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(54) Title: METHODS OF TREATING AND PREVENTING CANCER DRUG RESISTANCE

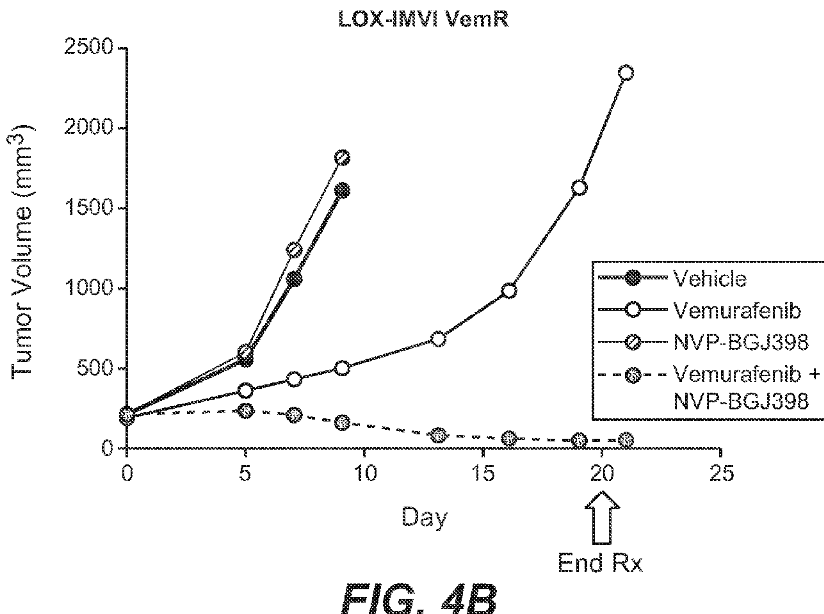

FIG. 4B

(57) Abstract: Provided herein are combination therapies for the treatment of pathological conditions, such as cancer, using an antagonist of FGFR signaling and a B-raf antagonist.

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METHODS OF TREATING AND PREVENTING CANCER DRUG RESISTANCE

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application No. 62/011,854, filed 13 June 2014, the disclosure of which is incorporated herein by reference in its entirety.

FIELD

[0002] Provided herein are combination therapies for the treatment of pathological conditions, such as cancer, using antagonists of FGFR signaling.

BACKGROUND

[0003] Cancer remains to be one of the most deadly threats to human health. In the U.S., cancer affects nearly 1.3 million new patients each year, and is the second leading cause of death after heart disease, accounting for approximately 1 in 4 deaths. For example, breast cancer is the second most common form of cancer and the second leading cancer killer among American women. It is also predicted that cancer may surpass cardiovascular diseases as the number one cause of death within 5 years. Solid tumors are responsible for most of those deaths. Although there have been significant advances in the medical treatment of certain cancers, the overall 5-year survival rate for all cancers has improved only by about 10% in the past 20 years. Cancers, or malignant tumors, metastasize and grow rapidly in an uncontrolled manner, making timely detection and treatment extremely difficult.

[0004] The relatively rapid acquisition of resistance to cancer drugs remains a key obstacle to successful cancer therapy. Substantial efforts to elucidate the molecular basis for such drug resistance have revealed a variety of mechanisms, including drug efflux, acquisition of drug binding-deficient mutants of the target, engagement of alternative survival pathways, epigenetic alterations). For example, RAF inhibitors are used to target malignant melanomas harboring B-raf V600E mutations; however, their clinical success is dampened by acquired resistance. Accordingly, new treatment methods are needed to successfully address heterogeneity within cancer cell populations and the emergence of cancer cells resistant to drug treatments.

SUMMARY

[0005] Provided herein are combination therapies using antagonists of FGFR signaling and antagonists of B-raf. In specific embodiments, the combination therapies use antagonists of FGFR1 signaling and antagonists of B-raf.

[0006] In particular, provided herein are methods of treating cancer in an individual comprising concomitantly administering to the individual (a) an antagonist of FGFR signaling and (b) a B-raf

antagonist. In some embodiments, the respective amounts of the antagonist of FGFR signaling and the B-raf antagonist are effective to increase the period of cancer sensitivity and/or delay the development of cancer resistance to the B-raf antagonist. In some embodiments, the respective amounts of the antagonist of FGFR signaling and the B-raf antagonist are effective to increase efficacy of a cancer treatment comprising a B-raf antagonist. For example, in some embodiments, the respective amounts of the antagonist of FGFR signaling and the B-raf antagonist are effective to increased efficacy compared to a standard treatment comprising administering an effective amount of B-raf antagonist without (in the absence of) the antagonist of FGFR signaling. In some embodiments, the respective amounts of the antagonist of FGFR signaling and the B-raf antagonist are effective to increased response (*e.g.*, complete response) compared to a standard treatment comprising administering an effective amount of the B-raf antagonist without (in the absence of) the antagonist of FGFR signaling. In some embodiments, the respective amounts of the antagonist of FGFR signaling and the B-raf antagonist are effective to increase cancer sensitivity and/or restore sensitivity to the B-raf antagonist.

[0007] Provided herein are also methods of treating a cancer cell, wherein the cancer cell is resistant to treatment with a B-raf antagonist in an individual comprising administering to the individual an effective amount of an antagonist of FGFR signaling and an effective amount of the B-raf antagonist. In addition, provided herein are methods of treating cancer resistant to a B-raf antagonist in an individual comprising administering to the individual an effective amount of an antagonist of FGFR signaling and an effective amount of the B-raf antagonist.

[0008] Provided herein are methods of increasing sensitivity and/or restoring sensitivity to a B-raf antagonist comprising administering to the individual an effective amount of an antagonist of FGFR signaling and an effective amount of the B-raf antagonist.

[0009] Also provided herein are methods of increasing efficacy of a cancer treatment comprising a B-raf antagonist in an individual comprises concomitantly administering to the individual (a) an effective amount of an antagonist of FGFR signaling and (b) an effective amount of the B-raf antagonist.

[0010] Provided herein are methods of treating cancer in an individual wherein the cancer treatment comprises concomitantly administering to the individual (a) an effective amount of an antagonist of FGFR signaling and (b) an effective amount of a B-raf antagonist, wherein the cancer treatment has increased efficacy compared to a standard treatment comprising administering an effective amount of the B-raf antagonist without (in the absence of) antagonist of FGFR signaling.

[0011] In addition, provided herein are methods of delaying and/or preventing development of cancer resistance to a B-raf antagonist in an individual, comprising concomitantly administering to the individual (a) an effective amount of an antagonist of FGFR signaling and (b) an effective amount of the B-raf antagonist.

[0012] Provided herein are methods of treating an individual with cancer who has increased likelihood of developing resistance to a B-raf antagonist comprising concomitantly administering to the individual (a) an effective amount of an antagonist of FGFR signaling and (b) an effective amount of the B-raf antagonist.

[0013] Further provided herein are methods of increasing sensitivity to a B-raf antagonist in an individual with cancer comprising concomitantly administering to the individual (a) an effective amount of an antagonist of FGFR signaling and (b) an effective amount of the B-raf antagonist.

[0014] Provided herein are also methods extending the period of sensitivity to a B-raf antagonist in an individual with cancer comprising concomitantly administering to the individual (a) an effective amount of an antagonist of FGFR signaling and (b) an effective amount of the B-raf antagonist.

[0015] Provided herein are methods of extending the duration of response to a B-raf antagonist in an individual with cancer comprising concomitantly administering to the individual (a) an effective amount of an antagonist of FGFR signaling and (b) an effective amount of the B-raf antagonist.

[0016] In some embodiments of any of the methods, the antagonist of FGFR signaling is an antibody inhibitor, a small molecule inhibitor, a binding polypeptide inhibitor, and/or a polynucleotide antagonist. In some embodiments, the antagonist of FGFR signaling is a binding polypeptide inhibitor. In some embodiments, the binding polypeptide inhibitor comprises a region of the extracellular domain of FGFR linked to a Fc domain (*e.g.*, a region of the extracellular domain of FGFR linked to an immoglobulin hinge and Fc domains). In some embodiments, the antagonist of FGFR signaling is an antagonist of FGFR1 signaling. In some embodiments, the antagonist of FGFR signaling is an antagonist of FGFR2 signaling. In some embodiments, the antagonist of FGFR signaling is an antagonist of FGFR3 signaling. In some embodiments, the antagonist of FGFR signaling is an antagonist of FGFR4 signaling. In some embodiments, the antagonist of FGFR signaling is a small molecule. In some embodiments, the antagonist of FGFR signaling is an antibody.

[0017] In some embodiments, the antagonist of FGFR1 signaling only binds to and/or inhibits FGFR1.

[0018] In some embodiments, the antagonist of FGFR1 signaling binds to and/or inhibits one or more of FGFR1b, FGFR1c, FGF1, FGF2, FGF3, FGF4, FGF5, FGF6, and FGF10. In some embodiments, the small molecule is N-[2-[[4-(diethylamino)butyl]amino]-6-(3,5-dimethoxyphenyl)pyrido[2,3-d]pyrimidin-7-yl]-N'-(1,1-dimethylethyl)-urea or pharmaceutically acceptable salt thereof. In some embodiments, the small molecule is BGJ398 (Novartis), AZD4547 (AstraZeneca), and/or FF284 (Chugai/Debiopharm (Debio 1347). In some embodiments, the antagonist of FGFR1 signaling is an anti-FGF2 antibody. In some embodiments, the antagonist of FGFR1 signaling is an anti-FGFR1 antibody. In some embodiments, the antagonist of FGFR1 signaling is an anti-FGFR1-IIIb antibody. In some embodiments, the antagonist of FGFR1 signaling is an anti-FGFR1-IIIc antibody. In some embodiments the antagonist of FGFR signaling is an anti-FGFR antibody capable of binding more than one FGFR polypeptide.

[0019] In some embodiments, the B-raf antagonist is one or more of sorafenib, PLX4720, PLX-3603, GSK2118436, GDC-0879, N-(3-(5-(4-chlorophenyl)-1H-pyrrolo[2,3-b]pyridine-3-carbonyl)-2,4-difluorophenyl)propane-1-sulfonamide, vemurafenib, GSK 2118436, RAF265 (Novartis), XL281, ARQ736, BAY73-4506. In further embodiments, the B-raf antagonist is vemurafenib. In further embodiments, the B-raf antagonist is GSK 2118436. The B-raf antagonist may be selective for B-raf V600E.

[0020] In some embodiments, the patient's cancer has been shown to express B-raf biomarker. B-raf biomarker may be mutant B-raf. Mutant B-raf is constitutively activated B-raf. In some embodiments, mutant B-raf is B-raf V600. B-raf V600 may be B-raf V600E. A non-limiting exemplary list of mutant B-raf is: B-raf V600K (GTG>AAG), V600R (GTG>AGG), V600E (GTG>GAA) and/or V600D (GTG>GAT). In some embodiments, mutant B-raf polypeptide is detected. In some embodiment, mutant B-raf nucleic acid is detected. "V600E" refers to a mutation in B-RAF (T>A) at nucleotide position 1799 that results in substitution of a glutamine for a valine at amino acid position 600 of B-raf. "V600E" is also known as "V599E" (1796T>A) under a previous numbering system (Kumar et al., Clin. Cancer Res. 9:3362-3368, 2003).

[0021] In specific embodiments, provided herein are methods of treating cancer in an individual comprising concomitantly administering to the individual (a) an FGFR1 antagonist and (b) a B-raf antagonist. In some embodiments, the respective amounts of the FGFR1 antagonist and the B-raf antagonist are effective to increase the period of cancer sensitivity and/or delay the development of cancer resistance to the B-raf antagonist. In some embodiments, the respective amounts of the FGFR1 antagonist and the B-raf antagonist are effective to increase efficacy of a cancer treatment comprising a B-raf antagonist. For example, in some embodiments, the

respective amounts of the FGFR1 antagonist and the B-raf antagonist are effective to increased efficacy compared to a standard treatment comprising administering an effective amount of B-raf antagonist without (in the absence of) the antagonist of FGFR signaling. In some embodiments, the respective amounts of the FGFR1 antagonist and the B-raf antagonist are effective to increased response (*e.g.*, complete response) compared to a standard treatment comprising administering an effective amount of the B-raf antagonist without (in the absence of) the antagonist of FGFR signaling. In some embodiments, the respective amounts of the FGFR1 antagonist and the B-raf antagonist are effective to increase cancer sensitivity and/or restore sensitivity to the B-raf antagonist.

[0022] In specific embodiments, provided herein are also methods of treating a cancer cell, wherein the cancer cell is resistant to treatment with a B-raf antagonist in an individual comprising administering to the individual an effective amount of an FGFR1 antagonist and an effective amount of the B-raf antagonist. In addition, provided herein are methods of treating cancer resistant to a B-raf antagonist in an individual comprising administering to the individual an effective amount of an FGFR1 antagonist and an effective amount of the B-raf antagonist.

[0023] In specific embodiments, provided herein are methods of increasing sensitivity and/or restoring sensitivity to a B-raf antagonist comprising administering to the individual an effective amount of an FGFR1 antagonist and an effective amount of the B-raf antagonist.

[0024] In specific embodiments, provided herein are methods of increasing efficacy of a cancer treatment comprising a B-raf antagonist in an individual comprises concomitantly administering to the individual (a) an effective amount of an FGFR1 antagonist and (b) an effective amount of the B-raf antagonist.

[0025] Provided herein are methods of treating cancer in an individual wherein the cancer treatment comprises concomitantly administering to the individual (a) an effective amount of an antagonist of FGFR1 signaling and (b) an effective amount of a B-raf antagonist, wherein the cancer treatment has increased efficacy compared to a standard treatment comprising administering an effective amount of the B-raf antagonist without (in the absence of) antagonist of FGFR signaling.

[0026] In specific embodiments, provided herein are methods of delaying and/or preventing development of cancer resistance to a B-raf antagonist in an individual, comprising concomitantly administering to the individual (a) an effective amount of an antagonist of FGFR1 signaling and (b) an effective amount of the B-raf antagonist.

[0027] In specific embodiments, provided herein are methods of treating an individual with cancer who has increased likelihood of developing resistance to a B-raf antagonist comprising

concomitantly administering to the individual (a) an effective amount of an antagonist of FGFR1 signaling and (b) an effective amount of the B-raf antagonist.

[0028] In specific embodiments, provided herein are methods of increasing sensitivity to a B-raf antagonist in an individual with cancer comprising concomitantly administering to the individual (a) an effective amount of an antagonist of FGFR1 signaling and (b) an effective amount of the B-raf antagonist.

[0029] In specific embodiments, provided herein are also methods extending the period of sensitivity to a B-raf antagonist in an individual with cancer comprising concomitantly administering to the individual (a) an effective amount of an antagonist of FGFR1 signaling and (b) an effective amount of the B-raf antagonist.

[0030] In specific embodiments, provided herein are methods of extending the duration of response to a B-raf antagonist in an individual with cancer comprising concomitantly administering to the individual (a) an effective amount of an antagonist of FGFR signaling and (b) an effective amount of the B-raf antagonist.

[0031] In some embodiments, the B-raf antagonist is one or more of sorafenib, PLX4720, PLX-3603, GSK2118436, GDC-0879, N-(3-(5-(4-chlorophenyl)-1H-pyrrolo[2,3-b]pyridine-3-carbonyl)-2,4-difluorophenyl)propane-1-sulfonamide, vemurafenib, GSK 2118436, RAF265 (Novartis), XL281, ARQ736, BAY73-4506. In further embodiments, the B-raf antagonist is vemurafenib. In further embodiments, the B-raf antagonist is GSK 2118436. The B-raf antagonist may be selective for B-raf V600E.

[0032] In specific embodiments of any of the methods, the B-raf antagonist is vemurafenib (Daiichi Sankyo).

[0033] In specific embodiments of any of the methods, the antagonist of FGFR1 signaling is an antibody inhibitor, a small molecule inhibitor, a binding polypeptide inhibitor, and/or a polynucleotide antagonist. In some embodiments, the antagonist of FGFR1 signaling is a binding polypeptide inhibitor. In some embodiments, the binding polypeptide inhibitor comprises a region of the extracellular domain of FGFR1 linked to a Fc domain (*e.g.*, a region of the extracellular domain of FGFR1 linked to an immoglobulin hinge and Fc domains). In some embodiments, the antagonist of FGFR1 signaling is a small molecule. In some embodiments, the antagonist of FGFR1 signaling is an antibody.

[0034] In specific embodiments, the antagonist of FGFR1 signaling binds to and/or inhibits one or more of FGFR1b, FGFR1c, FGF1, FGF2, FGF3, FGF4, FGF5, FGF6, and FGF10. In some embodiments, the small molecule is N-[2-[[4-(diethylamino)butyl]amino]-6-(3,5-dimethoxyphenyl)pyrido[2,3-d]pyrimidin-7-yl]-N'-(1,1-dimethylethyl)-urea or pharmaceutically

acceptable salt thereof. In some embodiments, the small molecule is BGJ398 (Novartis), AZD4547 (AstraZeneca), and/or FF284 (Chugai/Debiopharm (Debio 1347).

[0035] In specific embodiments, the antagonist of FGFR1 signaling is an anti-FGFR1 antibody.

[0036] In some embodiments, the antagonist of FGFR1 signaling only binds to and/or inhibits FGFR1.

[0037] In some embodiments, the antagonist of FGFR1 signaling is an anti-FGFR1-IIIb antibody. In some embodiments, the antagonist of FGFR1 signaling is an anti-FGFR1-IIIc antibody. In some embodiments the antagonist of FGFR1 signaling is an anti-FGFR1 antibody capable of binding more than one FGFR polypeptide. In some embodiments the antagonist of FGFR signaling is an anti-FGFR1 antibody that specifically binds FGFR1 and does not bind any other FGFR polypeptide.

[0038] The B-raf antagonist and the antagonist of FGFR signalling may be administered simultaneously. The B-raf antagonist and the antagonist of FGFR signalling may be administered sequentially. In some embodiments, the B-raf antagonist is administered prior to the antagonist of FGFR signalling. In some embodiments, the antagonist of FGFR signalling is administered prior to the B-raf antagonist.

[0039] In some embodiments of any of the methods, the cancer is lung cancer. In some embodiments, the lung cancer is NSCLC. In some embodiments, the cancer is breast cancer. In some embodiments, the cancer is HER2+ breast cancer. In some embodiments, the cancer has undergone epithelial-mesenchymal transition.

BRIEF DESCRIPTION OF THE FIGURES

[0040] Figure 1A-D. | Factors secreted by tumor cells and/or the tumor microenvironment contribute to drug resistance through activation of cell-surface receptors. A, A screen of 447 secreted factors across ten melanoma cell lines revealed FGFs, HGF, NRG1 and EGFs contribute towards resistance to B-raf and MEK antagonists. B, As shown in a melanoma cell line, FGF2, HGF and NRG1 rescued the tumor cells from resistance to B-raf- and MEK antagonists most broadly and potently (R = 50-100% rescue; PR = 25-50% rescue). C, Small molecule inhibitors targeting Met, FGFR and ERBB receptors show that ligand-mediated resistance is specific to the cognate receptor. D, Secreted growth factors which promote resistance to PLX4032 reactivate MAPK and PI3K pathways. Activation of MAPK by FGF2, MAPK and AKT by HGF and AKT by NRG1 and SCF are shown in 624 MEL cells in the presence of PLX4032. Treatments were 5μM PLX4032 for 24 hours and 50ng.mL FGF2, HGF, NRGB1, or SCF for 10 minutes.

[0041] Figure 2A-E. | A cell line ("LOX-IMVI VemR") was engineered to be resistant to vemurafienib. A, An image of an immunoblot of 11 cell lines probed for FGFR1 expression. B,

The LOX-IMVI VemR cell line is not affected by 5μM vemurafenib (*i.e.*, "PLX") as shown in the DMSO plot; however, the cell line is affected by 5μM vemurafenib in combination with an antagonist of FGFR signalling (*i.e.*, BGJ398, PD173074, and AP24534). Thus, the LOX-IMVI VemR cell line were found to be resensitized to vemurafenib by inhibiting FGFRs. C, A plot of the pg/mL of FGFR2 in the parental LOX-IMVI cell line compared to the vemurafenib resistant LOX-IMVI VemR cell line shows that the LOX-IMVI VemR cell line is characterized by an increased secretion of FGF2. D and E, RNAi knockdown and 5μM PLX4032 screening suggests that the vemurafenib resistance of the LOX-IMVI VemR cell line is FGFR1-dependent and driven by FGFR1/FGF2.

[0042] Figure 3A-B. | FGFR-inhibition prevents Vem-resistant cell outgrowth. A, An *in vitro* study showed the synergistic effect of vemurafenib (PLX4032) and an antagonist of FGFR signalling (BGJ398) on three (3) cancer cell lines. The LOX-IMVI VemR cells show a minimal response to treatment with vemurafenib and BGJ398 alone but a high response to a combination treatment of vemurafenib and BGJ398. The SK-MEL-3 and SK-MEL-24 cell lines show an augmented response to PLX4032 when combined with BGJ980. B, Expression patterns of select proteins are shown on West Blots in the presence of vemurafenib (PLX4032), an antagonist of FGFR signalling (NV-BGJ398), and/or FGF2.

[0043] Figure 4A-C. | The LOX-IMVI VemR cell line has FGFR-mediated vemurafenib resistance *in vivo*. A, LOX-IMVI cells (parental cell line) are sensitive to vemurafenib. B, A combination of vemurafenib with NVP-BGJ398 shows potent efficacy in the vemurafenib resistant LOX-IMVI VemR tumors. C, Re-emergence of LOX-IMVI (originally vemurafenib sensitive) tumors following the end of treatment with NVP-BGJ398 can be prevented by co-targeting FGFRs and B-raf (*i.e.*, co-treatment with BGJ398 and vemurafenib).

[0044] Figure 5A-D. | FGFR1 mediates FGF2 rescue in melanoma. A, siRNA knock down of FGFR subtypes in the 624 MEL cell line. B, A chart showing the defect of siRNA targeting FGFR1, FGFR2, FGFR3, FGFR4, FGFR1/4, and FGFR2/3 in seven cell lines. C, FGFR1 expression is increased in melanoma (n=49) with the V600E B-raf mutation. D, FGFR1 is increased in TCGA melanoma samples (n=247) of unknown B-raf mutations.

[0045] Figure 6 A-B. | FGFR1 mRNA levels correlate with FGF2 rescue in melanoma.

[0046] Figure 7 A-D. | Reactivation of MEK/ERK downstream of B-raf is a core mechanism of resistance in B-raf-mutant melanomas. A, MAPK signalling is required for FGF2-mediated resistance as shown by immunoblots. Reactivation of MAPK signalling is a common feature of RTK-mediated resistance as indicated the immunoblot wherein FGF2-mediated rescue activates MEK and ERK in the presence of PLX4032 (vemurafenib). B, Immunoblot showing the

activation of RAF1 (C-raf) suggests addition RAF-family members may mediate MAPK reactivation. C, A synthetic lethal chemical screen was utilized to identify signalling pathways mediating resistance to PLX4032 in 12 acquired-resistance melanoma cell lines. The table shows changes in sensitivity to PLX4032 when co-treated with inhibitors of MEK and ERK indicating a reactivation of the pathway downstream of B-raf. D, Examples of the synthetic lethal chemical screen shown in Figure 7C on specific cell lines.

