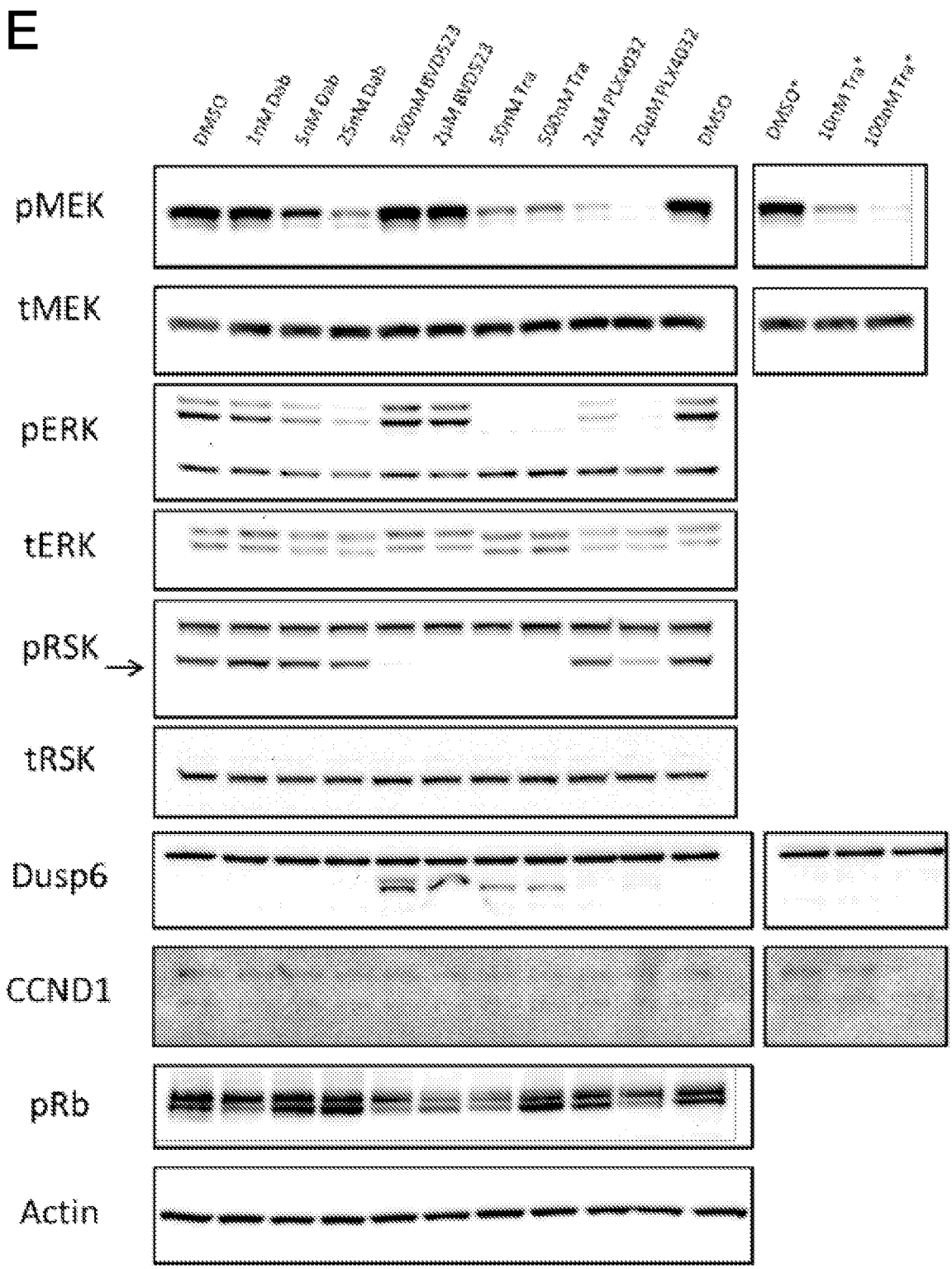

E
DMSO
1nM Dab
5nM Dab
25nM Dab
500nM BVD523
2μM BVD523
50nM Tra
500nM Tra
2μM PLX4032
20μM PLX4032
DMSO
DMSO*
10nM Tra*
100nM Tra*
pMEK
tMEK
pERK
tERK
pRSK
tRSK
Dusp6
CCND1
pRb
Actin