[0047] Figure 8 A-C. | Activation of PI3K represents an alternative mechanism of B-raf-mutant melanomas. A, A synthetic lethal chemical screen identified PI3K-dependent resistance to PLX4032. B and C, 624 melanoma cells made resistant to PLX4032 ("634 mel VemR") showed activation of MET (phosphorylation) and showed an increase in pAKT when treated with PLX4032 (vemurafenib). Co-treatment with a MET inhibitor was needed to growth arrest the 624 ml VemR cells in the presence of PLX4032. Similar reliance on PI3K signalling was observed in G361 cells (data not shown).

[0048] Figure 9 A-C. | Pro-survival mechanisms, independent of MAPK and PI3K promote drug resistance in B-RAF mutant melanomas. A and B, A small molecule screen identified SRC family activation in COLO800 and UACC-62 cells. Cell lines which exhibited a SRC-dependent resistence were also re-sensitized by inhibition of PI3K signalling. C, BCL-XL and BCL-2, members of the anti-apoptotic pathway, were identified. As shown in the graphs, G-361 cells that have an acquired resistance to PLX4032 were resistant to the BCL-XL and BCL-2 inhibitors but a variant of the G-361 cell line that is resistant to PLX4032 and MEKi (GDC-0973) are sensitive to the BCL-XL and BCL-2 inhibitors.

[0049] Figure 10 A-C. | LOX-IMVI became resistant to PLX4032 by an FGFR-mediated mechanism. A, LOX-IMVI vemR (vemurafenib resistant cell line) were shown to be dependent on FGFR-activity. B, LOX-IMVI vemR cells that were made resistant to an FGFR inhibitor became dependent on EGFR-activity. B and C, LOX-IMVI vemR cells that were made resistant to an FGFR and an EGFR inhibitor showed re-sensitization with MET and MEK inhibitors with concomitant increase in secreted HGF.

[0050] Figure 11. | Secreted factors can promote resistance to drug therapies. The graph in Fig. 11 shows a comparison of untreated cells (Con), drug treated cells (Drug), and cells that were treated with drug and a secreted factor. As shown, a drug such as vemurafenib can decrease (*i.e.*, kill) cell number but that resistance to the drug is acquired when cell secreted factors (*e.g.*, FGFs) are added.

[0051] Figure 12. | A screen for secreted factors that promote resistance to cancer therapies in HER2+ breast cancer cells was performed wherein the cells were treated with one of six

therapies (lapatinib, GDC-0032, GDC-0941, GDC-0349, T-DM1, or T-DM1 plus Pertuzumab). The enhanced killing or rescue that was correlated to each secreted factor was measured.

[0052] Figure 13. | A screen for secreted factors that promote resistance to cancer therapies in B-raf mutant melanoma cells was performed wherein the cells were treated with one of three therapies (PLX4032 (*i.e.*, vemurafenib), GDC-0973, or GDC-0623). The enhanced killing or rescue that was correlated to each secreted factor was measured.

[0053] Figure 14. | Immunoblots detecting p-Akt, Akt, pERK, ERK, and β-actin (control) on nine different cell lines were performed to detect downstream mechanisms of secreted factor-mediated drug resistance.

[0054] Figure 15 A-C. | Screen of 10 melanoma cell lines and 10 breast cancer lines was performed to determine the role of FGF signalling in drug resistance. A and B, A robust z-score was observed in the melanoma and breast cancer cell lines. C, Summary of FGF receptors, their subfamily, and their ligands.

[0055] Figure 16 A-B. | FGF2 reactivates key signalling pathways to promote resistance and stimulates sustained activation of downstream signaling. A, An immunoblot of cells exposed to FGF2 for 10 min compared to cells absent exposure. B, An immunoblot of cells exposed to FGF2 for 24 hrs compared to cells absent FGF2 exposure.

[0056] Figure 17 A-C. | The kinetics of FGF secreted factor-mediated signalling in melanoma cell lines. A, Cell lines were treated with PLX4032 (vemurafenib) for 4 hrs and an FGF for 10 min. B, The 624 MEL cell line was treated with PLX4032 for 24 hrs and an FGF for 24hrs. C, The 928 MEL cell line was treated with PLX4032 for 24 hrs and an FGF for 24hrs.

[0057] Figure 18 A-B. | FGFR targeting effectively blocks FGF2 rescue. A, Effective blocking of downstream pathways often does not overcome FGF2-rescue. B, Immunoblots of AU565 cells treated with lapatinib, MEKi, SMI, and FGF-2 (similar results also observed in the HCC1954 and UACC-893 cell lines).

[0058] Figure 19 A-D. | FGFR4 mediates FGF2 rescue in HER2+ breast cancer. A, Percent rescue of cells treated with lapatinib and FGF2. B, Immunoblot of cells treated with lapatinib and FGF2. C, TCGA breast cancer samples (n=913) show high FGFR1 levels in breast cancer. D, HER2+ breast cancer cells are enriched for high FGFR4.

[0059] Figure 20 A-C. | HER2+ breast cancer models of innate resistance. A, FGFR inhibitor (BGJ398) sensitizes HCC1569 cells to lapatinib. B, FGFR inhibitor (BGJ398) sensitizes MDA-MB-453 cells to lapatinib. C, Tumor volume decreases with the combination treatment of lapatinib and an FGFR inhibitor (BGJ398).

[0060] Figure 21 A-B. | Additional mechanism of acquired resistance include sensitivity to ERK/MEK inhibitors (A) and insensitivity to ERK/MEK inhibitors (B).

[0061] Figure 22 A-B. | Secreted factor-mediated resistance mechanisms are evident in acquired drug resistant models. A, Table of single drug resistant lines. B, Table of dual drug resistant lines.

[0062] Figure 23 A-C. | Vemurafenib resistant and sensitive cell lines can be used to determine and anticipate paths to resistance in patients. LOX-IMVI cells were rescued by FGF1, FGF2, EGF, and HGF in the screen. A, LOX-IMVI VemR cells were re-sensitized to PLX4032 by FGFR inhibition. B, Dual resistant LOX-IMVI VemR/FGFRi (*i.e.*, resistant to vemurafenib and FGFR inhibitor) cells were re-sensitized to PLX4032 by EGFR inhibition. C, Triple resistant LOX-IMVI VemR/FGFRi/Erlotinib cells were re-sensitized to PLX4032 by MET inhibition.

DETAILED DESCRIPTION

I. Definitions

[0063] An "antagonist" (interchangeably termed "inhibitor") of a polypeptide of interest is an agent that interferes with activation or function of the polypeptide of interest, *e.g.*, partially or fully blocks, inhibits, or neutralizes a biological activity mediated by a polypeptide of interest. For example, an antagonist of polypeptide X may refers to any molecule that partially or fully blocks, inhibits, or neutralizes a biological activity mediated by polypeptide X. Examples of inhibitors include antibodies; ligand antibodies; small molecule antagonists; antisense and inhibitory RNA (*e.g.,* shRNA) molecules. Preferably, the inhibitor is an antibody or small molecule which binds to the polypeptide of interest. In a particular embodiment, an inhibitor has a binding affinity (dissociation constant) to the polypeptide of interest of about 1,000 nM or less. In another embodiment, inhibitor has a binding affinity to the polypeptide of interest of about 100 nM or less. In another embodiment, an inhibitor has a binding affinity to the polypeptide of interest of about 50 nM or less. In a particular embodiment, an inhibitor is covalently bound to the polypeptide of interest. In a particular embodiment, an inhibitor inhibits signaling of the polypeptide of interest with an IC_{50} of 1,000 nM or less. In another embodiment, an inhibitor inhibits signaling of the polypeptide of interest with an IC_{50} of 500 nM or less. In another embodiment, an inhibitor inhibits signaling of the polypeptide of interest with an IC_{50} of 50 nM or less. In certain embodiments, the antagonist reduces or inhibits, by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or more, the expression level or biological activity of the polypeptide of interest. In some embodiments, the polypeptide of interest is FGFR receptor (*e.g.*,

FGFR1, FGFR2, FGFR3, and/or FGFR4) or FGF (*e.g.*, FGF1-23). In some embodiments, the polypeptide of interest is EGFR.

[0064] The term “polypeptide” as used herein, refers to any native polypeptide of interest from any vertebrate source, including mammals such as primates (*e.g.*, humans) and rodents (*e.g.*, mice and rats), unless otherwise indicated. The term encompasses “full-length,” unprocessed polypeptide as well as any form of the polypeptide that results from processing in the cell. The term also encompasses naturally occurring variants of the polypeptide, *e.g.*, splice variants or allelic variants.

[0065] "Polynucleotide," or "nucleic acid," as used interchangeably herein, refer to polymers of nucleotides of any length, and include DNA and RNA. The nucleotides can be deoxyribonucleotides, ribonucleotides, modified nucleotides or bases, and/or their analogs, or any substrate that can be incorporated into a polymer by DNA or RNA polymerase, or by a synthetic reaction. A polynucleotide may comprise modified nucleotides, such as methylated nucleotides and their analogs. If present, modification to the nucleotide structure may be imparted before or after assembly of the polymer. The sequence of nucleotides may be interrupted by non-nucleotide components. A polynucleotide may be further modified after synthesis, such as by conjugation with a label. Other types of modifications include, for example, "caps", substitution of one or more of the naturally occurring nucleotides with an analog, internucleotide modifications such as, for example, those with uncharged linkages (*e.g.*, methyl phosphonates, phosphotriesters, phosphoamidates, carbamates, etc.) and with charged linkages (*e.g.*, phosphorothioates, phosphorodithioates, etc.), those containing pendant moieties, such as, for example, proteins (*e.g.*, nucleases, toxins, antibodies, signal peptides, ply-L-lysine, etc.), those with intercalators (*e.g.*, acridine, psoralen, etc.), those containing chelators (*e.g.*, metals, radioactive metals, boron, oxidative metals, etc.), those containing alkylators, those with modified linkages (*e.g.*, alpha anomeric nucleic acids, etc.), as well as unmodified forms of the polynucleotide(s). Further, any of the hydroxyl groups ordinarily present in the sugars may be replaced, for example, by phosphonate groups, phosphate groups, protected by standard protecting groups, or activated to prepare additional linkages to additional nucleotides, or may be conjugated to solid or semi-solid supports. The 5' and 3' terminal OH can be phosphorylated or substituted with amines or organic capping group moieties of from 1 to 20 carbon atoms. Other hydroxyls may also be derivatized to standard protecting groups. Polynucleotides can also contain analogous forms of ribose or deoxyribose sugars that are generally known in the art, including, for example, 2'-O-methyl-, 2'-O-allyl, 2'-fluoro- or 2'-azido-ribose, carbocyclic sugar analogs, α-anomeric sugars, epimeric sugars such as arabinose, xyloses or lyxoses, pyranose

sugars, furanose sugars, sedoheptuloses, acyclic analogs and abasic nucleoside analogs such as methyl riboside. One or more phosphodiester linkages may be replaced by alternative linking groups. These alternative linking groups include, but are not limited to, embodiments wherein phosphate is replaced by P(O)S("thioate"), P(S)S ("dithioate"), "(O)NR_2 ("amidate"), P(O)R, P(O)OR', CO or CH_2 ("formacetal"), in which each R or R' is independently H or substituted or unsubstituted alkyl (1-20 C) optionally containing an ether (-O-) linkage, aryl, alkenyl, cycloalkyl, cycloalkenyl or araldyl. Not all linkages in a polynucleotide need be identical. The preceding description applies to all polynucleotides referred to herein, including RNA and DNA.

[0066] The term "small molecule" refers to any molecule with a molecular weight of about 2000 daltons or less, preferably of about 500 daltons or less.

[0067] An "isolated" antibody is one which has been separated from a component of its natural environment. In some embodiments, an antibody is purified to greater than 95% or 99% purity as determined by, for example, electrophoretic (*e.g.*, SDS-PAGE, isoelectric focusing (IEF), capillary electrophoresis) or chromatographic (*e.g.*, ion exchange or reverse phase HPLC). For review of methods for assessment of antibody purity, *see, e.g.*, Flatman *et al.*, *J. Chromatogr. B* 848:79-87 (2007).

[0068] The term "antibody" herein is used in the broadest sense and encompasses various antibody structures, including but not limited to monoclonal antibodies, polyclonal antibodies, multispecific antibodies (*e.g.*, bispecific antibodies), and antibody fragments so long as they exhibit the desired antigen-binding activity.

[0069] The terms anti-polypeptide of interest antibody and “an antibody that binds to” a polypeptide of interest refer to an antibody that is capable of binding a polypeptide of interest with sufficient affinity such that the antibody is useful as a diagnostic and/or therapeutic agent in targeting a polypeptide of interest. In one embodiment, the extent of binding of an anti-polypeptide of interest antibody to an unrelated, non- polypeptide of interest protein is less than about 10% of the binding of the antibody to a polypeptide of interest as measured, *e.g.*, by a radioimmunoassay (RIA). In certain embodiments, an antibody that binds to a polypeptide of interest has a dissociation constant (Kd) of $\leq 1\mu M$, ≤ 100 nM, ≤ 10 nM, ≤ 1 nM, ≤ 0.1 nM, ≤ 0.01 nM, or ≤ 0.001 nM (*e.g.*, 10^{-8} M or less, *e.g.*, from 10^{-8} M to 10^{-13} M, *e.g.*, from 10^{-9} M to 10^{-13} M). In certain embodiments, an anti- polypeptide of interest antibody binds to an epitope of a polypeptide of interest that is conserved among polypeptides of interest from different species. In some embodiments, the polypeptide of interest is FGFR (e.g., FGFR1, FGFR2, FGFR3, and/or FGFR4) and/or FGF (e.g., FGF1-23). In some embodiments, the polypeptide of interest is EGFR.

[0070] A "blocking antibody" or an "antagonist antibody" is one which inhibits or reduces biological activity of the antigen it binds. Preferred blocking antibodies or antagonist antibodies substantially or completely inhibit the biological activity of the antigen.

[0071] "Affinity" refers to the strength of the sum total of noncovalent interactions between a single binding site of a molecule (*e.g.*, an antibody) and its binding partner (*e.g.*, an antigen). Unless indicated otherwise, as used herein, "binding affinity" refers to intrinsic binding affinity which reflects a 1:1 interaction between members of a binding pair (*e.g.*, antibody and antigen). The affinity of a molecule X for its partner Y can generally be represented by the dissociation constant (Kd). Affinity can be measured by common methods known in the art, including those described herein. Specific illustrative and exemplary embodiments for measuring binding affinity are described in the following.

[0072] An "antibody fragment" refers to a molecule other than an intact antibody that comprises a portion of an intact antibody that binds the antigen to which the intact antibody binds. Examples of antibody fragments include but are not limited to Fv, Fab, Fab', Fab'-SH, $F(ab')_2$; diabodies; linear antibodies; single-chain antibody molecules (*e.g.,* scFv); and multispecific antibodies formed from antibody fragments.

[0073] An "antibody that binds to the same epitope" as a reference antibody refers to an antibody that blocks binding of the reference antibody to its antigen in a competition assay by 50% or more, and conversely, the reference antibody blocks binding of the antibody to its antigen in a competition assay by 50% or more.

[0074] The term "chimeric" antibody refers to an antibody in which a portion of the heavy and/or light chain is derived from a particular source or species, while the remainder of the heavy and/or light chain is derived from a different source or species.

[0075] The terms "full length antibody," "intact antibody," and "whole antibody" are used herein interchangeably to refer to an antibody having a structure substantially similar to a native antibody structure or having heavy chains that contain an Fc region.

[0076] The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, *i.e.*, the individual antibodies comprising the population are identical and/or bind the same epitope, except for possible variant antibodies, *e.g.*, containing naturally occurring mutations or arising during production of a monoclonal antibody preparation, such variants generally being present in minor amounts. In contrast to polyclonal antibody preparations, which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody of a monoclonal antibody preparation is directed against a single determinant on an antigen. Thus, the modifier

"monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by a variety of techniques, including but not limited to the hybridoma method, recombinant DNA methods, phage-display methods, and methods utilizing transgenic animals containing all or part of the human immunoglobulin loci, such methods and other exemplary methods for making monoclonal antibodies.

[0077] A "human antibody" is one which possesses an amino acid sequence which corresponds to that of an antibody produced by a human or a human cell or derived from a non-human source that utilizes human antibody repertoires or other human antibody-encoding sequences. This definition of a human antibody specifically excludes a humanized antibody comprising non-human antigen-binding residues.

[0078] A "humanized" antibody refers to a chimeric antibody comprising amino acid residues from non-human HVRs and amino acid residues from human FRs. In certain embodiments, a humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the HVRs (*e.g.*, CDRs) correspond to those of a non-human antibody, and all or substantially all of the FRs correspond to those of a human antibody. A humanized antibody optionally may comprise at least a portion of an antibody constant region derived from a human antibody. A "humanized form" of an antibody, *e.g.*, a non-human antibody, refers to an antibody that has undergone humanization.

[0079] An "immunoconjugate" is an antibody conjugated to one or more heterologous molecule(s), including but not limited to a cytotoxic agent.

[0080] "PLX4032" and "vemurafenib" are used interchangeably herein and refer to *N*-(3-{[5-(4-chlorophenyl)-1*H*-pyrrolo[2,3-b]pyridin-3-yl]carbonyl}-2,4-difluorophenyl)propane-1-sulfonamide.

[0081] "B-raf activation" refers to activation, or phosphorylation, of the B-raf kinase. Generally, B-raf activation results in signal transduction.

[0082] The term "B-raf", as used herein, refers, unless indicated otherwise, to any native or variant (whether native or synthetic) B-raf polypeptide. The term "wild type B-raf" generally refers to a polypeptide comprising the amino acid sequence of a naturally occurring B-raf protein.

[0083] The term "B-raf variant" as used herein refers to a B-raf polypeptide which includes one or more amino acid mutations in the native B-raf sequence. Optionally, the one or more amino acid mutations include amino acid substitution(s).

[0084] A "B-raf antagonist" (interchangeably termed "B-raf inhibitor") is an agent that interferes with B-raf activation or function. In a particular embodiment, a B-raf inhibitor has a binding affinity (dissociation constant) to B-raf of about 1,000 nM or less. In another embodiment, a B-raf inhibitor has a binding affinity to B-raf of about 100 nM or less. In another embodiment, a B-raf inhibitor has a binding affinity to B-raf of about 50 nM or less. In another embodiment, a B-raf inhibitor has a binding affinity to B-raf of about 10 nM or less. In another embodiment, a B-raf inhibitor has a binding affinity to B-raf of about 1 nM or less. In a particular embodiment, a B-raf inhibitor inhibits B-raf signaling with an IC50 of 1,000 nM or less. In another embodiment, a B-raf inhibitor inhibits B-raf signaling with an IC50 of 500 nM or less. In another embodiment, a B-raf inhibitor inhibits B-raf signaling with an IC50 of 50 nM or less. In another embodiment, a B-raf inhibitor inhibits B-raf signaling with an IC50 of 10 nM or less. In another embodiment, a B-raf inhibitor inhibits B-raf signaling with an IC50 of 1 nM or less.

[0085] "V600E" refers to a mutation in the B-RAF gene which results in substitution of a glutamine for a valine at amino acid position 600 of B-Raf. "V600E" is also known as "V599E" under a previous numbering system (Kumar et al., Clin. Cancer Res. 9:3362-3368, 2003).

[0086] The term "constitutive" or "constitutively" as used herein, as for example applied to receptor kinase activity, refers to continuous signaling activity of a receptor that is not dependent on the presence of a ligand or other activating molecules. Depending on the nature of the receptor, all of the activity may be constitutive or the activity of the receptor may be further activated by the binding of other molecules (e. g. ligands). Cellular events that lead to activation of receptors are well known among those of ordinary skill in the art. For example, activation may include oligomerization, e.g., dimerization, trimerization, etc., into higher order receptor complexes. Complexes may comprise a single species of protein, i.e., a homomeric complex. Alternatively, complexes may comprise at least two different protein species, i.e., a heteromeric complex. Complex formation may be caused by, for example, overexpression of normal or mutant forms of receptor on the surface of a cell. Complex formation may also be caused by a specific mutation or mutations in a receptor.

[0087] "Individual response" or "response" can be assessed using any endpoint indicating a benefit to the individual, including, without limitation, (1) inhibition, to some extent, of disease progression (*e.g.*, cancer progression), including slowing down and complete arrest; (2) a reduction in tumor size; (3) inhibition (*i.e.*, reduction, slowing down or complete stopping) of cancer cell infiltration into adjacent peripheral organs and/or tissues; (4) inhibition (*i.e.* reduction, slowing down or complete stopping) of metasisis; (5) relief, to some extent, of one or more symptoms associated with the disease or disorder (*e.g.*, cancer); (6) increase in the length of

progression free survival; and/or (9) decreased mortality at a given point of time following treatment.

[0088] The term “substantially the same,” as used herein, denotes a sufficiently high degree of similarity between two numeric values, such that one of skill in the art would consider the difference between the two values to be of little or no biological and/or statistical significance within the context of the biological characteristic measured by said values (*e.g.*, Kd values or expression). The difference between said two values is, for example, less than about 50%, less than about 40%, less than about 30%, less than about 20%, and/or less than about 10% as a function of the reference/comparator value.

[0089] The phrase “substantially different,” as used herein, denotes a sufficiently high degree of difference between two numeric values such that one of skill in the art would consider the difference between the two values to be of statistical significance within the context of the biological characteristic measured by said values (*e.g.*, Kd values). The difference between said two values is, for example, greater than about 10%, greater than about 20%, greater than about 30%, greater than about 40%, and/or greater than about 50% as a function of the value for the reference/comparator molecule.

[0090] An "effective amount" of a substance/molecule, *e.g.,* pharmaceutical composition, refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic or prophylactic result.

[0091] A “therapeutically effective amount” of a substance/molecule may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the substance/molecule to elicit a desired response in the individual. A therapeutically effective amount is also one in which any toxic or detrimental effects of the substance/molecule are outweighed by the therapeutically beneficial effects. A "prophylactically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired prophylactic result. Typically but not necessarily, since a prophylactic dose is used in subjects prior to or at an earlier stage of disease, the prophylactically effective amount will be less than the therapeutically effective amount.

[0092] The term "pharmaceutical formulation" refers to a preparation which is in such form as to permit the biological activity of an active ingredient contained therein to be effective, and which contains no additional components which are unacceptably toxic to a subject to which the formulation would be administered.

[0093] A "pharmaceutically acceptable carrier" refers to an ingredient in a pharmaceutical formulation, other than an active ingredient, which is nontoxic to a subject., A pharmaceutically acceptable carrier includes, but is not limited to, a buffer, excipient, stabilizer, or preservative.

[0094] The phrase "pharmaceutically acceptable salt" as used herein, refers to pharmaceutically acceptable organic or inorganic salts of a compound.

[0095] As used herein, "treatment" (and grammatical variations thereof such as "treat" or "treating") refers to clinical intervention in an attempt to alter the natural course of the individual being treated, and can be performed either for prophylaxis or during the course of clinical pathology. Desirable effects of treatment include, but are not limited to, preventing occurrence or recurrence of disease, alleviation of symptoms, diminishment of any direct or indirect pathological consequences of the disease, preventing metastasis, decreasing the rate of disease progression, amelioration or palliation of the disease state, and remission or improved prognosis. In some embodiments, antibodies of the invention are used to delay development of a disease or to slow the progression of a disease.