FIG. 27, Con't

F

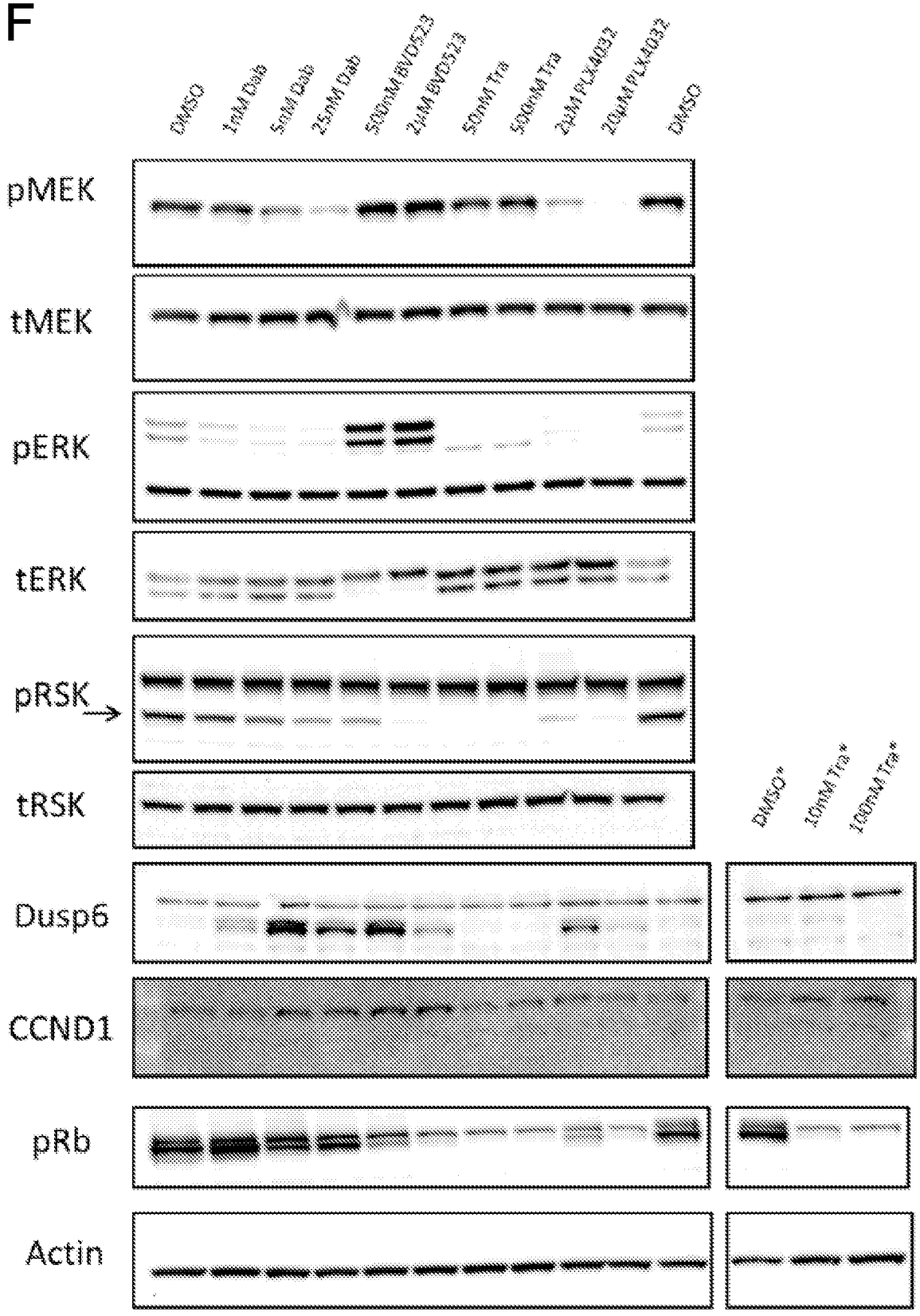

DMSO
1nM Dab
5nM Dab
25nM Dab
500nM BVD523
2μM BVD523
50nM Tra
500nM Tra
2μM PI3K4033
20μM PI3K4033
DMSO
pMEK
tMEK
pERK
tERK
pRSK
tRSK
DMSO*
10nM Tra*
100nM Tra*
Dusp6
CCND1
pRb
Actin