[0096] A "platinum agent" is a chemotherapeutic agent that comprises platinum, for example carboplatin, cisplatin, and oxaliplatin.

[0097] The term "cytotoxic agent" or "chemotherapeutic agent" is a biological (e.g., large molecule) or chemical (e.g., small molecule) compound useful in the treatment of cancer, regardless of mechanism of action. The term as used herein refers to a substance that inhibits or prevents a cellular function and/or causes cell death or destruction. The term is intended to include radioactive isotopes (*e.g.*, At^{211}, I^{131}, I^{125}, Y^{90}, Re^{186}, Re^{188}, Sm^{153}, Bi^{212}, P^{32}, Pb^{212}, and radioactive isotopes of Lu), chemotherapeutic agents or drugs (*e.g.,* methotrexate, adriamicin, vinca alkaloids (vincristine, vinblastine, etoposide), doxorubicin, melphalan, mitomycin C, chlorambucil, daunorubicin or other intercalating agents), growth inhibitory agents, enzymes and fragments thereof such as nucleolytic enzymes, antibiotics, and toxins such as small molecule toxins or enzymatically active toxins of bacterial, fungal, plant or animal origin, including fragments and/or variants thereof, and the various antitumor or anticancer agents disclosed below. Other cytotoxic agents are described below. A tumoricidal agent causes destruction of tumor cells.

[0098] An "individual" or "subject" is a mammal. Mammals include, but are not limited to, domesticated animals (*e.g.*, cows, sheep, cats, dogs, and horses), primates (*e.g.*, humans and non-human primates such as monkeys), rabbits, and rodents (*e.g.*, mice and rats). In certain embodiments, the individual or subject is a human.

[0099] The terms “cancer” and “cancerous” refer to or describe the physiological condition in mammals that is typically characterized by unregulated cell growth. Included in this definition are benign and malignant cancers. By “early stage cancer” or “early stage tumor” is meant a cancer that is not invasive or metastatic or is classified as a Stage 0, I, or II cancer. Examples of cancer include, but are not limited to, carcinoma, lymphoma, blastoma (including medulloblastoma and retinoblastoma), sarcoma (including liposarcoma and synovial cell sarcoma), neuroendocrine tumors (including carcinoid tumors, gastrinoma, and islet cell cancer), mesothelioma, schwannoma (including acoustic neuroma), meningioma, adenocarcinoma, melanoma, and leukemia or lymphoid malignancies. More particular examples of such cancers include melanoma, colorectal cancer, thyroid cancer (for example, papillary thyroid carcinoma), non-small cell lung cancer (NSCLC), cancer of the peritoneum, hepatocellular cancer, gastric or stomach cancer including gastrointestinal cancer, pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, breast cancer (including metastatic breast cancer), colon cancer, rectal cancer, colorectal cancer, endometrial or uterine carcinoma, salivary gland carcinoma, kidney or renal cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma, anal carcinoma, penile carcinoma, testicular cancer, esophageal cancer, tumors of the biliary tract, as well as head and neck cancer. In some embodiments, the cancer is melanoma; colorectal cancer; thyroid cancer, e.g., papillary thyroid cancer; or ovarian cancer.

[00100] The term “concomitantly” is used herein to refer to administration of two or more therapeutic agents, give in close enough temporal proximity where their individual therapeutic effects overlap in time. Accordingly, concurrent administration includes a dosing regimen when the administration of one or more agent(s) continues after discontinuing the administration of one or more other agent(s). In some embodiments, the concomitantly administration is concurrently, sequentially, and/or simultaneously.

[00101] By “reduce or inhibit” is meant the ability to cause an overall decrease of 20%, 30%, 40%, 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, or greater. Reduce or inhibit can refer to the symptoms of the disorder being treated, the presence or size of metastases, or the size of the primary tumor.

[00102] The term “package insert” is used to refer to instructions customarily included in commercial packages of therapeutic products, that contain information about the indications, usage, dosage, administration, combination therapy, contraindications and/or warnings concerning the use of such therapeutic products.

[00103] An "article of manufacture" is any manufacture (*e.g.*, a package or container) or kit comprising at least one reagent, *e.g.*, a medicament for treatment of a disease or disorder (*e.g.*, cancer), or a probe for specifically detecting a biomarker described herein. In certain embodiments, the manufacture or kit is promoted, distributed, or sold as a unit for performing the methods described herein.

[00104] As is understood by one skilled in the art, reference to "about" a value or parameter herein includes (and describes) embodiments that are directed to that value or parameter per se. For example, description referring to "about X" includes description of "X".

[00105] It is understood that aspect and embodiments of the invention described herein include "consisting" and/or "consisting essentially of" aspects and embodiments. As used herein, the singular form "a", "an", and "the" includes plural references unless indicated otherwise.

II. Methods and Uses

[00106] Provided herein are methods utilizing an antagonist of FGFR signaling and a B-raf antagonist.

[00107] In particular, provided herein are methods of treating cancer in an individual comprising concomitantly administering to the individual (a) an antagonist of FGFR signaling and (b) a B-raf antagonist. In some embodiments, the respective amounts of the antagonist of FGFR signaling and the B-raf antagonist are effective to increase the period of cancer sensitivity and/or delay the development of cancer resistance to the B-raf antagonist. In some embodiments, the respective amounts of the antagonist of FGFR signaling and the B-raf antagonist are effective to increase efficacy of a cancer treatment comprising B-raf antagonist. For example, in some embodiments, the respective amounts of the antagonist of FGFR signaling and the B-raf antagonist are effective to increased efficacy compared to a standard treatment comprising administering an effective amount of B-raf antagonist without (in the absence of) the antagonist of FGFR signaling. In some embodiments, the respective amounts of the antagonist of FGFR signaling and the B-raf antagonist are effective to increased response (*e.g.*, complete response) compared to a standard treatment comprising administering an effective amount of the B-raf antagonist without (in the absence of) the antagonist of FGFR signaling. In some embodiments, the respective amounts of the antagonist of FGFR signaling and the B-raf antagonist are effective to increase cancer sensitivity and/or restoring sensitivity to the B-raf antagonist. In some embodiments, the antagonist of FGFR signaling is an antagonist of FGFR1 signaling. In some embodiments, the antagonist of FGFR1 signaling binds to and/or inhibits one or more of FGFR1b, FGFR1c, FGF1, FGF2, FGF3, FGF4, FGF5, FGF6, and FGF10. In certain embodiments, the B-raf antagonist is one or more of vemurafenib (*i.e.*, PLX4032), sorafenib, PLX4720, PL-3603, GSK2118436,

GDC-0879, N-(3-(5-(4-chlorophenyl)-1H-pyrrolo[2,3-b]pyridine-3-carbonyl)-2,4-difluorophenyl)propane-1-sulfonamide,, GSK 2118436, RAF265 (Novartis), XL281, ARQ736, BAY73-4506. In certain embodiments, B-raf antagonist may be selective for B-raf V600E. In some embodiments, the B-raf antagonist is vemurafenib (*i.e.*, PLX4032).

[00108] Provided herein are methods of treating a cancer cell, wherein the cancer cell is resistant to treatment with a B-raf antagonist in an individual comprising administering to the individual an effective amount of an antagonist of FGFR signaling and an effective amount of the B-raf antagonist. Also provided herein are methods of treating cancer resistant to a B-raf antagonist in an individual comprising administering to the individual an effective amount of an antagonist of FGFR signaling and an effective amount of the B-raf antagonist. In some embodiments, the antagonist of FGFR signaling is an antagonist of FGFR1 signaling. In some embodiments, the antagonist of FGFR1 signaling binds to and/or inhibits one or more of FGFR1b, FGFR1c, FGF1, FGF2, FGF3, FGF4, FGF5, FGF6, and FGF10. In certain embodiments, the B-raf antagonist is one or more of vemurafenib (*i.e.*, PLX4032), sorafenib, PLX4720, PL-3603, GSK2118436, GDC-0879, N-(3-(5-(4-chlorophenyl)-1H-pyrrolo[2,3-b]pyridine-3-carbonyl)-2,4-difluorophenyl)propane-1-sulfonamide,, GSK 2118436, RAF265 (Novartis), XL281, ARQ736, BAY73-4506. In certain embodiments, B-raf antagonist may be selective for B-raf V600E. In some embodiments, the B-raf antagonist is vemurafenib (*i.e.*, PLX4032).

[00109] Provided herein are also methods of increasing sensitivity and/or restoring sensitivity to a B-raf antagonist comprising administering to the individual an effective amount of an antagonist of FGFR signaling and an effective amount of the B-raf antagonist. In some embodiments, the antagonist of FGFR signaling is an antagonist of FGFR1 signaling. In some embodiments, the antagonist of FGFR1 signaling binds to and/or inhibits one or more of FGFR1b, FGFR1c, FGF1, FGF2, FGF3, FGF4, FGF5, FGF6, and FGF10. In certain embodiments, the B-raf antagonist is one or more of vemurafenib (*i.e.*, PLX4032), sorafenib, PLX4720, PL-3603, GSK2118436, GDC-0879, N-(3-(5-(4-chlorophenyl)-1H-pyrrolo[2,3-b]pyridine-3-carbonyl)-2,4-difluorophenyl)propane-1-sulfonamide,, GSK 2118436, RAF265 (Novartis), XL281, ARQ736, BAY73-4506. In certain embodiments, B-raf antagonist may be selective for B-raf V600E. In some embodiments, the B-raf antagonist is vemurafenib (*i.e.*, PLX4032).

[00110] Further provided herein are methods of increasing efficacy of a cancer treatment comprising a B-raf antagonist in an individual comprises concomitantly administering to the individual (a) an effective amount of an antagonist of FGFR signaling and (b) an effective amount of the B-raf antagonist. In some embodiments, the antagonist of FGFR signaling is an antagonist of FGFR1 signaling. In some embodiments, the antagonist of FGFR1 signaling binds

to and/or inhibits one or more of FGFR1b, FGFR1c, FGF1, FGF2, FGF3, FGF4, FGF5, FGF6, and FGF10. In certain embodiments, the B-raf antagonist is one or more of vemurafenib (*i.e.*, PLX4032), sorafenib, PLX4720, PL-3603, GSK2118436, GDC-0879, N-(3-(5-(4-chlorophenyl)-1H-pyrrolo[2,3-b]pyridine-3-carbonyl)-2,4-difluorophenyl)propane-1-sulfonamide,, GSK 2118436, RAF265 (Novartis), XL281, ARQ736, BAY73-4506. In certain embodiments, B-raf antagonist may be selective for B-raf V600E. In some embodiments, the B-raf antagonist is vemurafenib (*i.e.*, PLX4032).

[00111] Provided herein of treating cancer in an individual wherein cancer treatment comprising concomitantly administering to the individual (a) an effective amount of an antagonist of FGFR signaling and (b) an effective amount of a B-raf antagonist, wherein the cancer treatment has increased efficacy compared to a standard treatment comprising administering an effective amount of the B-raf antagonist without (in the absence of) the antagonist of FGFR signaling. In addition, provided herein are methods of delaying and/or preventing development of cancer resistant to a B-raf antagonist in an individual, comprising concomitantly administering to the individual (a) an effective amount of an antagonist of FGFR signaling and (b) an effective amount of the B-raf antagonist. In some embodiments, the antagonist of FGFR signaling is an antagonist of FGFR1 signaling. In some embodiments, the antagonist of FGFR1 signaling binds to and/or inhibits one or more of FGFR1b, FGFR1c, FGF1, FGF2, FGF3, FGF4, FGF5, FGF6, and FGF10. In certain embodiments, the B-raf antagonist is one or more of vemurafenib (*i.e.*, PLX4032), sorafenib, PLX4720, PL-3603, GSK2118436, GDC-0879, N-(3-(5-(4-chlorophenyl)-1H-pyrrolo[2,3-b]pyridine-3-carbonyl)-2,4-difluorophenyl)propane-1-sulfonamide,, GSK 2118436, RAF265 (Novartis), XL281, ARQ736, BAY73-4506. In certain embodiments, B-raf antagonist may be selective for B-raf V600E. In some embodiments, the B-raf antagonist is vemurafenib (*i.e.*, PLX4032).

[00112] Provided herein are methods of treating an individual with cancer who has increased likelihood of developing resistance to a B-raf antagonist comprising concomitantly administering to the individual (a) an effective amount of an antagonist of FGFR signaling and (b) an effective amount of the B-raf antagonist. In some embodiments, the antagonist of FGFR signaling is an antagonist of FGFR1 signaling. In some embodiments, the antagonist of FGFR1 signaling binds to and/or inhibits one or more of FGFR1b, FGFR1c, FGF1, FGF2, FGF3, FGF4, FGF5, FGF6, and FGF10. In certain embodiments, the B-raf antagonist is one or more of vemurafenib (*i.e.*, PLX4032), sorafenib, PLX4720, PL-3603, GSK2118436, GDC-0879, N-(3-(5-(4-chlorophenyl)-1H-pyrrolo[2,3-b]pyridine-3-carbonyl)-2,4-difluorophenyl)propane-1-sulfonamide,, GSK 2118436, RAF265 (Novartis), XL281, ARQ736, BAY73-4506. In certain embodiments, B-raf

antagonist may be selective for B-raf V600E. In some embodiments, the B-raf antagonist is vemurafenib (*i.e.*, PLX4032).

[00113] Further provided herein are methods of increasing sensitivity to a B-raf antagonist in an individual with cancer comprising concomitantly administering to the individual (a) an effective amount of an antagonist of FGFR signaling and (b) an effective amount of the B-raf antagonist.In addition, provided herein are methods of extending the period of a B-raf antagonist sensitivity in an individual with cancer comprising concomitantly administering to the individual (a) an effective amount of an antagonist of FGFR signaling and (b) an effective amount of the B-raf antagonist. In some embodiments, the antagonist of FGFR signaling is an antagonist of FGFR1 signaling. In some embodiments, the antagonist of FGFR1 signaling binds to and/or inhibits one or more of FGFR1b, FGFR1c, FGF1, FGF2, FGF3, FGF4, FGF5, FGF6, and FGF10. In certain embodiments, the B-raf antagonist is one or more of vemurafenib (*i.e.*, PLX4032), sorafenib, PLX4720, PL-3603, GSK2118436, GDC-0879, N-(3-(5-(4-chlorophenyl)-1H-pyrrolo[2,3-b]pyridine-3-carbonyl)-2,4-difluorophenyl)propane-1-sulfonamide,, GSK 2118436, RAF265 (Novartis), XL281, ARQ736, BAY73-4506. In certain embodiments, B-raf antagonist may be selective for B-raf V600E. In some embodiments, the B-raf antagonist is vemurafenib (*i.e.*, PLX4032).

[00114] Provided herein are also methods of extending the duration of response to a B-raf antagonist in an individual with cancer comprising concomitantly administering to the (a) an effective amount of an antagonist of FGFR signaling and (b) an effective amount of the B-raf antagonist. In some embodiments, the antagonist of FGFR signaling is an antagonist of FGFR1 signaling. In some embodiments, the antagonist of FGFR1 signaling binds to and/or inhibits one or more of FGFR1b, FGFR1c, FGF1, FGF2, FGF3, FGF4, FGF5, FGF6, and FGF10. In certain embodiments, the B-raf antagonist is one or more of vemurafenib (*i.e.*, PLX4032), sorafenib, PLX4720, PL-3603, GSK2118436, GDC-0879, N-(3-(5-(4-chlorophenyl)-1H-pyrrolo[2,3-b]pyridine-3-carbonyl)-2,4-difluorophenyl)propane-1-sulfonamide,, GSK 2118436, RAF265 (Novartis), XL281, ARQ736, BAY73-4506. In certain embodiments, B-raf antagonist may be selective for B-raf V600E. In some embodiments, the B-raf antagonist is vemurafenib (*i.e.*, PLX4032).

[00115] In some embodiments of any of the methods, the antagonist of FGFR signaling is an antibody inhibitor, a small molecule inhibitor, a binding polypeptide inhibitor, and/or a polynucleotide antagonist. In some embodiments, the antagonist of FGFR signaling is a binding polypeptide inhibitor. In some embodiments, the binding polypeptide inhibitor comprises a region of the extracellular domain of FGFR linked to a Fc (*e.g.*, FP-1039 (Five Prime)). In some

embodiments, the antagonist of FGFR signaling is an antagonist of FGFR1 signaling. In some embodiments, the antagonist of FGFR signaling is an antagonist of FGFR2 signaling. In some embodiments, the antagonist of FGFR signaling is an antagonist of FGFR3 signaling. In some embodiments, the antagonist of FGFR signaling is an antagonist of FGFR4 signaling. In some embodiments, the antagonist of FGFR signaling is a small molecule. In some embodiments, the antagonist of FGFR signaling is an antibody.

[00116] In some embodiments, the antagonist of FGFR1 signaling binds to and/or inhibits one or more of FGFR1b, FGFR1c, FGF1, FGF2, FGF3, FGF4, FGF5, FGF6, and FGF10. In some embodiments, the small molecule is N-[2-[[4-(diethylamino)butyl]amino]-6-(3,5-dimethoxyphenyl)pyrido[2,3-d]pyrimidin-7-yl]-N'-(1,1-dimethylethyl)-urea or pharmaceutically acceptable salt thereof. In some embodiments, the small molecule is BGJ398 (Novartis), AZD4547 (AstraZeneca), and/or FF284 (Chugai/Debiopharm (Debio 1347). In some embodiments, the antagonist of FGFR1 signaling is an anti-FGF2 antibody. In some embodiments, the antagonist of FGFR1 signaling is an anti-FGFR1 antibody. In some embodiments, the antagonist of FGFR1 signaling is an anti-FGFR1-IIIb antibody. In some embodiments, the antagonist of FGFR1 signaling is an anti-FGFR1-IIIc antibody. In some embodiments the antagonist of FGFR signaling is an anti-FGFR antibody capable of binding more than one FGFR polypeptide.

[00117] Cancer having resistance to a therapy as used herein includes a cancer which is not responsive and/or reduced ability of producing a significant response (*e.g.*, partial response and/or complete response) to the therapy. Resistance may be acquired resistance which arises in the course of a treatment method. In some embodiments, the acquired drug resistance is transcient and/or reversible drug tolerance. Transcient and/or reversible drug resistance to a therapy includes wherein the drug resistance is capable of regaining sensitivity to the therapy after a break in the treatment method. In some embodiments, the acquired resistance is permanent resistance. Permanent resistance to a therapy includes a genetic change conferring drug resistance.

[00118] Cancer having sensitivity to a therapy as used herein includes cancer which is responsive and/or capable of producing a significant response (*e.g.*, partial response and/or complete response).

[00119] Methods of determining of assessing acquisition of resistance and/or maintenance of sensitivity to a therapy are known in the art and described in the Examples. Changes in acquisition of resistance and/or maintenance of sensitivity such as drug tolerance may be assessed by assaying the growth of drug tolerant persisters as described in the Examples and

Sharma et al. Changes in acquisition of resistance and/or maintenance of sensitivity such as permanent resistance and/or expanded resisters may be assessed by assaying the growth of drug tolerant expanded persisters as described in the Examples and Sharma et al. In some embodiments, resistance may be indicated by a change in IC_{50}, EC_{50} or decrease in tumor growth in drug tolerant persisters and/or drug tolerant expanded persisters. In some embodiments, the change is greater than about any of 50%, 100%, and/or 200%. In addition, changes in acquisition of resistance and/or maintenance of sensitivity may be assessed in vivo for examples by assessing response, duration of response, and/or time to progression to a therapy, *e.g.*, partial response and complete response. Changes in acquisition of resistance and/or maintenance of sensitivity may be based on changes in response, duration of response, and/or time to progression to a therapy in a population of individuals, *e.g.*, number of partial responses and complete responses.

[00120] In some embodiments of any of the methods, the cancer is a solid tumor cancer. In some embodiments, the cancer is lung cancer (*e.g.*, non-small cell lung cancer (NSCLC)). In some embodiments the cancer is breast cancer (*e.g.*, HER2 positive breast cancer). In some embodiments, the cancer is melanoma. In some embodiments, the cancer is cancer of epithelial tissue. In some embodiments, the cancer is adenocarcinoma. The cancer in any of the combination therapies methods described herein when starting the method of treatment comprising the antagonist of FGFR signaling and the B-raf antagonist may be sensitive (examples of sensitive include, but are not limited to, responsive and/or capable of producing a significant response (*e.g.*, partial response and/or complete response)) to a method of treatment comprising the B-raf antagonist alone. The cancer in any of the combination therapies methods described herein when starting the method of treatment comprising the antagonist of FGFR signaling and the B-raf antagonist may not be resistant (examples of resistance include, but are not limited to, not responsive and/or reduced ability and/or incapable of producing a significant response (*e.g.*, partial response and/or complete response)) to a method of treatment comprising the B-raf antagonist alone. In some embodiments, the cancer has undergone epithelial-mesenchymal transition (EMT). In some embodiments, EMT is detected by assaying expression of epithelial-associated proteins/RNAs (*e.g.*, E-cadherin) and/or mesenchymal-associate proteins/RNAs (*e.g.*, vimentin). In some embodiments, the cancer has wild-type B-raf (*i.e.*, the cancer does not have a mutation in B-raf). In some embodiments, the cancer has a mutation in B-raf. In some embodiments, mutant B-raf is constitutively activated B-raf. In some embodiments, mutant B-raf is B-raf V600. In some embodiments, B-raf V600 is B-raf V600E. In some embodiments, mutant B-raf is one or more of B-raf V600K (GTG>AAG), V600R (GTG>AGG), V600E (GTG>GAA) and/or V600D (GTG>GAT).

[00121] In some embodiments of any of the methods, the individual according to any of the above embodiments may be a human.

[00122] In some embodiments of any of the methods, the combination therapies noted above encompass combined administration (where two or more therapeutic agents are included in the same or separate formulations), and separate administration, in which case, administration of the antagonist of the invention can occur prior to, simultaneously, sequentially, concurrently, and/or following, administration of the additional therapeutic agent and/or adjuvant. In some embodiments, the combination therapy further comprises radiation therapy and/or additional therapeutic agents.

[00123] An antagonist of FGFR signaling and a B-raf antagonist can be administered by any suitable means, including oral, parenteral, intrapulmonary, and intranasal, and, if desired for local treatment, intralesional administration. Parenteral infusions include intramuscular, intravenous, intraarterial, intraperitoneal, or subcutaneous administration. Dosing can be by any suitable route, *e.g.,* by injections, such as intravenous or subcutaneous injections, depending in part on whether the administration is brief or chronic. Various dosing schedules including but not limited to single or multiple administrations over various time-points, bolus administration, and pulse infusion are contemplated herein.

[00124] Antagonists of FGFR signaling (*e.g.*, an antibody, binding polypeptide, and/or small molecule) and a B-raf antagonist described herein may be formulated, dosed, and administered in a fashion consistent with good medical practice. Factors for consideration in this context include the particular disorder being treated, the particular mammal being treated, the clinical condition of the individual patient, the cause of the disorder, the site of delivery of the agent, the method of administration, the scheduling of administration, and other factors known to medical practitioners. The antagonist of FGFR signaling and a B-raf antagonist need not be, but is optionally formulated with one or more agents currently used to prevent or treat the disorder in question. The effective amount of such other agents depends on the amount of the antagonist of FGFR signaling and a B-raf antagonist present in the formulation, the type of disorder or treatment, and other factors discussed above. These are generally used in the same dosages and with administration routes as described herein, or about from 1 to 99% of the dosages described herein, or in any dosage and by any route that is empirically/clinically determined to be appropriate.

[00125] For the prevention or treatment of disease, the appropriate dosage of an antagonist of FGFR signaling and a B-raf antagonist described herein (when used alone or in combination with one or more other additional therapeutic agents) will depend on the type of disease to be treated,

the severity and course of the disease, whether the antagonist of FGFR signaling and a B-raf antagonist is administered for preventive or therapeutic purposes, previous therapy, the patient's clinical history and response to the antagonist of FGFR signaling and a B-raf antagonist, and the discretion of the attending physician. The antagonist of FGFR signaling and a B-raf antagonist is suitably administered to the patient at one time or over a series of treatments. For repeated administrations over several days or longer, depending on the condition, the treatment would generally be sustained until a desired suppression of disease symptoms occurs. Such doses may be administered intermittently, *e.g.*, every week or every three weeks (*e.g.*, such that the patient receives from about two to about twenty, or *e.g.,* about six doses of the antagonist of FGFR signaling and a B-raf antagonist. An initial higher loading dose, followed by one or more lower doses may be administered. An exemplary dosing regimen comprises administering. However, other dosage regimens may be useful. The progress of this therapy is easily monitored by conventional techniques and assays.

[00126] It is understood that any of the above formulations or therapeutic methods may be carried out using an immunoconjugate as the antagonist of FGFR signaling and/or a B-raf antagonist.

III. Therapeutic Compositions

[00127] Provided herein are combinations comprising an antagonist of FGFR signaling and a B-raf antagonist. In certain embodiments, the combination increases the efficacy of the targeted therapeutic administered alone. In certain embodiments, the combination delays and/or prevents development of cancer resistance to the targeted therapeutic. In certain embodiments, the combination extends the period of the targeted therapeutic sensitivity in an individual with cancer.

[00128] Provided herein are antagonists of FGFR signaling and a B-raf antagonist useful in the combination therapy methods described herein. In some embodiments, the antagonists of FGFR signaling and/or B-raf antagonists are an antibody, binding polypeptide, binding small molecule, and/or polynucleotide.

[00129] Amino acid sequences of various FGFRs and FGFs are known in the art and are publicly available. *See e.g.*, FGFR1 (*e.g.*, UniProtKB/Swiss-Prot P11362-1, P11362-2, P11362-3, P11362-4, P11362-5, P11362-6, P11362-7, P11362-8, P11362-9, P11362-10, P11362-11, P11362-12, P11362-13, P11362-14, P11362-15, P11362-16, P11362-17, P11362-18, P11362-19, P11362-20, and/or P11362-21), FGFR2 (*e.g.*, UniProtKB/Swiss-Prot P21802-1 (*i.e.*, FGFR2-IIIc), P21802-2, P21802-3 (*i.e.*, FGFR2-IIIb), P21802-4, P21802-5, P21802-6, P21802-7, P21802-8, P21802-9, P21802-10, P21802-11, P21802-12, P21802-13, P21802-14, P21802-15, P21802-16, P21802-17, P21802-18, P21802-19, P21802-20, P21802-21, P21802-

22, and/or P21802-23), FGFR3 (*e.g.*, UniProtKB/Swiss-Prot P22607-1 (*i.e.*, FGFR3-IIIc), P22607-2 (*i.e.*, FGFR3-IIIb), P22607-3, and/or P22607-4), FGFR4 (*e.g.*, UniProtKB/Swiss-Prot P22455-1 and/or P22455-2), FGF1 (*e.g.*, UniProtKB/Swiss-Prot P05230-1 and/or P05230-2), FGF2 (*e.g.*, UniProtKB/Swiss-Prot P09038-1, P09038-2, P09038-3, and/or P09038-4), FGF3 (*e.g.*, UniProtKB/Swiss-Prot P11487), FGF4 (*e.g.*, UniProtKB/Swiss-Prot P08620), FGF5 (*e.g.*, UniProtKB/Swiss-Prot P12034-1 and/or P12034-2), FGF6 (*e.g.*, UniProtKB/Swiss-Prot 10767), FGF7 (*e.g.*, UniProtKB/Swiss-Prot P21781), FGF8 (*e.g.*, UniProtKB/Swiss-Prot P55075-1, P55075-2, P55075-3 and/or P55075-4), FGF9 (*e.g.*, UniProtKB/Swiss-Prot P31371), FGF10 (*e.g.*, UniProtKB/Swiss-Prot O15520), FGF11 (*e.g.*, UniProtKB/Swiss-Prot Q92914), FGF12 (*e.g.*, UniProtKB/Swiss-Prot P61328-1 and/or P61328-2), FGF13 (*e.g.*, UniProtKB/Swiss-Prot Q92913-1, Q92913-2, Q92913-3, Q92913-4, and/or Q92913-5), FGF14 (*e.g.*, UniProtKB/Swiss-Prot Q92915-1 and/or Q92915-2), FGF16 (*e.g.*, UniProtKB/Swiss-Prot O43320), FGF17 (*e.g.*, UniProtKB/Swiss-Prot O60258-1 and/or O60258-2), FGF18 (*e.g.*, UniProtKB/Swiss-Prot O76093), FGF19 (*e.g.*, UniProtKB/Swiss-Prot O95750), FGF20 (*e.g.*, UniProtKB/Swiss-Prot Q9NP95), FGF21 (*e.g.*, UniProtKB/Swiss-Prot Q9NSA1), FGF22 (*e.g.*, UniProtKB/Swiss-Prot Q9HCT0), and/or FGF23 (*e.g.*, UniProtKB/Swiss-Prot Q9GZV9).

[00130] In some embodiments of any of the methods, the antagonist of FGFR signaling is an antibody inhibitor, a small molecule inhibitor, a binding polypeptide inhibitor, and/or a polynucleotide antagonist. In some embodiments, the antagonist of FGFR signaling is a binding polypeptide inhibitor. In some embodiments, the binding polypeptide inhibitor comprises a region of the extracellular domain of FGFR linked to a Fc. In some embodiments, the antagonist of FGFR signaling is a small molecule. In some embodiments, the antagonist of FGFR signaling is an antibody.

[00131] In some embodiments of any of the methods, the antagonist of FGFR signaling is an antagonist of FGFR1 signaling. In some embodiments, the antagonist of FGFR1 signaling binds to and/or inhibits one or more of FGFR1-IIIb, FGFR1-IIIc, FGF1, FGF2, FGF3, FGF4, FGF5, FGF6, and FGF10. In some embodiments, the antagonist of FGFR1 signaling binds to and/or inhibits FGFR1 (*e.g.*, FGFR1-IIIb and/or FGFR1-IIIc). In some embodiments, the antagonist of FGFR1 signaling binds to and/or inhibits FGF2. In some embodiments, the antagonist of FGFR1 signaling binds to and/or inhibits FGF5.

[00132] In some embodiments of any of the methods, the antagonist of FGFR1 signaling is a binding polypeptide. In some embodiments, the binding polypeptide is an FGFR1 fusion protein comprising an extracellular domain of an FGFR1 polypeptide and a fusion partner. In some embodiments, the FGFR1 is FGFR1-IIIb. In some embodiments, the FGFR1 is FGFR1-IIIb. In

some embodiments, the extracellular domain comprises of amino acids 22 to 360 or 22 to 592 of FGFR1-IIIc. In some embodiments, the FGFR1 fusion protein is a protein described in US7678890, which is hereby incorporated by reference in its entirety.

[00133] In some embodiments of any of the methods, the antagonist of FGFR1 signaling is an antibody. In some embodiments, the antagonist of FGFR1 signaling is an anti-FGF2 antibody. In In some embodiments, the fusion partner is an Fc polypeptide. In some embodiments, the antibody is an FGF2 antibody, for example as described in US20090304707, which is hereby incorporated by reference in its entirety, for example the antibody produced by hybridoma PTA-8864 and/or a humanized antibody thereof. In some embodiments, the antagonist of FGFR1 signaling is an anti-FGFR1 antibody. In some embodiments, the antagonist of FGFR1 signaling is an anti-FGFR1-IIIb antibody. In some embodiments, the antagonist of FGFR1 signaling is an anti-FGFR1-IIIc antibody. In some embodiments the antagonist of FGFR1 signaling is an anti-FGFR1 antibody capable of binding more than one FGFR polypeptide.

[00134] In some embodiments, the antagonist of FGFR1 signaling is a small molecule. In some embodiments, the antagonist of FGFR1 signaling is N-[2-[[4-(diethylamino)butyl]amino]-6-(3,5-dimethoxyphenyl)pyrido[2,3-d]pyrimidin-7-yl]-N'-(1,1-dimethylethyl)-urea or pharmaceutically acceptable salt thereof. In some embodiments, the antagonist of FGFR1 signaling is BGJ398 (Novartis, *i.e.*, 3-(2,6-Dichloro-3,5-dimethoxy-phenyl)-1-{6-[4-(4-ethyl-piperazin-1-yl)-phenylamino]-pyrimidin-4-yl}-1-methyl-urea and/or a pharmaceutically acceptable salt thereof; CAS# 872511-34-7). In some embodiments, the antagonist of FGFR1 signaling is AZD4547 (AstraZeneca; *i.e.*, N-(5-(3,5-dimethoxyphenethyl)-1H-pyrazol-3-yl)-4-((3S,5R)-3,5-dimethylpiperazin-1-yl)benzamide and/or pharmaceutically acceptable salts thereof). In some embodiments, the antagonist of FGFR1 signaling is FF284 (Chugai/Debiopharm (Debio 1347).

[00135] In some embodiments of any of the methods, the antagonist of FGFR signaling is an antagonist of FGFR2 signaling. In some embodiments, the antagonist of FGFR2 signaling binds to and/or inhibits one or more of FGFR2-IIIb, FGFR2-IIIc, FGF1, FGF2, FGF3, FGF4, FGF6, FGF7, FGF9, FGF10, FGF17, FGF18 and FGF22. In some embodiments, the antagonist of FGFR2 signaling binds to and/or inhibits FGFR2 (*e.g.*, FGFR2-IIIb and/or FGFR2-IIIc). In some embodiments, the antagonist of FGFR2 signaling binds to and/or inhibits FGF2. In some embodiments, the antagonist of FGFR2 signaling binds to and/or inhibits FGF9.

[00136] In some embodiments of any of the methods, the antagonist of FGFR2 signaling is a binding polypeptide. In some embodiments, the binding polypeptide is an FGFR2 fusion protein comprising an extracellular domain of an FGFR2 polypeptide and a fusion partner. Examples include, but are not limited to, those described in WO2008/065543 and WO2007/014123, which

are incorporated by reference in their entirety. In some embodiments, the antagonist of FGFR2 signaling is an anti-FGFR2 antibody. In some embodiments, the antagonist of FGFR2 signaling is an anti-FGFR2-IIIb antibody. In some embodiments, the antagonist of FGFR2 signaling is an anti-FGFR2-IIIc antibody. In some embodiments the antagonist of FGFR2 signaling is an anti-FGFR2 antibody capable of binding more than one FGFR polypeptide. Examples of FGFR2 antibodies are known in the art and include, but are not limited to the antibodies described in US 8,101,723, US 8,101,721, WO2001/79266, WO2007/144893, and WO2010/054265, which are incorporated by reference in their entirety.

[00137] In some embodiments, the antagonist of FGFR2 signaling is a small molecule. In some embodiments, the antagonist of FGFR2 signaling is BGJ398 (Novartis, *i.e.*, 3-(2,6-Dichloro-3,5-dimethoxy-phenyl)-1-{6-[4-(4-ethyl-piperazin-1-yl)-phenylamino]-pyrimidin-4-yl}-1-methyl-urea and/or a pharmaceutically acceptable salt thereof; CAS# 872511-34-7). In some embodiments, the antagonist of FGFR2 signaling is AZD4547 (AstraZeneca; *i.e.*, N-(5-(3,5-dimethoxyphenethyl)-1H-pyrazol-3-yl)-4-((3S,5R)-3,5-dimethylpiperazin-1-yl)benzamide and/or pharmaceutically acceptable salts thereof). In some embodiments, the antagonist of FGFR2 signaling is FF284 (Chugai/Debiopharm (Debio 1347).

[00138] In some embodiments of any of the methods, the antagonist of FGFR signaling is an antagonist of FGFR3 signaling. In some embodiments, the antagonist of FGFR3 signaling binds to and/or inhibits one or more of FGFR3-IIIb, FGFR3-IIIc, FGF1, FGF2, FGF4, FGF8, FGF9, FGF17, FGF18 and FGF23. In some embodiments, the antagonist of FGFR3 signaling binds to and/or inhibits FGFR3 (*e.g.*, FGFR3-IIIb and/or FGFR3-IIIc). In some embodiments, the antagonist of FGFR3 signaling binds to and/or inhibits FGF2. In some embodiments, the antagonist of FGFR3 signaling binds to and/or inhibits FGF9.

[00139] In some embodiments of any of the methods, the antagonist of FGFR3 signaling is a binding polypeptide. In some embodiments, the binding polypeptide is an FGFR3 fusion protein comprising an extracellular domain of an FGFR3 polypeptide and a fusion partner. In some embodiments, the antagonist of FGFR3 signaling is an anti-FGFR3 antibody. In some embodiments, the antagonist of FGFR3 signaling is an anti-FGFR3-IIIb antibody. In some embodiments, the antagonist of FGFR3 signaling is an anti-FGFR3-IIIc antibody. In some embodiments the antagonist of FGFR3 signaling is an anti-FGFR3 antibody capable of binding more than one FGFR polypeptide. Examples of FGFR3 antibodies are known in the art and include, but are not limited to the antibodies described in US 8,101,721, WO2010/111367, WO2001/79266, WO2002/102854, WO2002/10972, WO2007/144893, WO2010/002862, and/or WO2010/048026, which are incorporated by reference in their entirety.

[00140] In some embodiments, the antagonist of FGFR3 signaling is a small molecule. In some embodiments, the antagonist of FGFR3 signaling is BGJ398 (Novartis, *i.e.*, 3-(2,6-Dichloro-3,5-dimethoxy-phenyl)-1-{6-[4-(4-ethyl-piperazin-1-yl)-phenylamino]-pyrimidin-4-yl}-1-methyl-urea and/or a pharmaceutically acceptable salt thereof; CAS# 872511-34-7). In some embodiments, the antagonist of FGFR3 signaling is AZD4547 (AstraZeneca; *i.e.*, N-(5-(3,5-dimethoxyphenethyl)-1H-pyrazol-3-yl)-4-((3S,5R)-3,5-dimethylpiperazin-1-yl)benzamide and/or pharmaceutically acceptable salts thereof). In some embodiments, the antagonist of FGFR3 signaling is FF284 (Chugai/Debiopharm (Debio 1347). In some embodiments of any of the methods, the FGFR3 antagonist is Brivanib, Dovitinib (TKI-258), and/or HM-80871A.

[00141] In some embodiments of any of the methods, the antagonist of FGFR signaling is an antagonist of FGFR4 signaling. In some embodiments, the antagonist of FGFR4 signaling binds to and/or inhibits one or more of FGFR4-IIIb, FGFR4-IIIc, FGF1, FGF2, FGF4, FGF6, FGF8, FGF9, FGF16, FGF17, FGF18, and FGF19. In some embodiments, the antagonist of FGFR4 signaling binds to and/or inhibits FGFR4 (*e.g.*, FGFR4-IIIb and/or FGFR4-IIIc). In some embodiments, the antagonist of FGFR4 signaling binds to and/or inhibits FGF2. In some embodiments, the antagonist of FGFR4 signaling binds to and/or inhibits FGF9.

[00142] In some embodiments of any of the methods, the antagonist of FGFR4 signaling is a binding polypeptide. In some embodiments, the binding polypeptide is an FGFR4 fusion protein comprising an extracellular domain of an FGFR4 polypeptide and a fusion partner. In some embodiments, the antagonist of FGFR4 signaling is an anti-FGFR4 antibody. In some embodiments the antagonist of FGFR4 signaling is an anti-FGFR4 antibody capable of binding more than one FGFR polypeptide. Examples of FGFR4 antibodies are known in the art and include, but are not limited to the antibodies described in WO2008/052796 and WO2005/037235, which are incorporated by reference in their entirety.

[00143] In some embodiments, the antagonist of FGFR4 signaling is a small molecule. In some embodiments, a weak antagonist of FGFR4 signaling is BGJ398 (Novartis, *i.e.*, 3-(2,6-Dichloro-3,5-dimethoxy-phenyl)-1-{6-[4-(4-ethyl-piperazin-1-yl)-phenylamino]-pyrimidin-4-yl}-1-methyl-urea and/or a pharmaceutically acceptable salt thereof; CAS# 872511-34-7). In some embodiments, a weak antagonist of FGFR4 is AZD4547 (AstraZeneca; *i.e.*, N-(5-(3,5-dimethoxyphenethyl)-1H-pyrazol-3-yl)-4-((3S,5R)-3,5-dimethylpiperazin-1-yl)benzamide and/or pharmaceutically acceptable salts thereof). In some embodiments, a weak antagonist of FGFR4 is FF284 (Chugai/Debiopharm (Debio 1347).

[00144] Exemplary FGFR antagonists are known in the art and include, but are not limited to, US5288855, US6344546, WO94/21813, US20070274981, WO2005/066211, WO2011/068893,

US5229501, US6656728, US7678890, WO95/021258, US6921763, US6713474, US6610688, US6297238, US20130053376, US20130039855, US2013004492, US20120316137, US20120251538, US20120195851, US20110129524, US20110053932, US20050227921, EP1761505, WO2012/125124, WO2012/123585, WO2011/099576, WO2011/035922, WO2009148928, WO2008/149521, WO2005/079390, WO2003/080064, WO2008/075068 (in particular Example 80), WO2005/080330, which are incorporated by reference in their entirety.

[00145] In some embodiments, the antagonist of FGFR signaling may be a specific inhibitor for FGFR/FGF, for example a specific inhibitor of FGFR1. In some embodiments, the inhibitor may be a dual inhibitor or pan inhibitor wherein the antagonist of FGFR signaling inhibits FGFR/FGF and one or more other target polypeptides and/or one or more FGFRs/FGFs.

[00146] Provided here are also B-raf antagonists useful in the methods described herein.

[00147] Exemplary B-raf antagonists include those known in the art, for example, vemurafenib (also known as Zelobraf® and PLX4032) sorafenib, PLX4720, PLX3603, GSK2118436, GDC-0879, N-(3-(5-(4-chlorophenyl)-1H-pyrrolo[2,3-b]pyridine-3-carbonyl)-2,4-difluorophenyl)propane-1-sulfonamide, and those described in WO2007/002325, WO2007/002433, WO2009111278, WO2009111279, WO2009111277, WO2009111280 and U.S. Pat. No. 7,491,829. Other B-raf antagonists include, GSK 2118436, RAF265 (Novartis), XL281, ARQ736, BAY73-4506. In some embodiments, the B-raf antagonist is a selective B-raf antagonist. In some embodiments, the B-raf antagonist is a selective antagonist of B-raf V600. In some embodiments, the B-raf antagonist is a selective antagonist of B-raf V600E. In some embodiments, B-raf V600 is B-raf V600E, B-raf V600K, and/or V600D. In some embodiments, B-raf V600 is B-raf V600R.

[00148] The B-raf antagonist may be a small molecule inhibitor. Small molecule inhibitors are preferably organic molecules other than polypeptides or antibodies as defined herein that bind, preferably specifically, to B-raf. In some embodiments, the B-raf antagonist is a kinase inhibitor. In some embodiments, the B-raf antagonist is an antibody, a peptide, a peptidomimetic, an aptomer or a polynubleotide.

[00149] Anti-B-raf antibodies that are useful in the methods include any antibody that binds with sufficient affinity and specificity to B-raf and can reduce or inhibit B-raf activity. The antibody selected will normally have a sufficiently strong binding affinity for B-raf, for example, the antibody may bind human B-raf with a Kd value of between 100 nM-1 pM. Antibody affinities may be determined by a surface plasmon resonance based assay (such as the BIAcore assay as described in PCT Application Publication No. WO2005/012359); enzyme-linked immunoabsorbent assay (ELISA); and competition assays (*e.g.*, RIA's), for example.

[00150] In some embodiments, the B-raf antagonist may be a specific inhibitor for B-raf. In some embodiments, the inhibitor may be a dual inhibitor or pan inhibitor wherein the B-raf antagonist inhibits B-raf and one or more other target polypeptides.

A. Antibodies

[00151] Provided herein isolated antibodies that bind to a polypeptide of interest, such as an FGFR (*e.g.*, FGFR1, FGFR2, FGFR3, and/or FGFR4), FGF (*e.g.*, FGF1-23), and/or B-raf for use in the methods described herein. In any of the above embodiments, an antibody is humanized. Further, the antibody according to any of the above embodiments is a monoclonal antibody, including a chimeric, humanized or human antibody. In one embodiment, the antibody is an antibody fragment, *e.g.*, a Fv, Fab, Fab', scFv, diabody, or $F(ab')_2$ fragment. In another embodiment, the antibody is a full length antibody, *e.g.*, an "intact IgG1" antibody or other antibody class or isotype as defined herein.

[00152] In a further aspect, an antibody according to any of the above embodiments may incorporate any of the features, singly or in combination, as described in Sections below:

1. Antibody Affinity

[00153] In certain embodiments, an antibody provided herein has a dissociation constant (Kd) of ≤ 1μM, ≤ 100 nM, ≤ 10 nM, ≤ 1 nM, ≤ 0.1 nM, ≤ 0.01 nM, or ≤ 0.001 nM (*e.g.*, 10^{-8} M or less, *e.g.*, from 10^{-8} M to 10^{-13} M, *e.g.*, from 10^{-9} M to 10^{-13} M). In one embodiment, Kd is measured by a radiolabeled antigen binding assay (RIA). In one embodiment, the RIA is performed with the Fab version of an antibody of interest and its antigen. For example, solution binding affinity of Fabs for antigen is measured by equilibrating Fab with a minimal concentration of (^{125}I)-labeled antigen in the presence of a titration series of unlabeled antigen, then capturing bound antigen with an anti-Fab antibody-coated plate (*see, e.g.*, Chen *et al.*, *J. Mol. Biol.* 293:865-881(1999)). To establish conditions for the assay, MICROTITER® multi-well plates (Thermo Scientific) are coated overnight with 5 μg/ml of a capturing anti-Fab antibody (Cappel Labs) in 50 mM sodium carbonate (pH 9.6), and subsequently blocked with 2% (w/v) bovine serum albumin in PBS for two to five hours at room temperature (approximately 23°C). In a non-adsorbent plate (Nunc #269620), 100 pM or 26 pM [^{125}I]-antigen are mixed with serial dilutions of a Fab of interest (*e.g.*, consistent with assessment of the anti-VEGF antibody, Fab-12, in Presta *et al.*, *Cancer Res.* 57:4593-4599 (1997)). The Fab of interest is then incubated overnight; however, the incubation may continue for a longer period (*e.g.*, about 65 hours) to ensure that equilibrium is reached. Thereafter, the mixtures are transferred to the capture plate for incubation at room temperature (*e.g.*, for one hour). The solution is then removed and the plate washed eight times with 0.1% polysorbate 20 (TWEEN-20®) in PBS. When the plates have dried, 150 μl/well

of scintillant (MICROSCINT-20 ™; Packard) is added, and the plates are counted on a TOPCOUNT ™ gamma counter (Packard) for ten minutes. Concentrations of each Fab that give less than or equal to 20% of maximal binding are chosen for use in competitive binding assays.