FIG. 27, Con't

G

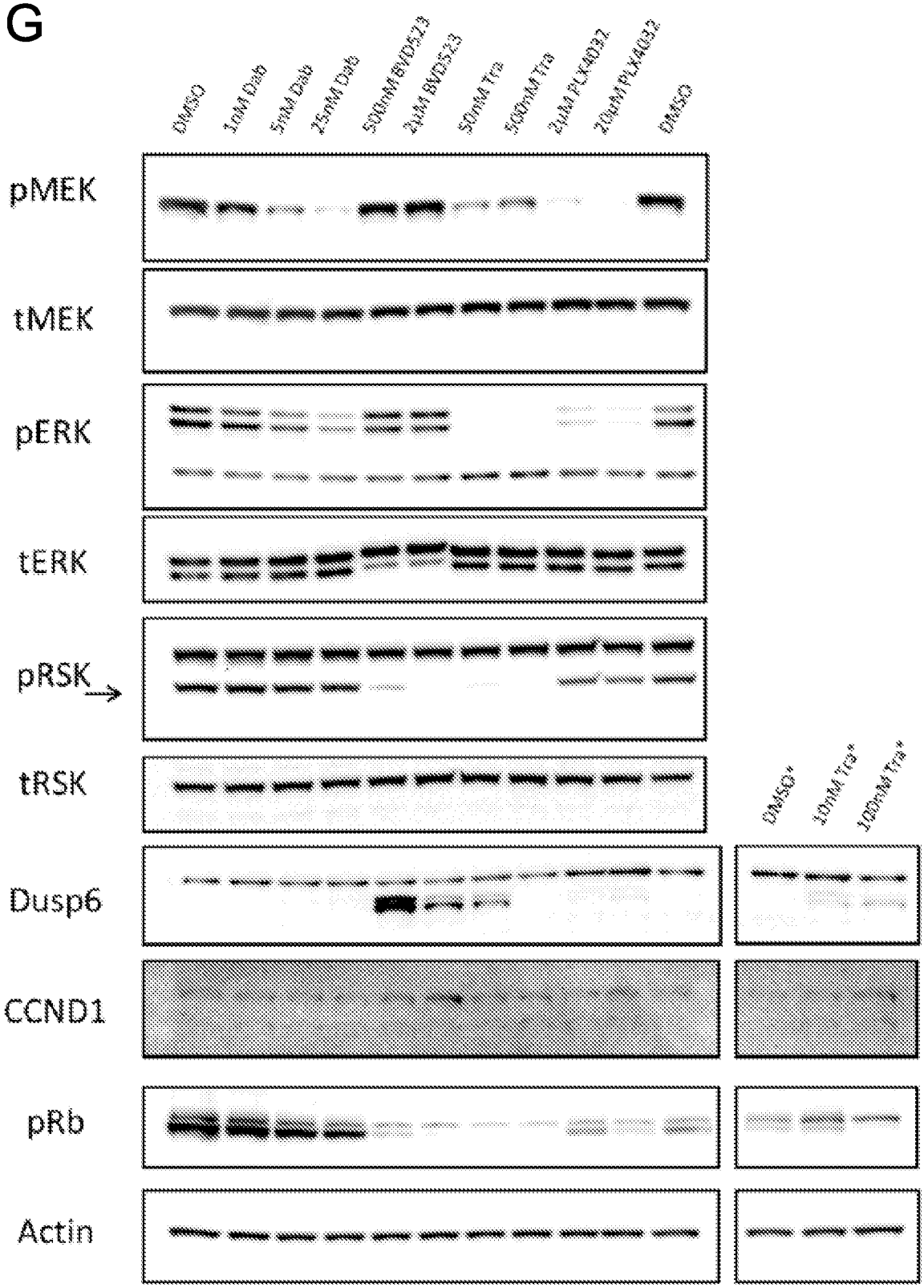

DMSO
1nM Dab
5nM Dab
25nM Dab
500nM BVD523
2uM BVD523
50nM Tra
500nM Tra
2uM PLK4032
20uM PLK4032
DMSO
pMEK
tMEK
pERK
tERK
pRSK
tRSK
DMSO*
10nM Tra*
100nM Tra*
Dusp6
CCND1
pRb
Actin