[00154] According to another embodiment, Kd is measured using a BIACORE® surface plasmon resonance assay. For example, an assay using a BIACORE®-2000 or a BIACORE ®-3000 (BIAcore, Inc., Piscataway, NJ) is performed at 25°C with immobilized antigen CM5 chips at ~10 response units (RU). In one embodiment, carboxymethylated dextran biosensor chips (CM5, BIACORE, Inc.) are activated with *N*-ethyl-*N'*- (3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) and *N*-hydroxysuccinimide (NHS) according to the supplier's instructions. Antigen is diluted with 10 mM sodium acetate, pH 4.8, to 5 μg/ml (~0.2 μM) before injection at a flow rate of 5 μl/minute to achieve approximately 10 response units (RU) of coupled protein. Following the injection of antigen, 1 M ethanolamine is injected to block unreacted groups. For kinetics measurements, two-fold serial dilutions of Fab (0.78 nM to 500 nM) are injected in PBS with 0.05% polysorbate 20 (TWEEN-20™) surfactant (PBST) at 25°C at a flow rate of approximately 25 μl/min. Association rates (k_{on}) and dissociation rates (k_{off}) are calculated using a simple one-to-one Langmuir binding model (BIACORE ® Evaluation Software version 3.2) by simultaneously fitting the association and dissociation sensorgrams. The equilibrium dissociation constant (Kd) is calculated as the ratio k_{off}/k_{on}. *See, e.g.*, Chen *et al.*, *J. Mol. Biol.* 293:865-881 (1999). If the on-rate exceeds 10^6 M^{-1} s^{-1} by the surface plasmon resonance assay above, then the on-rate can be determined by using a fluorescent quenching technique that measures the increase or decrease in fluorescence emission intensity (excitation = 295 nm; emission = 340 nm, 16 nm band-pass) at 25°C of a 20 nM anti-antigen antibody (Fab form) in PBS, pH 7.2, in the presence of increasing concentrations of antigen as measured in a spectrometer, such as a stop-flow equipped spectrophometer (Aviv Instruments) or a 8000-series SLM-AMINCO ™ spectrophotometer (ThermoSpectronic) with a stirred cuvette.

2. *Antibody Fragments*

[00155] In certain embodiments, an antibody provided herein is an antibody fragment. Antibody fragments include, but are not limited to, Fab, Fab', Fab'-SH, $F(ab')_2$, Fv, and scFv fragments, and other fragments described below. For a review of certain antibody fragments, *see* Hudson *et al. Nat. Med.* 9:129-134 (2003). For a review of scFv fragments, *see, e.g.*, Pluckthün, in *The Pharmacology of Monoclonal Antibodies*, vol. 113, Rosenburg and Moore eds., (Springer-Verlag, New York), pp. 269-315 (1994); *see* also WO 93/16185; and U.S. Patent Nos. 5,571,894 and 5,587,458. For discussion of Fab and $F(ab')_2$ fragments comprising salvage receptor binding epitope residues and having increased *in vivo* half-life, *see* U.S. Patent No. 5,869,046.

[00156] Diabodies are antibody fragments with two antigen-binding sites that may be bivalent or bispecific. *See,* for example, EP 404,097; WO 1993/01161; Hudson *et al.*, *Nat. Med.* 9:129-134 (2003); and Hollinger *et al.*, *Proc. Natl. Acad. Sci. USA* 90: 6444-6448 (1993). Triabodies and tetrabodies are also described in Hudson *et al.*, *Nat. Med.* 9:129-134 (2003).

[00157] Single-domain antibodies are antibody fragments comprising all or a portion of the heavy chain variable domain or all or a portion of the light chain variable domain of an antibody. In certain embodiments, a single-domain antibody is a human single-domain antibody (Domantis, Inc., Waltham, MA; *see, e.g.*, U.S. Patent No. 6,248,516).

[00158] Antibody fragments can be made by various techniques, including but not limited to proteolytic digestion of an intact antibody as well as production by recombinant host cells (*e.g., E. coli* or phage), as described herein.

3. Chimeric and Humanized Antibodies

[00159] In certain embodiments, an antibody provided herein is a chimeric antibody. Certain chimeric antibodies are described, *e.g.*, in U.S. Patent No. 4,816,567; and Morrison *et al., Proc. Natl. Acad. Sci. USA*, 81:6851-6855 (1984)). In one example, a chimeric antibody comprises a non-human variable region (*e.g.*, a variable region derived from a mouse, rat, hamster, rabbit, or non-human primate, such as a monkey) and a human constant region. In a further example, a chimeric antibody is a "class switched" antibody in which the class or subclass has been changed from that of the parent antibody. Chimeric antibodies include antigen-binding fragments thereof.

[00160] In certain embodiments, a chimeric antibody is a humanized antibody. Typically, a non-human antibody is humanized to reduce immunogenicity to humans, while retaining the specificity and affinity of the parental non-human antibody. Generally, a humanized antibody comprises one or more variable domains in which HVRs, *e.g.*, CDRs, (or portions thereof) are derived from a non-human antibody, and FRs (or portions thereof) are derived from human antibody sequences. A humanized antibody optionally will also comprise at least a portion of a human constant region. In some embodiments, some FR residues in a humanized antibody are substituted with corresponding residues from a non-human antibody (*e.g.*, the antibody from which the HVR residues are derived), *e.g.*, to restore or improve antibody specificity or affinity.

[00161] Humanized antibodies and methods of making them are reviewed, *e.g.*, in Almagro and Fransson, *Front. Biosci.* 13:1619-1633 (2008), and are further described, *e.g.*, in Riechmann *et al., Nature* 332:323-329 (1988); Queen *et al.*, *Proc. Nat'l Acad. Sci. USA* 86:10029-10033 (1989); US Patent Nos. 5, 821,337, 7,527,791, 6,982,321, and 7,087,409; Kashmiri *et al.*, *Methods* 36:25-34 (2005) (describing specificity-determining region (SDR) grafting); Padlan, *Mol. Immunol.* 28:489-498 (1991) (describing "resurfacing"); Dall'Acqua *et al.*, *Methods* 36:43-

60 (2005) (describing "FR shuffling"); and Osbourn *et al.*, *Methods* 36:61-68 (2005) and Klimka *et al.*, *Br. J. Cancer*, 83:252-260 (2000) (describing the "guided selection" approach to FR shuffling).

[00162] Human framework regions that may be used for humanization include but are not limited to: framework regions selected using the "best-fit" method (*see, e.g.*, Sims *et al. J. Immunol.* 151:2296 (1993)); framework regions derived from the consensus sequence of human antibodies of a particular subgroup of light or heavy chain variable regions (*see, e.g.*, Carter *et al. Proc. Natl. Acad. Sci. USA*, 89:4285 (1992); and Presta *et al. J. Immunol.*, 151:2623 (1993)); human mature (somatically mutated) framework regions or human germline framework regions (*see, e.g.*, Almagro and Fransson, *Front. Biosci.* 13:1619-1633 (2008)); and framework regions derived from screening FR libraries (*see, e.g.*, Baca *et al.*, *J. Biol. Chem.* 272:10678-10684 (1997) and Rosok *et al.*, *J. Biol. Chem.* 271:22611-22618 (1996)).

4. Human Antibodies

[00163] In certain embodiments, an antibody provided herein is a human antibody. Human antibodies can be produced using various techniques known in the art. Human antibodies are described generally in van Dijk and van de Winkel, *Curr. Opin. Pharmacol.* 5: 368-74 (2001) and Lonberg, *Curr. Opin. Immunol.* 20:450-459 (2008).

[00164] Human antibodies may be prepared by administering an immunogen to a transgenic animal that has been modified to produce intact human antibodies or intact antibodies with human variable regions in response to antigenic challenge. Such animals typically contain all or a portion of the human immunoglobulin loci, which replace the endogenous immunoglobulin loci, or which are present extrachromosomally or integrated randomly into the animal's chromosomes. In such transgenic mice, the endogenous immunoglobulin loci have generally been inactivated. For review of methods for obtaining human antibodies from transgenic animals, *see* Lonberg, *Nat. Biotech.* 23:1117-1125 (2005). *See* also, *e.g.*, U.S. Patent Nos. 6,075,181 and 6,150,584 describing XENOMOUSE™ technology; U.S. Patent No. 5,770,429 describing HuMab® technology; U.S. Patent No. 7,041,870 describing K-M MOUSE® technology, and U.S. Patent Application Publication No. US 2007/0061900, describing VelociMouse® technology). Human variable regions from intact antibodies generated by such animals may be further modified, *e.g.*, by combining with a different human constant region.

[00165] Human antibodies can also be made by hybridoma-based methods. Human myeloma and mouse-human heteromyeloma cell lines for the production of human monoclonal antibodies have been described. (*See, e.g.*, Kozbor *J. Immunol.*, 133: 3001 (1984); Brodeur *et al.*, Monoclonal Antibody Production Techniques and Applications, pp. 51-63 (Marcel Dekker, Inc., New York,

1987); and Boerner *et al.*, *J. Immunol.*, 147: 86 (1991).) Human antibodies generated via human B-cell hybridoma technology are also described in Li *et al.*, Proc. Natl. Acad. Sci. USA, 103:3557-3562 (2006). Additional methods include those described, for example, in U.S. Patent No. 7,189,826 (describing production of monoclonal human IgM antibodies from hybridoma cell lines) and Ni, Xiandai Mianyixue, 26(4):265-268 (2006) (describing human-human hybridomas). Human hybridoma technology (Trioma technology) is also described in Vollmers and Brandlein, *Hist. & Histopath.*, 20(3):927-937 (2005) and Vollmers and Brandlein, *Methods Find Exp. Clin. Pharmacol.*, 27(3):185-91 (2005).

[00166] Human antibodies may also be generated by isolating Fv clone variable domain sequences selected from human-derived phage display libraries. Such variable domain sequences may then be combined with a desired human constant domain. Techniques for selecting human antibodies from antibody libraries are described below.

5. Library-Derived Antibodies

[00167] Antibodies may be isolated by screening combinatorial libraries for antibodies with the desired activity or activities. For example, a variety of methods are known in the art for generating phage display libraries and screening such libraries for antibodies possessing the desired binding characteristics. Such methods are reviewed, *e.g.*, in Hoogenboom *et al. Methods Mol. Biol.* 178:1-37 (O'Brien *et al.*, ed., Human Press, Totowa, NJ, 2001) and further described, *e.g.*, in the McCafferty *et al.*, *Nature* 348:552-554; Clackson *et al.*, *Nature* 352: 624-628 (1991); Marks *et al.*, *J. Mol. Biol.* 222: 581-597 (1992); Marks and Bradbury, *Methods Mol. Biol.* 248:161-175 (Lo, ed., Human Press, Totowa, NJ, 2003); Sidhu *et al.*, J. Mol. Biol. 338(2): 299-310 (2004); Lee *et al.*, *J. Mol. Biol.* 340(5): 1073-1093 (2004); Fellouse, *Proc. Natl. Acad. Sci. USA* 101(34): 12467-12472 (2004); and Lee *et al.*, *J. Immunol. Methods* 284(1-2): 119-132(2004).

[00168] In certain phage display methods, repertoires of VH and VL genes are separately cloned by polymerase chain reaction (PCR) and recombined randomly in phage libraries, which can then be screened for antigen-binding phage as described in Winter *et al.*, *Ann. Rev. Immunol.*, 12: 433-455 (1994). Phage typically display antibody fragments, either as single-chain Fv (scFv) fragments or as Fab fragments. Libraries from immunized sources provide high-affinity antibodies to the immunogen without the requirement of constructing hybridomas. Alternatively, the naive repertoire can be cloned (*e.g.*, from human) to provide a single source of antibodies to a wide range of non-self and also self antigens without any immunization as described by Griffiths *et al.*, *EMBO J*, 12: 725-734 (1993). Finally, naive libraries can also be made synthetically by cloning unrearranged V-gene segments from stem cells, and using PCR primers containing

random sequence to encode the highly variable CDR3 regions and to accomplish rearrangement in vitro, as described by Hoogenboom and Winter, *J. Mol. Biol.*, 227: 381-388 (1992). Patent publications describing human antibody phage libraries include, for example: US Patent No. 5,750,373, and US Patent Publication Nos. 2005/0079574, 2005/0119455, 2005/0266000, 2007/0117126, 2007/0160598, 2007/0237764, 2007/0292936, and 2009/0002360.

[00169] Antibodies or antibody fragments isolated from human antibody libraries are considered human antibodies or human antibody fragments herein.

6. *Multispecific Antibodies*

[00170] In certain embodiments, an antibody provided herein is a multispecific antibody, *e.g.,* a bispecific antibody. Multispecific antibodies are monoclonal antibodies that have binding specificities for at least two different sites. In certain embodiments, one of the binding specificities is a polypeptide of interest, such as FGFR (*e.g.*, FGFR1, FGFR2, FGFR3, and/or FGFR4), FGF (*e.g.*, FGF1-23), and/or B-raf and the other is for any other antigen. In certain embodiments, bispecific antibodies may bind to two different epitopes of a polypeptide of interest, such as FGFR/FGF and/or B-raf. Bispecific antibodies may also be used to localize cytotoxic agents to cells which express a polypeptide of interest, such as FGFR (*e.g.*, FGFR1, FGFR2, FGFR3, and/or FGFR4), FGF (*e.g.*, FGF1-23), and/or B-raf. Bispecific antibodies can be prepared as full length antibodies or antibody fragments.

[00171] Techniques for making multispecific antibodies include, but are not limited to, recombinant co-expression of two immunoglobulin heavy chain-light chain pairs having different specificities (*see* Milstein and Cuello, *Nature* 305: 537 (1983)), WO 93/08829, and Traunecker *et al.*, *EMBO J.* 10: 3655 (1991)), and "knob-in-hole" engineering (*see, e.g.*, U.S. Patent No. 5,731,168). Multi-specific antibodies may also be made by engineering electrostatic steering effects for making antibody Fc-heterodimeric molecules (WO 2009/089004A1); cross-linking two or more antibodies or fragments (*see, e.g.*, US Patent No. 4,676,980, and Brennan *et al.*, Science, 229: 81 (1985)); using leucine zippers to produce bi-specific antibodies (*see, e.g.*, Kostelny *et al.*, *J. Immunol.*, 148(5):1547-1553 (1992)); using "diabody" technology for making bispecific antibody fragments (*see, e.g.*, Hollinger *et al.*, *Proc. Natl. Acad. Sci. USA*, 90:6444-6448 (1993)); and using single-chain Fv (sFv) dimers (*see,e.g.,* Gruber *et al.*, J. Immunol., 152:5368 (1994)); and preparing trispecific antibodies as described, *e.g.*, in Tutt *et al. J. Immunol.* 147: 60 (1991).

[00172] Engineered antibodies with three or more functional antigen binding sites, including "Octopus antibodies," are also included herein (*see, e.g.,* US 2006/0025576A1).

[00173] The antibody or fragment herein also includes a "Dual Acting FAb" or "DAF" comprising an antigen binding site that binds to a polypeptide of interest, such as FGFR (*e.g.*, FGFR1, FGFR2, FGFR3, and/or FGFR4), FGF (*e.g.*, FGF1-23), and/or B-raf as well as another, different antigen (*see,* US 2008/0069820, for example).

7. Antibody Variants

a) Glycosylation variants

[00174] In certain embodiments, an antibody provided herein is altered to increase or decrease the extent to which the antibody is glycosylated. Addition or deletion of glycosylation sites to an antibody may be conveniently accomplished by altering the amino acid sequence such that one or more glycosylation sites is created or removed.

[00175] Where the antibody comprises an Fc region, the carbohydrate attached thereto may be altered. Native antibodies produced by mammalian cells typically comprise a branched, biantennary oligosaccharide that is generally attached by an N-linkage to Asn297 of the CH2 domain of the Fc region. *See, e.g.*, Wright *et al. TIBTECH* 15:26-32 (1997). The oligosaccharide may include various carbohydrates, *e.g.*, mannose, N-acetyl glucosamine (GlcNAc), galactose, and sialic acid, as well as a fucose attached to a GlcNAc in the "stem" of the biantennary oligosaccharide structure. In some embodiments, modifications of the oligosaccharide in an antibody of the invention may be made in order to create antibody variants with certain improved properties.

[00176] In one embodiment, antibody variants are provided having a carbohydrate structure that lacks fucose attached (directly or indirectly) to an Fc region. For example, the amount of fucose in such antibody may be from 1% to 80%, from 1% to 65%, from 5% to 65% or from 20% to 40%. The amount of fucose is determined by calculating the average amount of fucose within the sugar chain at Asn297, relative to the sum of all glycostructures attached to Asn 297 (e. g. complex, hybrid and high mannose structures) as measured by MALDI-TOF mass spectrometry, as described in WO 2008/077546, for example. Asn297 refers to the asparagine residue located at about position 297 in the Fc region (Eu numbering of Fc region residues); however, Asn297 may also be located about ± 3 amino acids upstream or downstream of position 297, *i.e.*, between positions 294 and 300, due to minor sequence variations in antibodies. Such fucosylation variants may have improved ADCC function. *See, e.g.*, US Patent Publication Nos. US 2003/0157108 (Presta, L.); US 2004/0093621 (Kyowa Hakko Kogyo Co., Ltd). Examples of publications related to "defucosylated" or "fucose-deficient" antibody variants include: US 2003/0157108; WO 2000/61739; WO 2001/29246; US 2003/0115614; US 2002/0164328; US 2004/0093621; US 2004/0132140; US 2004/0110704; US 2004/0110282; US 2004/0109865; WO 2003/085119; WO

2003/084570; WO 2005/035586; WO 2005/035778; WO2005/053742; WO2002/031140; Okazaki *et al. J. Mol. Biol.* 336:1239-1249 (2004); Yamane-Ohnuki *et al.*, *Biotech. Bioeng.* 87: 614 (2004). Examples of cell lines capable of producing defucosylated antibodies include Lec13 CHO cells deficient in protein fucosylation (Ripka *et al. Arch. Biochem. Biophys.* 249:533-545 (1986); US Pat Appl No US 2003/0157108 A1, Presta, L; and WO 2004/056312 A1, Adams *et al.*, especially at Example 11), and knockout cell lines, such as alpha-1,6-fucosyltransferase gene, FUT8, knockout CHO cells (*see, e.g.*, Yamane-Ohnuki *et al. Biotech. Bioeng.* 87: 614 (2004); Kanda, Y. *et al.*, *Biotechnol. Bioeng.*, 94(4):680-688 (2006); and WO2003/085107).

[00177] Antibodies variants are further provided with bisected oligosaccharides, *e.g.*, in which a biantennary oligosaccharide attached to the Fc region of the antibody is bisected by GlcNAc. Such antibody variants may have reduced fucosylation and/or improved ADCC function. Examples of such antibody variants are described, *e.g.*, in WO 2003/011878 (Jean-Mairet *et al.*); US Patent No. 6,602,684 (Umana *et al.*); and US 2005/0123546 (Umana *et al.*). Antibody variants with at least one galactose residue in the oligosaccharide attached to the Fc region are also provided. Such antibody variants may have improved CDC function. Such antibody variants are described, *e.g.*, in WO 1997/30087 (Patel *et al.*); WO 1998/58964 (Raju, S.); and WO 1999/22764 (Raju, S.).

b) Fc region variants

[00178] In certain embodiments, one or more amino acid modifications may be introduced into the Fc region of an antibody provided herein, thereby generating an Fc region variant. The Fc region variant may comprise a human Fc region sequence (*e.g.*, a human IgG1, IgG2, IgG3 or IgG4 Fc region) comprising an amino acid modification (*e.g.,* a substitution) at one or more amino acid positions.

[00179] In certain embodiments, the invention contemplates an antibody variant that possesses some but not all effector functions, which make it a desirable candidate for applications in which the half life of the antibody *in vivo* is important yet certain effector functions (such as complement and ADCC) are unnecessary or deleterious. *In vitro* and/or *in vivo* cytotoxicity assays can be conducted to confirm the reduction/depletion of CDC and/or ADCC activities. For example, Fc receptor (FcR) binding assays can be conducted to ensure that the antibody lacks FcγR binding (hence likely lacking ADCC activity), but retains FcRn binding ability. The primary cells for mediating ADCC, NK cells, express FcγRIII only, whereas monocytes express FcγRI, FcγRII and FcγRIII. FcR expression on hematopoietic cells is summarized in Table 3 on page 464 of Ravetch and Kinet, *Annu. Rev. Immunol.* 9:457-492 (1991). Non-limiting examples of *in vitro* assays to assess ADCC activity of a molecule of interest is described in U.S. Patent

No. 5,500,362 (*see, e.g.,* Hellstrom, I. *et al. Proc. Nat'l Acad. Sci. USA* 83:7059-7063 (1986)) and Hellstrom, I *et al.*, *Proc. Nat'l Acad. Sci. USA* 82:1499-1502 (1985); 5,821,337 (*see* Bruggemann, M. *et al.*, *J. Exp. Med.* 166:1351-1361 (1987)). Alternatively, non-radioactive assays methods may be employed (*see,* for example, ACTI™ non-radioactive cytotoxicity assay for flow cytometry (CellTechnology, Inc. Mountain View, CA; and CytoTox 96® non-radioactive cytotoxicity assay (Promega, Madison, WI). Useful effector cells for such assays include peripheral blood mononuclear cells (PBMC) and Natural Killer (NK) cells. Alternatively, or additionally, ADCC activity of the molecule of interest may be assessed *in vivo*, *e.g.*, in an animal model such as that disclosed in Clynes *et al. Proc. Nat'l Acad. Sci. USA* 95:652-656 (1998). C1q binding assays may also be carried out to confirm that the antibody is unable to bind C1q and hence lacks CDC activity. *See, e.g.*, C1q and C3c binding ELISA in WO 2006/029879 and WO 2005/100402. To assess complement activation, a CDC assay may be performed (*see,* for example, Gazzano-Santoro *et al.*, *J. Immunol. Methods* 202:163 (1996); Cragg, M.S. *et al.*, *Blood* 101:1045-1052 (2003); and Cragg, M.S. and M.J. Glennie, *Blood* 103:2738-2743 (2004)). FcRn binding and *in vivo* clearance/half life determinations can also be performed using methods known in the art (*see, e.g.*, Petkova, S.B. *et al.*, *Int'l. Immunol.* 18(12):1759-1769 (2006)).

[00180] Antibodies with reduced effector function include those with substitution of one or more of Fc region residues 238, 265, 269, 270, 297, 327 and 329 (U.S. Patent No. 6,737,056). Such Fc mutants include Fc mutants with substitutions at two or more of amino acid positions 265, 269, 270, 297 and 327, including the so-called "DANA" Fc mutant with substitution of residues 265 and 297 to alanine (US Patent No. 7,332,581).