FIG. 27, Con't

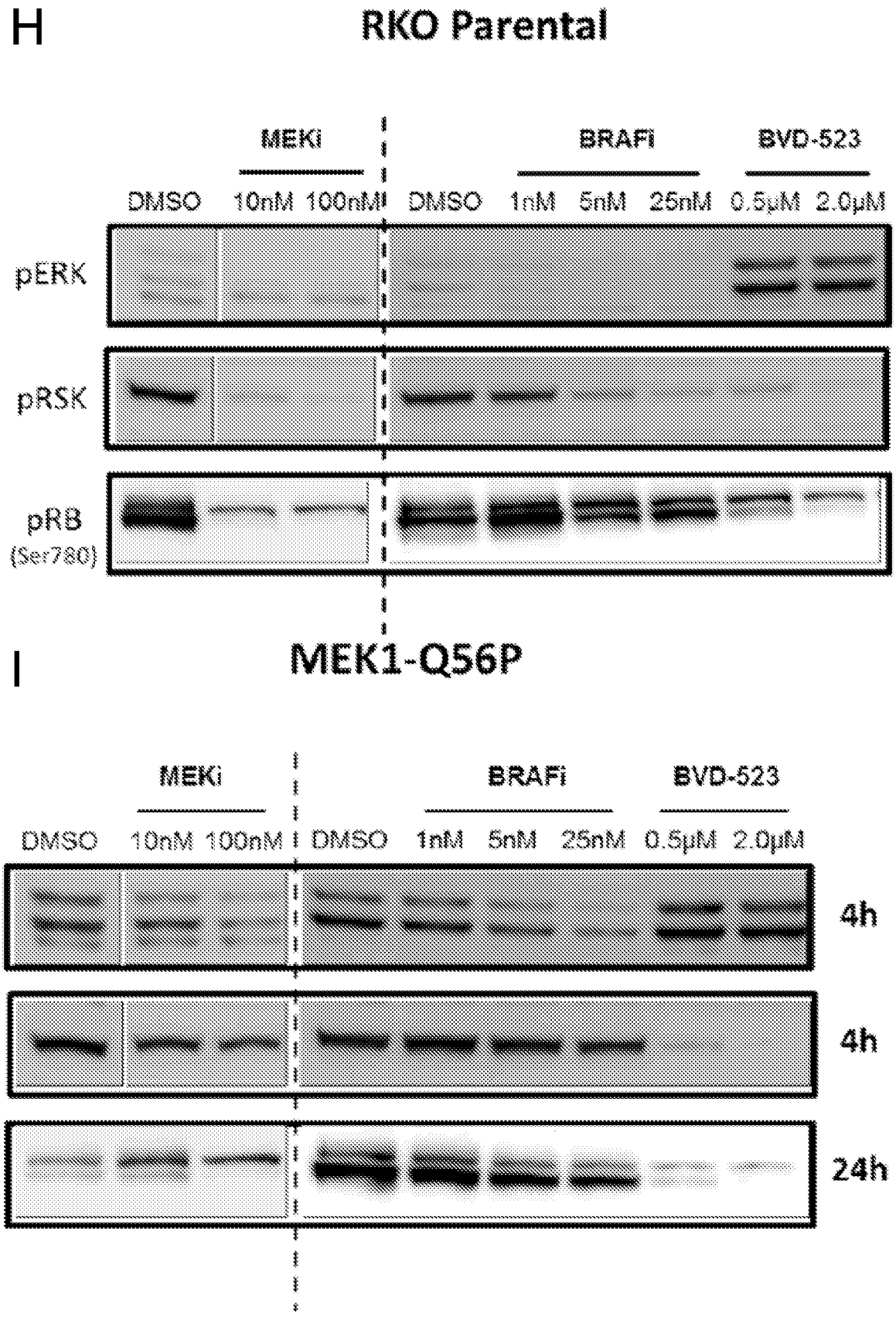