[00181] Certain antibody variants with improved or diminished binding to FcRs are described. (*See, e.g.*, U.S. Patent No. 6,737,056; WO 2004/056312, and Shields *et al.*, *J. Biol. Chem.* 9(2): 6591-6604 (2001).) In certain embodiments, an antibody variant comprises an Fc region with one or more amino acid substitutions which improve ADCC, *e.g.*, substitutions at positions 298, 333, and/or 334 of the Fc region (EU numbering of residues). In some embodiments, alterations are made in the Fc region that result in altered (*i.e.*, either improved or diminished) C1q binding and/or Complement Dependent Cytotoxicity (CDC), *e.g.*, as described in US Patent No. 6,194,551, WO 99/51642, and Idusogie *et al. J. Immunol.* 164: 4178-4184 (2000).

[00182] Antibodies with increased half-lives and improved binding to the neonatal Fc receptor (FcRn), which is responsible for the transfer of maternal IgGs to the fetus (Guyer *et al.*, *J. Immunol.* 117:587 (1976) and Kim *et al.*, *J. Immunol.* 24:249 (1994)), are described in US2005/0014934A1 (Hinton *et al.*). Those antibodies comprise an Fc region with one or more substitutions therein which improve binding of the Fc region to FcRn. Such Fc variants include

those with substitutions at one or more of Fc region residues: 238, 256, 265, 272, 286, 303, 305, 307, 311, 312, 317, 340, 356, 360, 362, 376, 378, 380, 382, 413, 424 or 434, *e.g.*, substitution of Fc region residue 434 (US Patent No. 7,371,826). *See* also Duncan & Winter, *Nature* 322:738-40 (1988); U.S. Patent No. 5,648,260; U.S. Patent No. 5,624,821; and WO 94/29351 concerning other examples of Fc region variants.

c) Cysteine engineered antibody variants

[00183] In certain embodiments, it may be desirable to create cysteine engineered antibodies, *e.g.*, by using the THIOMAB™ technology, in which one or more residues of an antibody are substituted with cysteine residues. In particular embodiments, the substituted residues occur at accessible sites of the antibody. By substituting those residues with cysteine, reactive thiol groups are thereby positioned at accessible sites of the antibody and may be used to conjugate the antibody to other moieties, such as drug moieties or linker-drug moieties, to create an immunoconjugate, as described further herein. In certain embodiments, any one or more of the following residues may be substituted with cysteine: V205 (Kabat numbering) of the light chain; A118 (EU numbering) of the heavy chain; and S400 (EU numbering) of the heavy chain Fc region. Additional antibodies can be designed with cysteine substitutions as described in U.S. Pat. Nos. 7,521,541 and U.S. Pat. Pub. No. 20110301334 which are incorporated in their entirety herein. Cysteine engineered antibodies may be generated as described, *e.g.*, in U.S. Patent No. 7,521,541.

B. Immunoconjugates

[00184] Further provided herein are immunoconjugates comprising antibodies which bind a polypeptide of interest such as FGFR (*e.g.*, FGFR1, FGFR2, FGFR3, and/or FGFR4), FGF (*e.g.*, FGF1-23), or B-raf, conjugated to one or more cytotoxic agents, such as chemotherapeutic agents or drugs, growth inhibitory agents, toxins (*e.g.*, protein toxins, enzymatically active toxins of bacterial, fungal, plant, or animal origin, or fragments thereof), or radioactive isotopes for use in the methods described herein.

[00185] In one embodiment, an immunoconjugate is an antibody-drug conjugate (ADC) in which an antibody is conjugated to one or more drugs, including but not limited to a maytansinoid (*see* U.S. Patent Nos. 5,208,020, 5,416,064 and European Patent EP 0 425 235 B1); an auristatin such as monomethylauristatin drug moieties DE and DF (MMAE and MMAF) (*see* U.S. Patent Nos. 5,635,483 and 5,780,588, and 7,498,298); a dolastatin; a calicheamicin or derivative thereof (*see* U.S. Patent Nos. 5,712,374, 5,714,586, 5,739,116, 5,767,285, 5,770,701, 5,770,710, 5,773,001, and 5,877,296; Hinman *et al.*, *Cancer Res.* 53:3336-3342 (1993); and Lode *et al.*, *Cancer Res.* 58:2925-2928 (1998)); an anthracycline such as daunomycin or doxorubicin (*see* Kratz *et al.*,

Current Med. Chem. 13:477-523 (2006); Jeffrey *et al.*, *Bioorganic & Med. Chem. Letters* 16:358-362 (2006); Torgov *et al.*, *Bioconj. Chem.* 16:717-721 (2005); Nagy *et al.*, *Proc. Natl. Acad. Sci. USA* 97:829-834 (2000); Dubowchik *et al.*, *Bioorg. & Med. Chem. Letters* 12:1529-1532 (2002); King *et al.*, *J. Med. Chem.* 45:4336-4343 (2002); and U.S. Patent No. 6,630,579); methotrexate; vindesine; a taxane such as docetaxel, paclitaxel, larotaxel, tesetaxel, and ortataxel; a trichothecene; and CC1065.

[00186] In another embodiment, an immunoconjugate comprises an antibody as described herein conjugated to an enzymatically active toxin or fragment thereof, including but not limited to diphtheria A chain, nonbinding active fragments of diphtheria toxin, exotoxin A chain (from Pseudomonas aeruginosa), ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, Aleurites fordii proteins, dianthin proteins, Phytolaca americana proteins (PAPI, PAPII, and PAP-S), momordica charantia inhibitor, curcin, crotin, sapaonaria officinalis inhibitor, gelonin, mitogellin, restrictocin, phenomycin, enomycin, and the tricothecenes.

[00187] In another embodiment, an immunoconjugate comprises an antibody as described herein conjugated to a radioactive atom to form a radioconjugate. A variety of radioactive isotopes are available for the production of radioconjugates. Examples include At^{211}, I^{131}, I^{125}, Y^{90}, Re^{186}, Re^{188}, Sm^{153}, Bi^{212}, P^{32}, Pb^{212} and radioactive isotopes of Lu. When the radioconjugate is used for detection, it may comprise a radioactive atom for scintigraphic studies, for example Tc^{99m} or I^{123}, or a spin label for nuclear magnetic resonance (NMR) imaging (also known as magnetic resonance imaging, mri), such as iodine-123 again, iodine-131, indium-111, fluorine-19, carbon-13, nitrogen-15, oxygen-17, gadolinium, manganese or iron.

[00188] Conjugates of an antibody and cytotoxic agent may be made using a variety of bifunctional protein coupling agents such as N-succinimidyl-3-(2-pyridyldithio) propionate (SPDP), succinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate (SMCC), iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCl), active esters (such as disuccinimidyl suberate), aldehydes (such as glutaraldehyde), bis-azido compounds (such as bis (p-azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as toluene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene). For example, a ricin immunotoxin can be prepared as described in Vitetta *et al.*, *Science* 238:1098 (1987). Carbon-14-labeled 1-isothiocyanatobenzyl-3-methyldiethylene triaminepentaacetic acid (MX-DTPA) is an exemplary chelating agent for conjugation of radionucleotide to the antibody. *See* WO94/11026. The linker may be a “cleavable linker” facilitating release of a cytotoxic drug in the cell. For example, an acid-labile linker, peptidase-sensitive linker, photolabile linker,

dimethyl linker or disulfide-containing linker (Chari *et al.*, *Cancer Res.* 52:127-131 (1992); U.S. Patent No. 5,208,020) may be used.

[00189] The immunuoconjugates or ADCs herein expressly contemplate, but are not limited to such conjugates prepared with cross-linker reagents including, but not limited to, BMPS, EMCS, GMBS, HBVS, LC-SMCC, MBS, MPBH, SBAP, SIA, SIAB, SMCC, SMPB, SMPH, sulfo-EMCS, sulfo-GMBS, sulfo-KMUS, sulfo-MBS, sulfo-SIAB, sulfo-SMCC, and sulfo-SMPB, and SVSB (succinimidyl-(4-vinylsulfone)benzoate) which are commercially available (*e.g.*, from Pierce Biotechnology, Inc., Rockford, IL., U.S.A).

C. Binding Polypeptides

[00190] Binding polypeptides are polypeptides that bind, preferably specifically, to FGFR (*e.g.*, FGFR1, FGFR2, FGFR3, and/or FGFR4), FGF (*e.g.*, FGF1-23), and/or B-raf are also provided for use in the methods described herein. In some embodiments, the binding polypeptides are FGFR (*e.g.*, FGFR1, FGFR2, FGFR3, and/or FGFR4) and/or FGF (*e.g.*, FGF1-23) antagonists and/or B-raf antagonists. Binding polypeptides may be chemically synthesized using known polypeptide synthesis methodology or may be prepared and purified using recombinant technology. Binding polypeptides are usually at least about 5 amino acids in length, alternatively at least about 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100 amino acids in length or more, wherein such binding polypeptides that are capable of binding, preferably specifically, to a target, *e.g.*, FGFR (*e.g.*, FGFR1, FGFR2, FGFR3, and/or FGFR4), FGF (*e.g.*, FGF1-23), or B-raf, as described herein. Binding polypeptides may be identified without undue experimentation using well known techniques. In this regard, it is noted that techniques for screening polypeptide libraries for binding polypeptides that are capable of specifically binding to a polypeptide target are well known in the art (*see, e.g.*, U.S. Patent Nos. 5,556,762, 5,750,373, 4,708,871, 4,833,092, 5,223,409, 5,403,484, 5,571,689, 5,663,143; PCT Publication Nos. WO 84/03506 and WO84/03564; Geysen *et al.*, *Proc. Natl. Acad. Sci. U.S.A.*, 81:3998-4002 (1984); Geysen *et al.*, *Proc. Natl. Acad. Sci. U.S.A.*, 82:178-182 (1985); Geysen *et al.*, in Synthetic Peptides as Antigens, 130-149 (1986); Geysen *et al.*, *J. Immunol. Meth*., 102:259-274 (1987); Schoofs *et al.*, *J. Immunol*., 140:611-616 (1988), Cwirla, S. E. *et al.* (1990) *Proc. Natl. Acad. Sci. USA*, 87:6378; Lowman, H.B. *et al.* (1991) *Biochemistry*, 30:10832; Clackson, T. *et al.* (1991) *Nature*, 352: 624; Marks, J. D. *et al.* (1991), *J. Mol. Biol.*, 222:581; Kang, A.S. *et al.* (1991) *Proc. Natl. Acad. Sci. USA*, 88:8363, and Smith, G. P. (1991) *Current Opin. Biotechnol.*, 2:668).

[00191] Methods of generating peptide libraries and screening these libraries are also disclosed in U.S. Patent Nos. 5,723,286, 5,432,018, 5,580,717, 5,427,908, 5,498,530, 5,770,434, 5,734,018, 5,698,426, 5,763,192, and 5,723,323.

D. Binding Small Molecules

[00192] Provided herein are binding small molecules for use as a small molecule antagonist of FGFR (*e.g.*, FGFR1, FGFR2, FGFR3, and/or FGFR4), FGF (*e.g.*, FGF1-23), and/or B-raf for use in the methods described above.

[00193] Binding small molecules are preferably organic molecules other than binding polypeptides or antibodies as defined herein that bind, preferably specifically, to FGFR (*e.g.*, FGFR1, FGFR2, FGFR3, and/or FGFR4), FGF (*e.g.*, FGF1-23), and/ or B-raf as described herein. Binding organic small molecules may be identified and chemically synthesized using known methodology (*see, e.g.*, PCT Publication Nos. WO00/00823 and WO00/39585). Binding organic small molecules are usually less than about 2000 daltons in size, alternatively less than about 1500, 750, 500, 250 or 200 daltons in size, wherein such organic small molecules that are capable of binding, preferably specifically, to a polypeptide as described herein may be identified without undue experimentation using well known techniques. In this regard, it is noted that techniques for screening organic small molecule libraries for molecules that are capable of binding to a polypeptide of interest are well known in the art (*see, e.g.*, PCT Publication Nos. WO00/00823 and WO00/39585). Binding organic small molecules may be, for example, aldehydes, ketones, oximes, hydrazones, semicarbazones, carbazides, primary amines, secondary amines, tertiary amines, N-substituted hydrazines, hydrazides, alcohols, ethers, thiols, thioethers, disulfides, carboxylic acids, esters, amides, ureas, carbamates, carbonates, ketals, thioketals, acetals, thioacetals, aryl halides, aryl sulfonates, alkyl halides, alkyl sulfonates, aromatic compounds, heterocyclic compounds, anilines, alkenes, alkynes, diols, amino alcohols, oxazolidines, oxazolines, thiazolidines, thiazolines, enamines, sulfonamides, epoxides, aziridines, isocyanates, sulfonyl chlorides, diazo compounds, acid chlorides, or the like.

E. Antagonist Polynucleotides

[00194] Provided herein are also polynucleotide antagonists for use in the methods described herein. The polynucleotide may be an antisense nucleic acid and/or a ribozyme. The antisense nucleic acids comprise a sequence complementary to at least a portion of an RNA transcript of a gene of interest, such as FGFR (*e.g.*, FGFR1, FGFR2, FGFR3, and/or FGFR4), FGF (*e.g.*, FGF1-23), and/or B-raf gene. However, absolute complementarity, although preferred, is not required.

[00195] A sequence "complementary to at least a portion of an RNA," referred to herein, means a sequence having sufficient complementarity to be able to hybridize with the RNA, forming a

stable duplex; in the case of double stranded antisense nucleic acids, a single strand of the duplex DNA may thus be tested, or triplex formation may be assayed. The ability to hybridize will depend on both the degree of complementarity and the length of the antisense nucleic acid. Generally, the larger the hybridizing nucleic acid, the more base mismatches with a RNA it may contain and still form a stable duplex (or triplex as the case may be). One skilled in the art can ascertain a tolerable degree of mismatch by use of standard procedures to determine the melting point of the hybridized complex.

[00196] Polynucleotides that are complementary to the 5' end of the message, *e.g.*, the 5' untranslated sequence up to and including the AUG initiation codon, should work most efficiently at inhibiting translation. However, sequences complementary to the 3' untranslated sequences of mRNAs have been shown to be effective at inhibiting translation of mRNAs as well. *See generally*, Wagner, R., 1994, *Nature* 372:333-335. Thus, oligonucleotides complementary to either the 5'- or 3'-non-translated, non-coding regions of the gene, could be used in an antisense approach to inhibit translation of endogenous mRNA. Polynucleotides complementary to the 5' untranslated region of the mRNA should include the complement of the AUG start codon. Antisense polynucleotides complementary to mRNA coding regions are less efficient inhibitors of translation but could be used in accordance with the invention. Whether designed to hybridize to the 5'-, 3'- or coding region of an mRNA, antisense nucleic acids should be at least six nucleotides in length, and are preferably oligonucleotides ranging from 6 to about 50 nucleotides in length. In specific aspects the oligonucleotide is at least 10 nucleotides, at least 17 nucleotides, at least 25 nucleotides or at least 50 nucleotides.

F. Antibody and Binding Polypeptide Variants

[00197] In certain embodiments, amino acid sequence variants of the antibodies and/or the binding polypeptides provided herein are contemplated. For example, it may be desirable to improve the binding affinity and/or other biological properties of the antibody and/or binding polypeptide. Amino acid sequence variants of an antibody and/or binding polypeptides may be prepared by introducing appropriate modifications into the nucleotide sequence encoding the antibody and/or binding polypeptide, or by peptide synthesis. Such modifications include, for example, deletions from, and/or insertions into and/or substitutions of residues within the amino acid sequences of the antibody and/or binding polypeptide. Any combination of deletion, insertion, and substitution can be made to arrive at the final construct, provided that the final construct possesses the desired characteristics, *e.g.*, antigen-binding.

[00198] In certain embodiments, antibody variants and/or binding polypeptide variants having one or more amino acid substitutions are provided. Sites of interest for substitutional mutagenesis

include the HVRs and FRs. Conservative substitutions are shown in Table 1 under the heading of "preferred substitutions." More substantial changes are provided in Table 1 under the heading of "exemplary substitutions," and as further described below in reference to amino acid side chain classes. Amino acid substitutions may be introduced into an antibody and/or binding polypeptide of interest and the products screened for a desired activity, *e.g.*, retained/improved antigen binding, decreased immunogenicity, or improved ADCC or CDC.

TABLE 1

Original Residue	Exemplary Substitutions	Preferred Substitutions
Ala (A)	Val; Leu; Ile	Val
Arg (R)	Lys; Gln; Asn	Lys
Asn (N)	Gln; His; Asp, Lys; Arg	Gln
Asp (D)	Glu; Asn	Glu
Cys (C)	Ser; Ala	Ser
Gln (Q)	Asn; Glu	Asn
Glu (E)	Asp; Gln	Asp
Gly (G)	Ala	Ala
His (H)	Asn; Gln; Lys; Arg	Arg
Ile (I)	Leu; Val; Met; Ala; Phe; Norleucine	Leu
Leu (L)	Norleucine; Ile; Val; Met; Ala; Phe	Ile
Lys (K)	Arg; Gln; Asn	Arg
Met (M)	Leu; Phe; Ile	Leu
Phe (F)	Trp; Leu; Val; Ile; Ala; Tyr	Tyr
Pro (P)	Ala	Ala
Ser (S)	Thr	Thr
Thr (T)	Val; Ser	Ser
Trp (W)	Tyr; Phe	Tyr
Tyr (Y)	Trp; Phe; Thr; Ser	Phe
Val (V)	Ile; Leu; Met; Phe; Ala; Norleucine	Leu

[00199] Amino acids may be grouped according to common side-chain properties:

(1) hydrophobic: Norleucine, Met, Ala, Val, Leu, Ile;

(2) neutral hydrophilic: Cys, Ser, Thr, Asn, Gln;

(3) acidic: Asp, Glu;

(4) basic: His, Lys, Arg;

(5) residues that influence chain orientation: Gly, Pro;

(6) aromatic: Trp, Tyr, Phe.

[00200] Non-conservative substitutions will entail exchanging a member of one of these classes for another class.

G. Antibody and Binding Polypeptide Derivatives

[00201] In certain embodiments, an antibody and/or binding polypeptide provided herein may be further modified to contain additional nonproteinaceous moieties that are known in the art and readily available. The moieties suitable for derivatization of the antibody and/or binding polypeptide include but are not limited to water soluble polymers. Non-limiting examples of water soluble polymers include, but are not limited to, polyethylene glycol (PEG), copolymers of ethylene glycol/propylene glycol, carboxymethylcellulose, dextran, polyvinyl alcohol, polyvinyl pyrrolidone, poly-1, 3-dioxolane, poly-1,3,6-trioxane, ethylene/maleic anhydride copolymer, polyaminoacids (either homopolymers or random copolymers), and dextran or poly(n-vinyl pyrrolidone)polyethylene glycol, propropylene glycol homopolymers, prolypropylene oxide/ethylene oxide co-polymers, polyoxyethylated polyols (*e.g.*, glycerol), polyvinyl alcohol, and mixtures thereof. Polyethylene glycol propionaldehyde may have advantages in manufacturing due to its stability in water. The polymer may be of any molecular weight, and may be branched or unbranched. The number of polymers attached to the antibody and/or binding polypeptide may vary, and if more than one polymer are attached, they can be the same or different molecules. In general, the number and/or type of polymers used for derivatization can be determined based on considerations including, but not limited to, the particular properties or functions of the antibody and/or binding polypeptide to be improved, whether the antibody derivative and/or binding polypeptide derivative will be used in a therapy under defined conditions, etc.

[00202] In another embodiment, conjugates of an antibody and/or binding polypeptide to nonproteinaceous moiety that may be selectively heated by exposure to radiation are provided. In one embodiment, the nonproteinaceous moiety is a carbon nanotube (Kam *et al.*, *Proc. Natl. Acad. Sci. USA* 102: 11600-11605 (2005)). The radiation may be of any wavelength, and includes, but is not limited to, wavelengths that do not harm ordinary cells, but which heat the nonproteinaceous moiety to a temperature at which cells proximal to the antibody and/or binding polypeptide-nonproteinaceous moiety are killed.

IV. Methods of Screening and/or Identifying Antagonists of FGFR signaling With Desired Function

[00203] Additional antagonists of a polypeptide of interest, such as FGFR (*e.g.*, FGFR1, FGFR2, FGFR3, and/or FGFR4), FGF (*e.g.*, FGF1-23), and/or B-raf for use in the methods described herein, including antibodies, binding polypeptides, and/or small molecules have been described above. Additional antagonists of such as antibodies, binding polypeptides, and/or binding small molecules provided herein may be identified, screened for, or characterized for their physical/chemical properties and/or biological activities by various assays known in the art.

[00204] In certain embodiments, a computer system comprising a memory comprising atomic coordinates of FGFR (*e.g.*, FGFR1, FGFR2, FGFR3, and/or FGFR4) and/or FGF (*e.g.*, FGF1-23), polypeptide are useful as models for rationally identifying compounds that a ligand binding site of FGFR signaling. Such compounds may be designed either de novo, or by modification of a known compound, for example. In other cases, binding compounds may be identified by testing known compounds to determine if the "dock" with a molecular model of FGFR (*e.g.*, FGFR1, FGFR2, FGFR3, and/or FGFR4) and/or FGF (*e.g.*, FGF1-23). Such docking methods are generally well known in the art.

[00205] FGFR signaling crystal structure data can be used in conjunction with computer-modeling techniques to develop models of binding of various FGFR (*e.g.*, FGFR1, FGFR2, FGFR3, and/or FGFR4) and/or FGF (*e.g.*, FGF1-23)-binding compounds by analysis of the crystal structure data. The site models characterize the three-dimensional topography of site surface, as well as factors including van der Waals contacts, electrostatic interactions, and hydrogen-bonding opportunities. Computer simulation techniques are then used to map interaction positions for functional groups including but not limited to protons, hydroxyl groups, amine groups, divalent cations, aromatic and aliphatic functional groups, amide groups, alcohol groups, etc. that are designed to interact with the model site. These groups may be designed into a pharmacophore or candidate compound with the expectation that the candidate compound will specifically bind to the site. Pharmacophore design thus involves a consideration of the ability of the candidate compounds falling within the pharmacophore to interact with a site through any or all of the available types of chemical interactions, including hydrogen bonding, van der Waals, electrostatic, and covalent interactions, although in general, pharmacophores interact with a site through non-covalent mechanisms.

[00206] The ability of a pharmacophore or candidate compound to bind to FGFR (*e.g.*, FGFR1, FGFR2, FGFR3, and/or FGFR4) and/or FGF (*e.g.*, FGF1-23) polypeptide can be analyzed in addition to actual synthesis using computer modeling techniques. Only those candidates that are

indicated by computer modeling to bind the target (*e.g.*, FGFR (*e.g.*, FGFR1, FGFR2, FGFR3, and/or FGFR4) and/or FGF (*e.g.*, FGF1-23) polypeptide binding site) with sufficient binding energy (in one example, binding energy corresponding to a dissociation constant with the target on the order of 10^{-2} M or tighter) may be synthesized and tested for their ability to bind to FGFR (*e.g.*, FGFR1, FGFR2, FGFR3, and/or FGFR4) and/or FGF (*e.g.*, FGF1-23), polypeptide and to inhibit FGFR signaling, if applicable, enzymatic function using enzyme assays known to those of skill in the art and/or as described herein. The computational evaluation step thus avoids the unnecessary synthesis of compounds that are unlikely to bind FGFR (*e.g.*, FGFR1, FGFR2, FGFR3, and/or FGFR4) and/or FGF (*e.g.*, FGF1-23) polypeptide with adequate affinity.