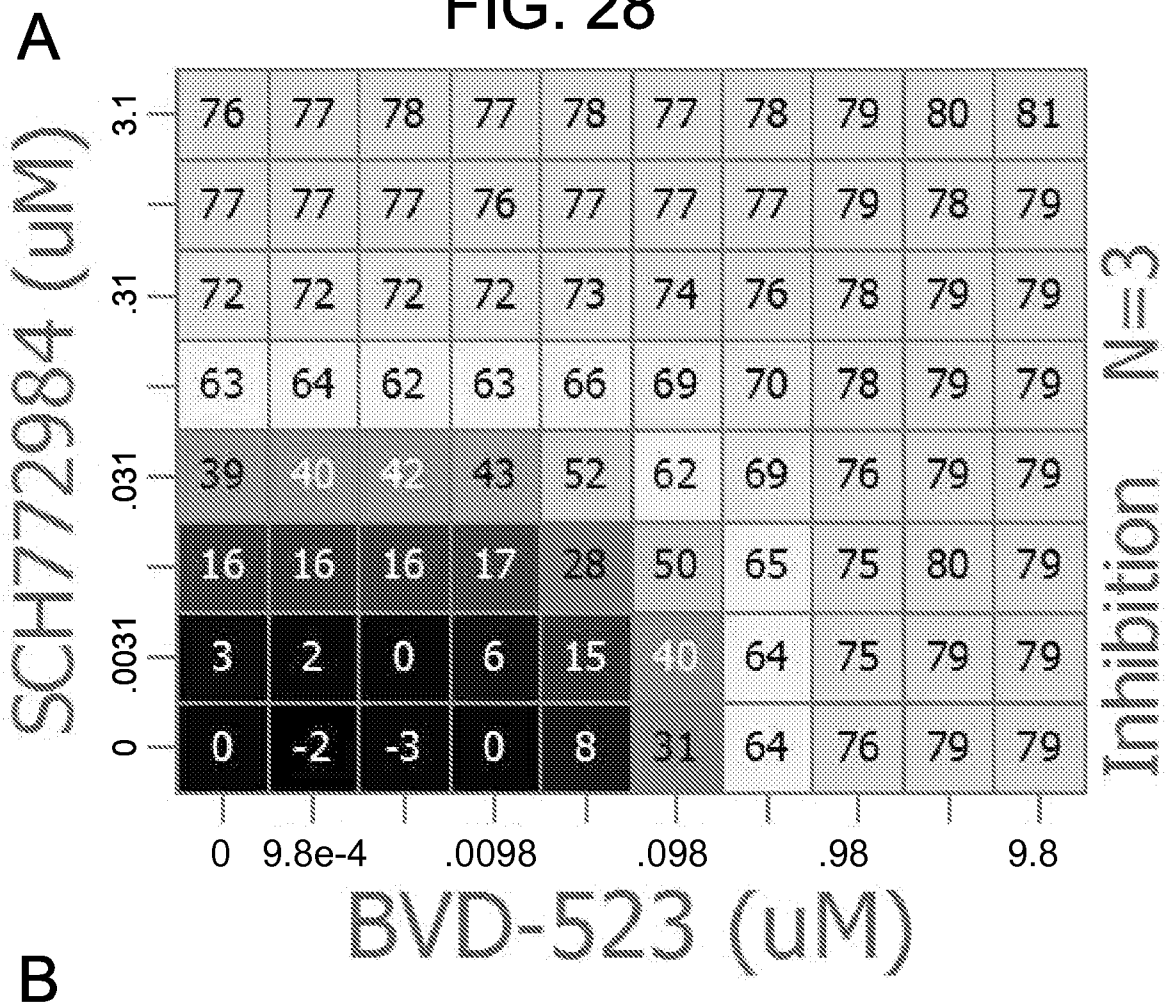
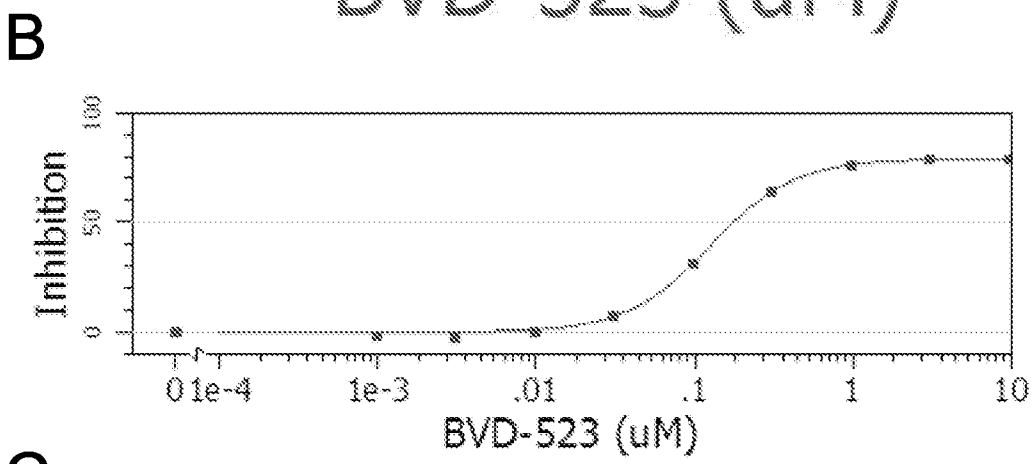