[00207] FGFR signaling pharmacophore or candidate compound may be computationally evaluated and designed by means of a series of steps in which chemical entities or fragments are screened and selected for their ability to associate with individual binding target sites on FGFR (*e.g.*, FGFR1, FGFR2, FGFR3, and/or FGFR4) and/or FGF (*e.g.*, FGF1-23) polypeptide. One skilled in the art may use one of several methods to screen chemical entities or fragments for their ability to associate with FGFR (*e.g.*, FGFR1, FGFR2, FGFR3, and/or FGFR4) and/or FGF (*e.g.*, FGF1-23) polypeptide, and more particularly with target sites on FGFR (*e.g.*, FGFR1, FGFR2, FGFR3, and/or FGFR4) and/or FGF (*e.g.*, FGF1-23) polypeptide. The process may begin by visual inspection of, for example a target site on a computer screen, based on FGFR (*e.g.*, FGFR1, FGFR2, FGFR3, and/or FGFR4) and/or FGF (*e.g.*, FGF1-23) polypeptide coordinates, or a subset of those coordinates known in the art.

[00208] To select for an antagonist which induces cancer cell death, loss of membrane integrity as indicated by, *e.g.*, propidium iodide (PI), trypan blue or 7AAD uptake may be assessed relative to a reference. A PI uptake assay can be performed in the absence of complement and immune effector cells. A tumor cells are incubated with medium alone or medium containing the appropriate combination therapy. The cells are incubated for a 3-day time period. Following each treatment, cells are washed and aliquoted into 35 mm strainer-capped 12 x 75 tubes (1 ml per tube, 3 tubes per treatment group) for removal of cell clumps. Tubes then receive PI (10 μg/ml). Samples may be analyzed using a FACSCAN® flow cytometer and FACSCONVERT® CellQuest software (Becton Dickinson). Those antagonists that induce statistically significant levels of cell death compared to media alone and/or monotherapy as determined by PI uptake may be selected as cell death-inducing antibodies, binding polypeptides or binding small molecules.

[00209] In some embodiments of any of the methods of screening and/or identifying, the candidate antagonist of FGFR (*e.g.*, FGFR1, FGFR2, FGFR3, and/or FGFR4) and/or FGF (*e.g.*,

FGF1-23) is an antibody, binding polypeptide, binding small molecule, or polynucleotide. In some embodiments, the antagonist of FGFR (*e.g.*, FGFR1, FGFR2, FGFR3, and/or FGFR4) and/or FGF (*e.g.*, FGF1-23) is an antibody. In some embodiments, the antagonist of FGFR (*e.g.*, FGFR1, FGFR2, FGFR3, and/or FGFR4) and/or FGF (*e.g.*, FGF1-23) is a small molecule.

V. Pharmaceutical Formulations

[00210] Pharmaceutical formulations of an antagonist of FGFR signaling and a B-raf antagonist as described herein are prepared by mixing such antibody having the desired degree of purity with one or more optional pharmaceutically acceptable carriers (*Remington's Pharmaceutical Sciences* 16th edition, Osol, A. Ed. (1980)), in the form of lyophilized formulations or aqueous solutions. In some embodiments, the antagonist of FGFR signaling and/or B-raf antagonist is a binding small molecule, an antibody, binding polypeptide, and/or polynucleotide. Pharmaceutically acceptable carriers are generally nontoxic to recipients at the dosages and concentrations employed, and include, but are not limited to: buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride; benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (*e.g.,* Zn-protein complexes); and/or non-ionic surfactants such as polyethylene glycol (PEG). Exemplary pharmaceutically acceptable carriers herein further include insterstitial drug dispersion agents such as soluble neutral-active hyaluronidase glycoproteins (sHASEGP), for example, human soluble PH-20 hyaluronidase glycoproteins, such as rHuPH20 (HYLENEX®, Baxter International, Inc.). Certain exemplary sHASEGPs and methods of use, including rHuPH20, are described in US Patent Publication Nos. 2005/0260186 and 2006/0104968. In one aspect, a sHASEGP is combined with one or more additional glycosaminoglycanases such as chondroitinases.

[00211] Exemplary lyophilized formulations are described in US Patent No. 6,267,958. Aqueous antibody formulations include those described in US Patent No. 6,171,586 and WO2006/044908, the latter formulations including a histidine-acetate buffer.

[00212] The formulation herein may also contain more than one active ingredients as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other. Such active ingredients are suitably present in combination in amounts that are effective for the purpose intended.

[00213] Active ingredients may be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules) or in macroemulsions. Such techniques are disclosed in *Remington's Pharmaceutical Sciences* 16th edition, Osol, A. Ed. (1980).

[00214] Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the antagonist of FGFR signaling and a B-raf antagonist, which matrices are in the form of shaped articles, *e.g.,* films, or microcapsules.

[00215] The formulations to be used for *in vivo* administration are generally sterile. Sterility may be readily accomplished, *e.g.*, by filtration through sterile filtration membranes.

VI. Articles of Manufacture

[00216] In another aspect of the invention, an article of manufacture containing materials useful for the treatment, prevention and/or diagnosis of the disorders described above is provided. The article of manufacture comprises a container and a label or package insert on or associated with the container. Suitable containers include, for example, bottles, vials, syringes, IV solution bags, etc. The containers may be formed from a variety of materials such as glass or plastic. The container holds a composition which is by itself or combined with another composition effective for treating, preventing and/or diagnosing the condition and may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). At least one active agent in the composition is an antagonist of FGFR signaling and a B-raf antagonist described herein. The label or package insert indicates that the composition is used for treating the condition of choice. Moreover, the article of manufacture may comprise (a) a first container with a composition contained therein, wherein the composition comprises an antagonist of FGFR signaling and a B-raf antagonist; and (b) a second container with a composition contained therein, wherein the composition comprises a further cytotoxic or otherwise therapeutic agent.

[00217] In some embodiments, the article of manufacture comprises a container, a label on said container, and a composition contained within said container; wherein the composition includes

one or more reagents (*e.g.*, primary antibodies that bind to one or more biomarkers or probes and/or primers to one or more of the biomarkers described herein), the label on the container indicating that the composition can be used to evaluate the presence of one or more biomarkers in a sample, and instructions for using the reagents for evaluating the presence of one or more biomarkers in a sample. The article of manufacture can further comprise a set of instructions and materials for preparing the sample and utilizing the reagents. In some embodiments, the article of manufacture may include reagents such as both a primary and secondary antibody, wherein the secondary antibody is conjugated to a label, *e.g.*, an enzymatic label. In some embodiments, the article of manufacture one or more probes and/or primers to one or more of the biomarkers described herein.

[00218] In some embodiments of any of the article of manufacture, the antagonist of FGFR signaling and/or a B-raf antagonist is an antibody, binding polypeptide, binding small molecule, or polynucleotide. In some embodiments, the antagonist of FGFR signaling and/or B-raf antagonist is a small molecule. In some embodiments, the antagonist of FGFR signaling and/or B-raf antagonist is an antibody. In some embodiments, the antibody is a monoclonal antibody. In some embodiments, the antibody is a human, humanized, or chimeric antibody. In some embodiments, the antibody is an antibody fragment and the antibody fragment binds FGFR signaling and/or inhibitor.

[00219] The article of manufacture in this embodiment of the invention may further comprise a package insert indicating that the compositions can be used to treat a particular condition. Alternatively, or additionally, the article of manufacture may further comprise a second (or third) container comprising a pharmaceutically-acceptable buffer, such as bacteriostatic water for injection (BWFI), phosphate-buffered saline, Ringer's solution and dextrose solution. It may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, and syringes.

[00220] Other optional components in the article of manufacture include one or more buffers (*e.g.*, block buffer, wash buffer, substrate buffer, etc), other reagents such as substrate (*e.g.*, chromogen) which is chemically altered by an enzymatic label, epitope retrieval solution, control samples (positive and/or negative controls), control slide(s) etc.

[00221] It is understood that any of the above articles of manufacture may include an immunoconjugate described herein in place of or in addition to an antagonist of FGFR signaling and a B-raf antagonist.

EXAMPLES

[00222] The following are examples of methods and compositions of the invention. It is understood that various other embodiments may be practiced, given the general description provided above.

Example 1

[00223] Two models of acquired resistance to B-raf inhibitors were studied to determine the secreted factors associated with B-raf inhibitor resistance. Specifically, HER2+ breast cancer and B-raf mutant melanoma cell lines were studied. The treatment regime for HER+ positive breast cancer consists of surgery, Herceptin, lapatinib, Pertuzumab, T-DM1, anthracyclines, taxanes, and capecitabine (Trastuzumab, Pertuzumab, and docetaxel as first-line treatment for metastatic breast cancer). HER2+ breast cancer makes up approximately 15-35% of breast cancers (approximately 40,000 cases per year). Combined targeting of HER receptors can improve survival by compensating for resistance mechanisms; however, despite the high initial response rates, the majority of patients eventually develop progressive disease. The treatment regime for B-raf mutant melanoma is surgery, Ipilimubab (CTLA4), vemurafenib, trametinib, dabrafenib, and darcarbazine. Approximately 50% of melanomas are characterized by the B-raf V600E mutation and there are approximately 108,000 new cases each year. While a majority of patients respond to vemurafenib, 10% of patients experience tumor progression early in therapy and the majority of patients have residual tumor following maximal response with relapse within 1 year.

[00224] As described herein, a screen for secreted factors that promote resistance to therapies such as vemurafenib were performed to determine the contributing causes of acquired resistance to B-raf inhibitors (**FIGURE 11**).

[00225] In order to determine which secreted factors promote drug resistance, secreted factor screens were run on HER2+ breast cancer cells and B-raf mutant melanoma cells (**FIGURES 12** and **13**). The results of the secreted factor screens show which secreted factors are associated with enhanced cell death (*i.e.*, enhanced killing by factor) and which secreted factors are associated with rescue (*i.e.*, acquired drug resistance).

[00226] Secreted factors were measured in HER2+ breast cancer cells in the presence of lapatinib, GDC-0032, GDC-0941, GDC-0349, T-DM1, or T-DM1 plus pertuzumab. Based on this screen, BTC, EGF, FGFs, HGF, HRG1, NRG1 (EGF), OSM, PRGN, and TGFA (EGF) were identified as possible secreted factors that lead to drug resistance in HER2+ breast cancer cells. Similarly, secreted factors were also measured in B-raf mutant melanoma cells in the absence of drug or in the presence of PLX4032, GDC-0973, or GDC-0623. Based on this screen, FGFs,

HGF, HRG1, NRG1 (EGF), OSM, TGFA (EGF), and TNFA were identified as possible secreted factors that lead to drug resistance in B-RAF mutant melanoma cells.

[00227] As a result of the secreted factor screen, a discrete number of factors were identified that promote rescue. For HER2+ breast cancer cell lines, ligands for FGFRs, EGFR, and HER3/4 were implicated as drivers of resistance. In a smaller subset of HER2+ breast cancer cell lines, ligands for MET and cc-chemokines were also implicated as drivers of resistance. For B-RAF mutant melanoma cell lines, ligands for FGFRs, MET, and HER3/4 were involved in resistance. In a smaller subset of B-RAF mutant melanoma cell lines, ligands for cKIT and EGFR were also implicated as drivers of resistance.

[00228] Based on the screen, the same subset of secreted factors that promote resistance were identified for all compounds (*i.e.*, drugs) tested. Accordingly, the secreted factors were cancer type dependent. It was also concluded that drug target, chemistry, and concentration influences strength of secreted factor driven resistance (*i.e.*, the selection of drug screening concentration was critical). It was determined that basal receptor protein expression status does not always predict secreted factor rescue (*i.e.*, acquired resistance). For example, while EGFR and MET do predict secreted factor rescue, HER3 and the FGFRs do not. Furthermore, there was no apparent receptor crosstalk-mediated rescue between EGFR, MET, and the FGFRs and targeting downstream (mTOR) signaling nodes overcame the majority of rescue.

Example 2

[00229] Downstream mechanisms of secreted factor mediated resistance was investigated. Specifically, common pathways that are reactivated by secreted factors were investigated to determine whether their inhibition can overcome the acquired drug resistance.

[00230] Based on an immunoblot screen of nine cell lines treated with one of five secreted factors, it was determined that no single downstream signal predicts all secreted factor mediated resistance (**FIGURE 14**). Furthermore, a ligand may rescue different cell lines by different mechanisms.

Example 3

[00231] FGF signalling and resistance was studied in 10 HER2+ breast cancer cell lines and in 10 B-raf mutant melanoma cell lines (**FIGURE 15**). 7 of the 10 HER2+ breast cancer cell lines were rescued by FGF2 (**FIGURE 15A**). 8 of the 10 B-raf mutant melanoma cell lines were rescued by FGF2 (**FIGURE 15B**). Subsequent analysis determined that 50-70% of the melanoma lines with the V600E mutation were rescued by FGF2 (n=30).

[00232] Furthermore, it was determined that FGF2 reactivates key signalling pathways to promote resistance (**FIGURE 16**). This was shown in a cell assay wherein HER2+ breast cancer

cells were exposed to FGF2 (50ng.mL) for 10 minutes in the presence or absence of lapatinib (2μM) (**FIGURE 16A**). Similarly, an assay was performed wherein HER2+ breast cancer cells were exposed to FGF2 (50ng/mL) for 24 hours in the presence or absence of lapatinib (2μM) (**FIGURE 16B**). Based on these experiments, it was determined that FGF2 stimulates sustained activation of downstream signalling.

Example 4

[00233] The kinetics and feedback mechanisms of secreted factor mediated signaling were also studied. It was shown that FGFR targeting effectively blocked FGF2 rescue.

[00234] Three cell lines (624 MEL, 928 MEL, and LOX IMVI) were exposed to (5μM) PLX4032 (*i.e.,* vemurafenib) for 4 hours and then exposed to secreted (50ng/mL) FGFs (subtypes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 16, 17, 18, 19, 20, 21, and 22) for 10 minutes. Immunoblots were prepared and probed for p-MEK and p-ERK. It was determined that many FGFs activate the MAPK pathway but do not promote resistance (**FIGURE17A**). The 624 MEL and 982 MEL cell lines were also exposed to (5μM) PLX4032 for 24 hours and (50ng/mL) FGFs (subtypes 1, 2, 4, 6, 8, 9, 17, and 18) for 24 hours and then processed for immunoblots. The immunoblots probed for p-MEK and p-ERK. It was determined that the longevity of signal may play a role but that additional factors are involved in acquired drug resistance (**FIGURE17B**).

[00235] The feedback mechanisms associated with downstream mediators of FGF-rescue were also studied (**FIGURE 18**). BT-474 breast cancer cells were treated with 2μM lapatinib or 2μM lapatinib plus 50ng/mL FGF2 in the presence or absence of one or more inhibitors of p38, PI3K, MEK, and FGFR (**FIGURE 18A**). Cells were also pre-treated with 2μM lapatinib, a MEK inhibitor, and/or a small molecule inhibitor of p38, PI3K, p38 and PI3K, or FGFR and then followed by a 10 minute stimulation with 50ng/mL FGF2. The pre-treated and stimulated cells were processed and an immunoblot was performed that probed for p-HER2, pMEK, MEK, p-ERK, ERK, p90RSK (pS380), p90 RSK, p-p38 MAPK (T180/Y182), p38 MAPK, p-Akt (S473), Akt, and β-actin (**FIGURE 18B**). Similar pre-treatment/stimulation experiments were also performed using HCC-1954 and UACC-893 breast cancer cell lines.

[00236] It was determined that effective blocking of downstream pathways often does not overcome FGF2-rescue and that multiple feedback and compensatory mechanisms are evident. Furthermore, it was determined that only FGFR targeting effectively blocked FGF2 rescue.

Example 5

[00237] Experiments were performed and it was shown that FGFR1 mediates FGF2 rescue in melanoma.

[00238] 624 MEL cells were treated with DMSO (control), 5μM PLX4032, or 5μM PLX4032/FGFb and exposed to siRNA targeting FGFR1, FGFR2, FGFR3, FGFR4, FGFR1 and FGFR4 (*i.e.*, FGFR1/4), FGFR2 and FGFR3 (*i.e.*, FGFR2/3), or FRS2 (**FIGURE 5A**). Similarly, a siRNA screen targeting FGFR1, FGFR2, FGFR3, FGFR4, FGFR1 and FGFR4, and FGFR2 and FGFR3 was performed on seven cell lines (624 MEL, 928 MEL, A-375, COLO 849, G361, LOX-IMVI, and UACC62) (**FIGURE 5B**). Only siRNA targeting FGFR1 and FGFR1/4 elicited a full block in cell growth/proliferation.

[00239] FGFR1, FGFR1, FGFR3, and FGFR4 expression levels were measured in WT and mutant (V600E) cell line melanoma samples (n=49) (**FIGURE 5C**). It was shown that FGFR1 had the highest expression in WT and V600E mutant melanoma cell samples. Analysis of TCGA melanoma samples of unknown B-raf status (n=247) were also analyzed (**FIGURE 5D**). The analysis showed that FGFR1 was more highly expressed than FGFR2, FGFR3, and FGFR4 (**$p<0.0001$).

Example 6

[00240] FGFR4 was shown to mediate FGF2 rescue in HER2+ breast cancer cell lines.

[00241] HER2+ breast cancer cell lines (AU565, BT-474, HCC1954, SK-BR-3, and UACC-893) were treated with lapatinib and FGF2. Thereafter, the cells were either exposed to siRNA targeting FGFR1, FGFR2, FGFR3, or FGFR4 or exposed to the FGFR pan inhibitor BGJ398 (**FIGURE 19A**). It was shown that the siRNA targeting FGFR4 and the pan inhibitor had the greatest percent rescue from acquired resistance to lapatinib and FGF2. Immunoblots were also performed to detect IP/pTyr/IB:FGFR4, FGFR4, pERK, ERK, and actin (control) in cells treated with lapatinib in the presence and absence of FGF2 (**FIGURE 19B**).

[00242] TCGA breast cancer samples were also analyzed (**FIGURES 19C** and **19D**). FGFR1 levels were shown to be high in breast cancer samples (n=913) (**FIGURE 19C**). When gated for HER2+ breast cancer, it was shown that HER2+ breast cancer FGFR4 is enriched for high FGFR4 (HER2 log2 RPKM cutoff = 8.0) (**FIGURE 19D**).

Example 7

[00243] Models of innate resistance in HER2+ breast cancer cell lines and acquired resistance in B-raf mutant melanoma cell lines were studied.

[00244] The innate resistant HER2+ breast cancer cell lines were HCC1569 and MDA-MB-453. HC1569 expressed FGFR2 (detected by Western blot) and secreted FGF2 (detected by ELISA). FGFR ECD chimeras from the HCC1569 line were sensitized to lapatinib. Furthermore, HC1569 cells that were treated with FGFR inhibitor(s) sensitized the cells to lapatinib. The MDA-MB-453 cell line had high phosphorylated FGFR4 expression (detected by Western blot) and did not

secrete FGF2 (no detection of FGF2 via ELISA). MDA-MB-453 cells that were treated with FGFR inhibitor(s) sensitized the cells to lapatinib.

[00245] HCC1569 and MDA-MB-453 cells were treated with 100nM afatinib, 100nM crizotinib, or 100nM BGJ398 in the presence or absence of 5μM lapatinib (**FIGURES 20A** and **20B**). As shown, the combination of lapatinib and BGJ398 rescue the cell lines from drug resistance and decrease tumor volume (**FIGURES 20A-C**).

[00246] The Lox-IMVI VemR cell line was used as the model of acquired resistance in B-raf mutant melanoma. 11 cell lines were tested for FGFR1 expression using a Western blot. Of the cell lines tested, FGFR1 expression was detected in the LOX-IMVI (vemurafenib sensitive) and LOX-IMVI VemR (vemurafenib resistant) lines (**FIGURE 2A**). The LOX-IMVI VemR cell line was further shown to be rescued (*i.e.*, resensitized to vemurafenib) with the addition of antagonists of FGFR signalling (1μM BGJ398, 1μM PD173074, and 1μM AP24534) (**FIGURE 2B**). FGF2 expression (pg/mL) was also measured in the LOX-IMVI ("parental") and LOX-IMVI VemR ("VemR") cell lines and showed that the LOX-IMVI VemR had an increased expression of FGF2 in comparison to the vemurafenib sensitive parental line (**FIGURE 2C**). Furthermore, an siRNA screen targeting FGFR1, FGFR2, FGFR3, FGFR4, FGFR1/4, FGFR2/3, and FRS2 demonstrated that FGFR1 knockdown in combination with vemurafenib resensitizes the LOX-IMVI VemR cell line to vemurafenib treatment (**FIGURE 2D**).

[00247] The vemurafenib resistance and recovery of the LOX-IMVI VemR cell line was also studied *in vivo*. The tumor volume (mm^3) was measured in LOX-IMVI (parental, vemurafenib sensitive) tumors and in LOX-IMVI VemR (vemurafenib resistant) tumors in the presence of vemurafenib, BGJ398, or vemurafenib and BGJ398 (**FIGURES 3A** and **3B**). As shown in the figures, tumor volume decreased when treated with vemurafenib (25mg/kg, BID) or vemurafenib (25mg/kg, BID) and BGJ398 (15mg/kg, QD) in the LOX-IMVI cells. In contrast, the LOX-IMVI VemR cells did not show a decrease in tumor volume when exposed to vemurafenib but did show a decrease in tumor volume when exposed to the combination treatment of vemurafenib and BGJ398.

[00248] Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, the descriptions and examples should not be construed as limiting the scope of the invention. The disclosures of all patent and scientific literature cited herein are expressly incorporated in their entirety by reference.

WHAT IS CLAIMED IS:

1) A method of treating cancer in an individual comprising concomitantly administering to the individual (a) an antagonist of FGFR signaling and (b) a B-raf antagonist.

2) The method of claim 1, wherein the respective amounts of the antagonist of FGFR signaling and the B-raf antagonist are effective to increase the period of cancer sensitivity and/or delay the development of cancer resistance to the B-raf antagonist.

3) The method of claim 1, wherein the respective amounts of the antagonist of FGFR signaling and the B-raf antagonist are effective to increase cancer sensitivity and/or restore sensitivity to the B-raf antagonist.

4) A method of treating a cancer cell, wherein the cancer cell is resistant to treatment with a B-raf antagonist in an individual comprising administering to the individual an effective amount of an antagonist of FGFR signaling and an effective amount of the B-raf antagonist.

5) A method of treating cancer resistant to a B-raf antagonist in an individual comprising administering to the individual an effective amount of an antagonist of FGFR signaling and an effective amount of the B-raf antagonist.

6) A method of increasing sensitivity and/or restoring sensitivity to a B-raf antagonist comprising administering to the individual an effective amount of an antagonist of FGFR signaling antagonist and an effective amount of the B-raf antagonist.

7) A method of increasing efficacy of a cancer treatment comprising a B-raf antagonist in an individual comprises concomitantly administering to the individual (a) an effective amount of an antagonist of FGFR signaling and (b) an effective amount of the B-raf antagonist.