H
RKO Parental
MEKi
BRAFi
BVD-523
DMSO
10nM
100nM
DMSO
1nM
5nM
25nM
0.5μM
2.0μM
pERK
pRSK
pRB
(Ser780)
I
MEK1-Q56P
MEKi
BRAFi
BVD-523
DMSO
10nM
100nM
DMSO
1nM
5nM
25nM
0.5μM
2.0μM
4h
4h
24h

FIG. 28


A
SCH772984 (uM)
3.1
.31
.031
.0031
0
76 77 78 77 78 77 78 79 80 81
77 77 77 76 77 77 77 79 78 79
72 72 72 72 73 74 76 78 79 79
63 64 62 63 66 69 70 78 79 79
39 40 42 43 52 62 69 76 79 79
16 16 16 17 28 50 65 75 80 79
3 2 0 6 15 40 64 75 79 79
0 -2 -3 0 8 31 64 76 79 79
Inhibition N=3
0 9.8e-4 .0098 .098 .98 9.8
BVD-523 (uM)


B
Inhibition
100
50
0
0 1e-4 1e-3 .01 .1 1 10
BVD-523 (uM)

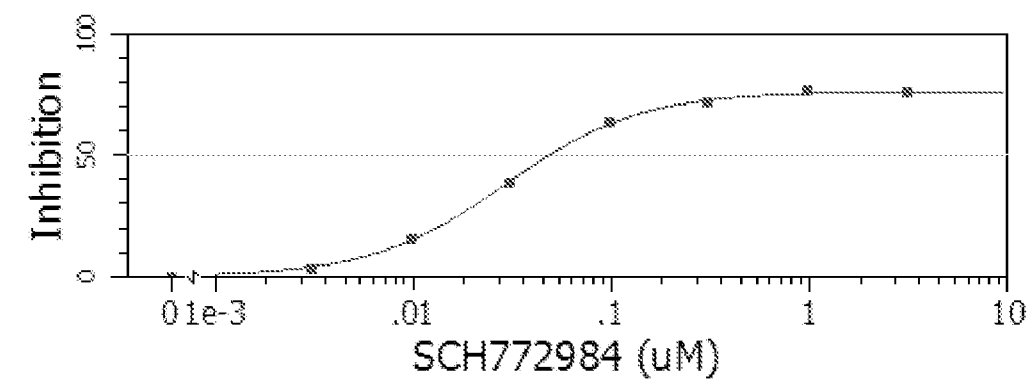
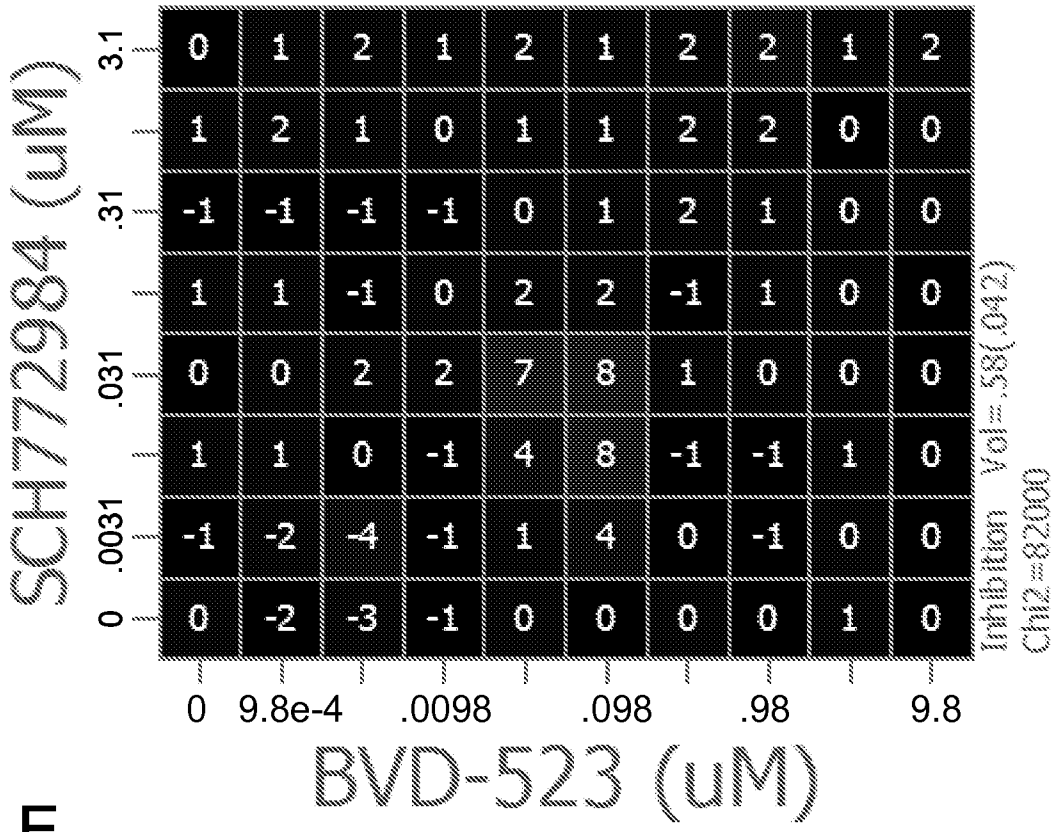

C
Inhibition
100
50
0
0 1e-3 .01 .1 1 10
SCH772984 (uM)

FIG. 28, Con't

D



E