8) A method of delaying and/or preventing development of cancer resistant to a B-raf antagonist in an individual, comprising concomitantly administering to the individual (a) an effective amount of an antagonist of FGFR signaling and (b) an effective amount of the B-raf antagonist.

9) A method of treating an individual with cancer who has increased likelihood of developing resistance to a B-raf antagonist comprising concomitantly administering to the individual (a) an effective amount of an antagonist of FGFR signaling and (b) an effective amount of the B-raf antagonist.

10) A method of increasing sensitivity to a B-raf antagonist in an individual with cancer comprising concomitantly administering to the individual (a) an effective amount of an antagonist of FGFR signaling and (b) an effective amount of the B-raf antagonist.

11) A method of extending the period of a B-raf antagonist sensitivity in an individual with cancer comprising concomitantly administering to the individual (a) an effective amount of an antagonist of FGFR signaling and (b) an effective amount of the B-raf antagonist.

12) A method of extending the duration of response to a B-raf antagonist in an individual with cancer comprising concomitantly administering to the individual (a) an effective amount of an antagonist of FGFR signaling and (b) an effective amount of the B-raf antagonist.

13) The method of any one of claims 1-12, wherein the cancer is lung cancer (*e.g.*, non-small cell lung cancer (NSCLC)), breast cancer, or melanoma.

14) The method of any one of claims 1-13, wherein the cancer has undergone epithelial-mesenchymal transition.

15) The method of any one of claims 1-14, wherein the antagonist of FGFR signaling is an antibody inhibitor, a small molecule inhibitor, a binding polypeptide inhibitor, and/or a polynucleotide antagonist.

16) The method of any one of claims 1-15, wherein the antagonist of FGFR signaling is an antagonist of FGFR1 signaling.

17) The method of any one of claim 1-15, wherein the antagonist of FGFR1 signaling binds to one or more of FGFR1b, FGFR1c, FGF1, FGF2, FGF3, FGF4, FGF5, FGF6, and FGF10.

18) The method of any one of claims 15-17, wherein the antagonist of FGFR signaling is a binding polypeptide inhibitor, and the binding polypeptide inhibitor comprises a region of the extracellular domain of FGFR linked to a Fc.

19) The method of any one of claims 15-17, wherein the antagonist of FGFR signaling is a small molecule and the small molecule is N-[2-[[4-(diethylamino)butyl]amino]-6-(3,5-dimethoxyphenyl)pyrido[2,3-d]pyrimidin-7-yl]-N'-(1,1-dimethylethyl)-urea or pharmaceutically acceptable salt thereof.

20) The method of any one of claims 15-17, wherein the antagonist of FGFR signaling is an anti-FGFR1 antibody.

21) The method of any one of claims 1-19, wherein the B-raf antagonist is *N*-(3-{[5-(4-chlorophenyl)-1*H*-pyrrolo[2,3-b]pyridin-3-yl]carbonyl}-2,4-difluorophenyl)propane-1-sulfonamide or a pharmaceutically acceptable salt thereof.

22) The method of any one of claims 1-21, wherein the antagonist of FGFR signaling and the B-raf antagonist provide a synergistic effect.

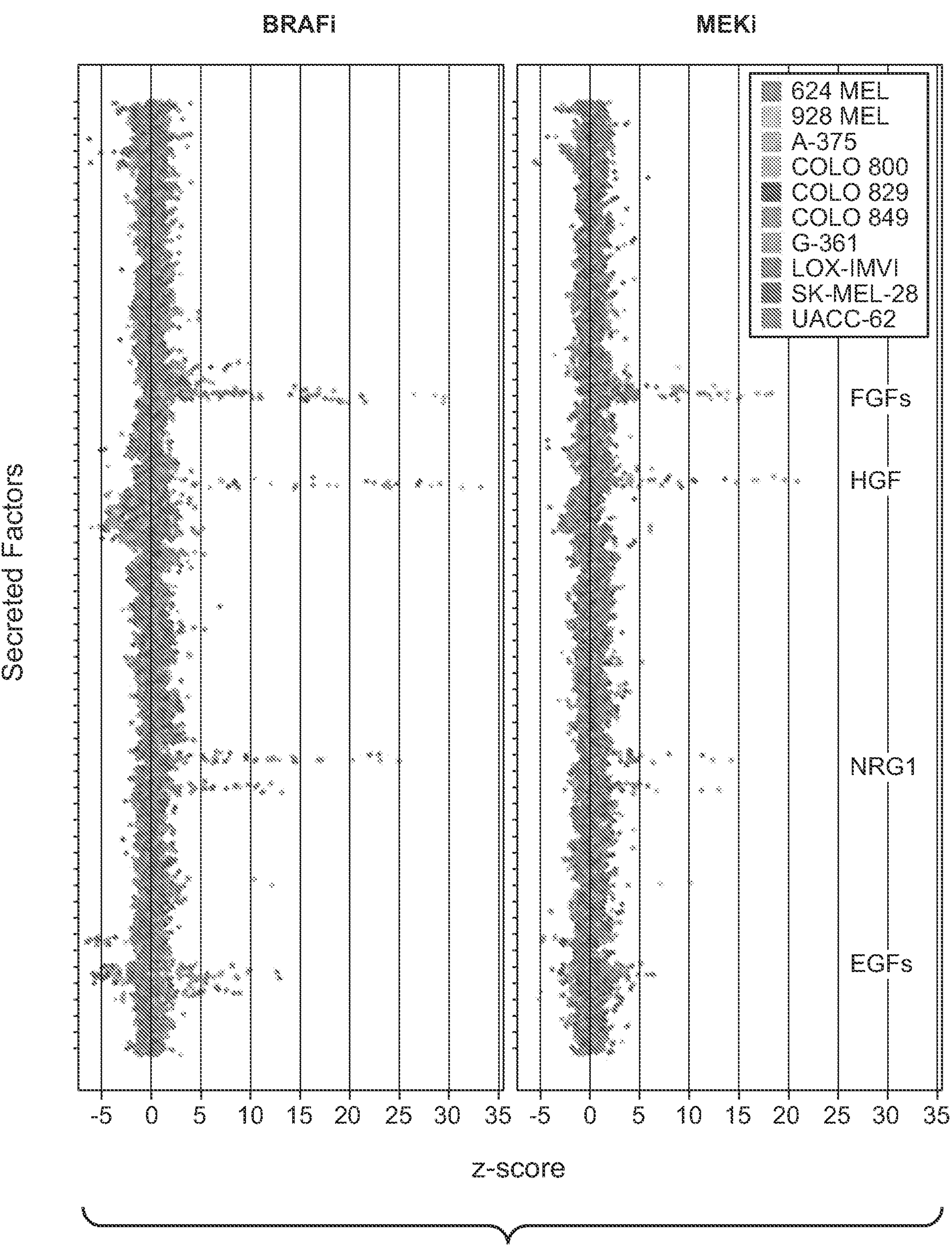


FIG. 1A

		624 MEL	928 MEL	A-375	COLO 800	COLO 829	COLO 849	G-361	LOX-IMVI	SK-MEL-28	UACC-62
FGF2	Screen										
	Confirmation	R	R	PR		PR	PR	R	R		
HGF	Screen										
	Confirmation	R	R	PR		PR	PR	R	PR	PR	
NRG1	Screen										
	Confirmation				PR	PR	PR	R		PR	

FIG. 1B

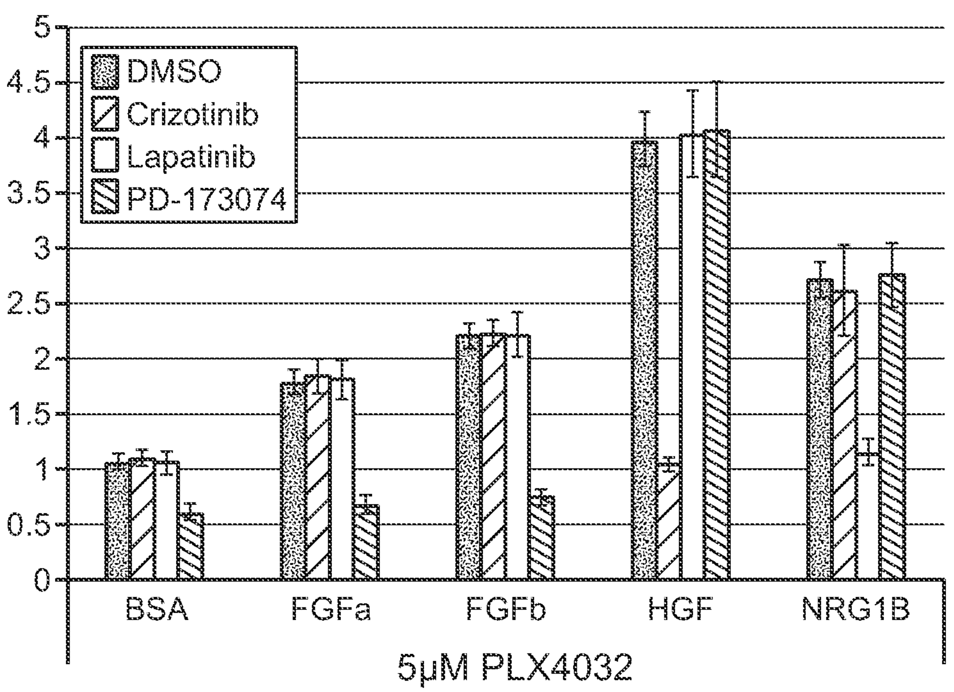


FIG. 1C

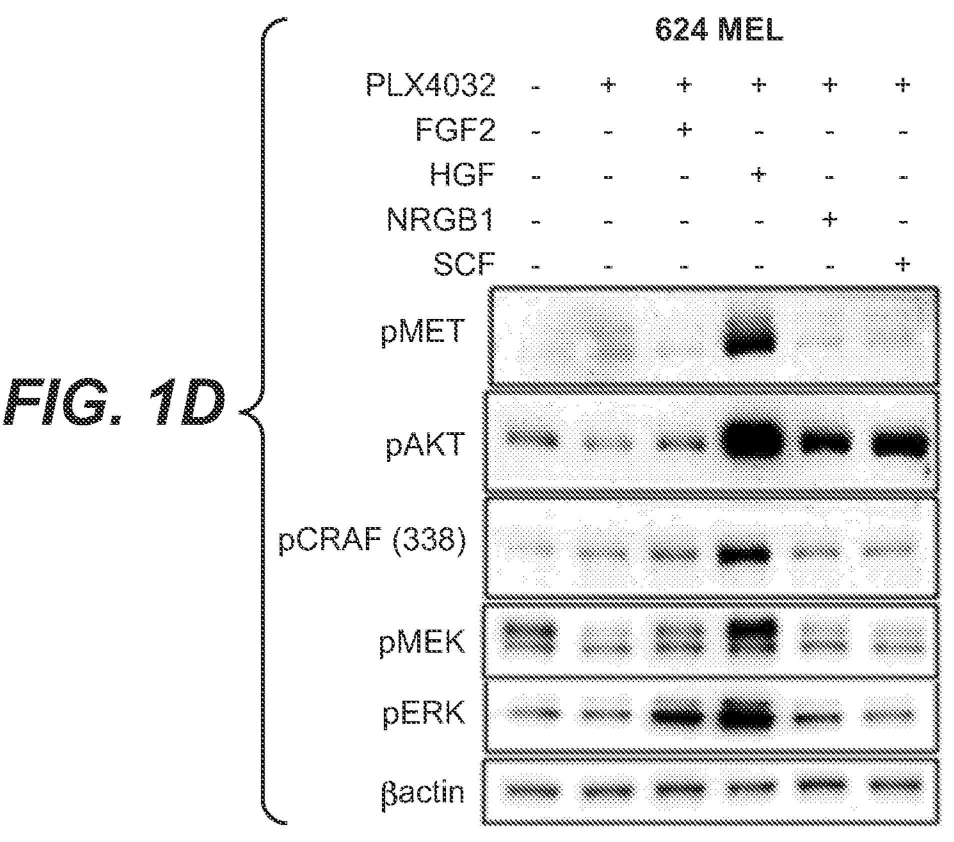


FIG. 1D

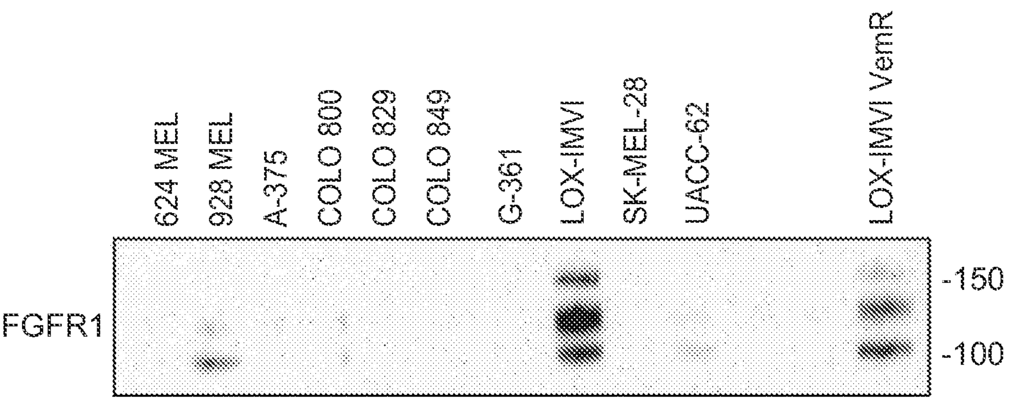


FIG. 2A

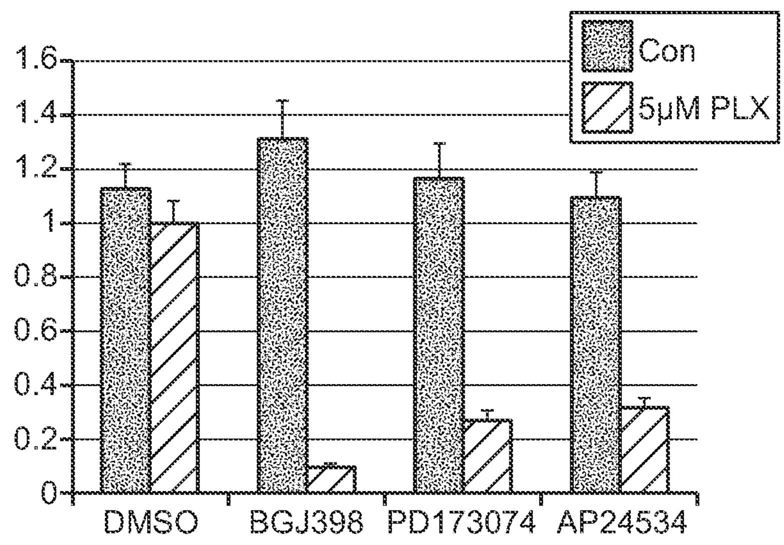


FIG. 2B

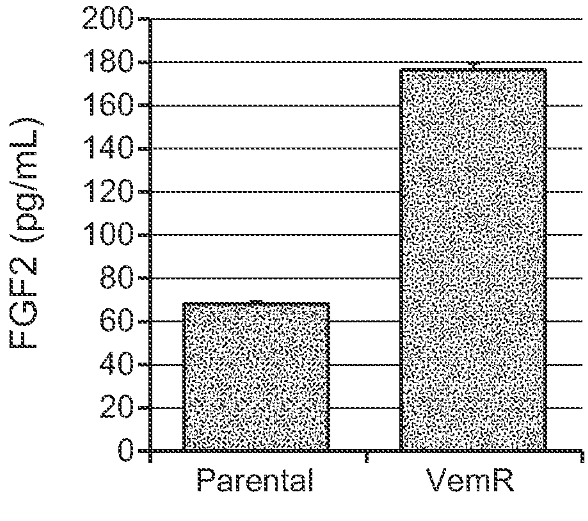


FIG. 2C

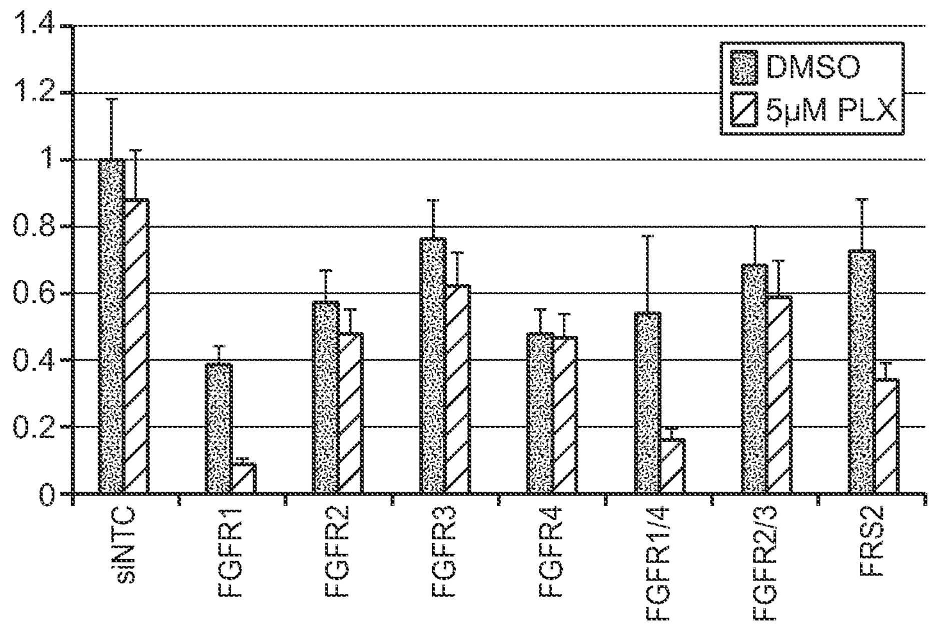


FIG. 2D

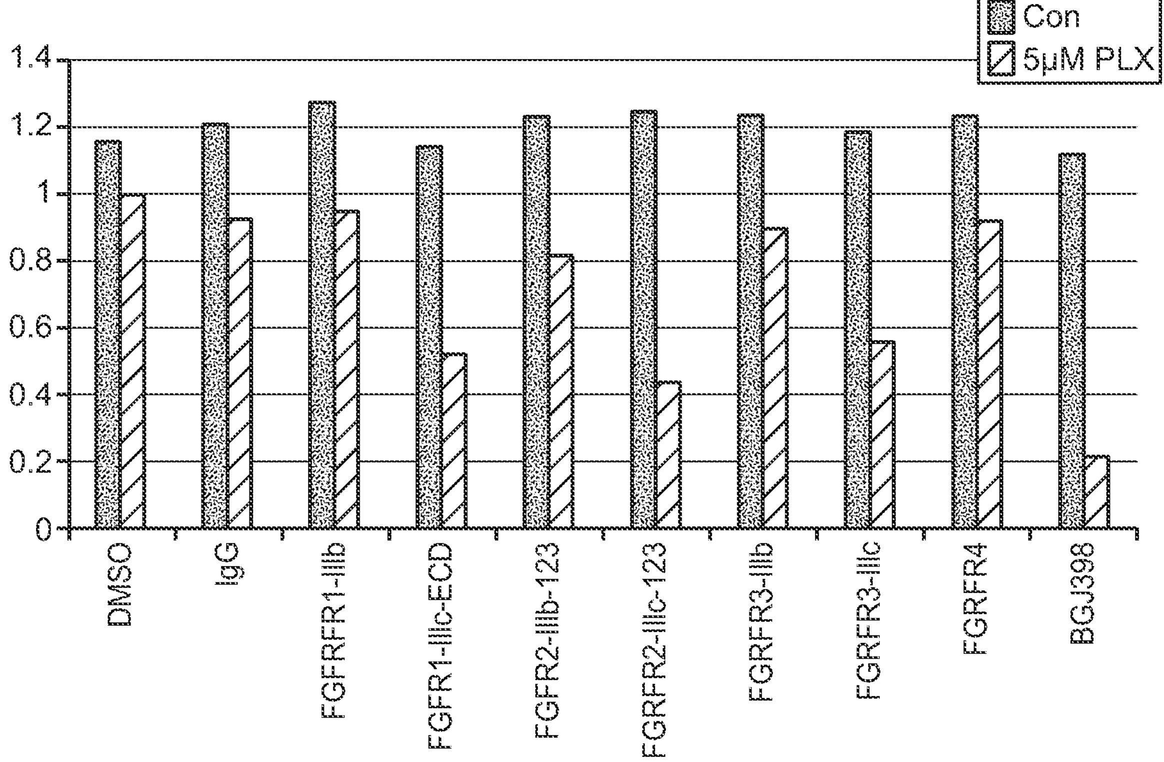


FIG. 2E

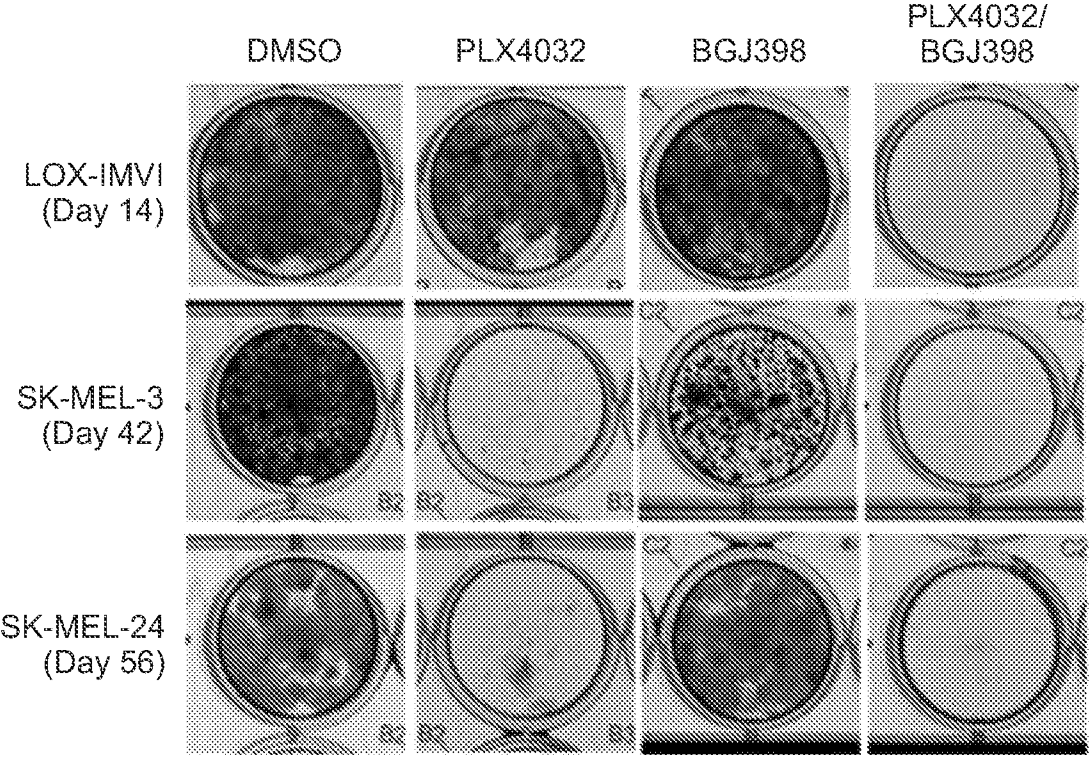


FIG. 3A

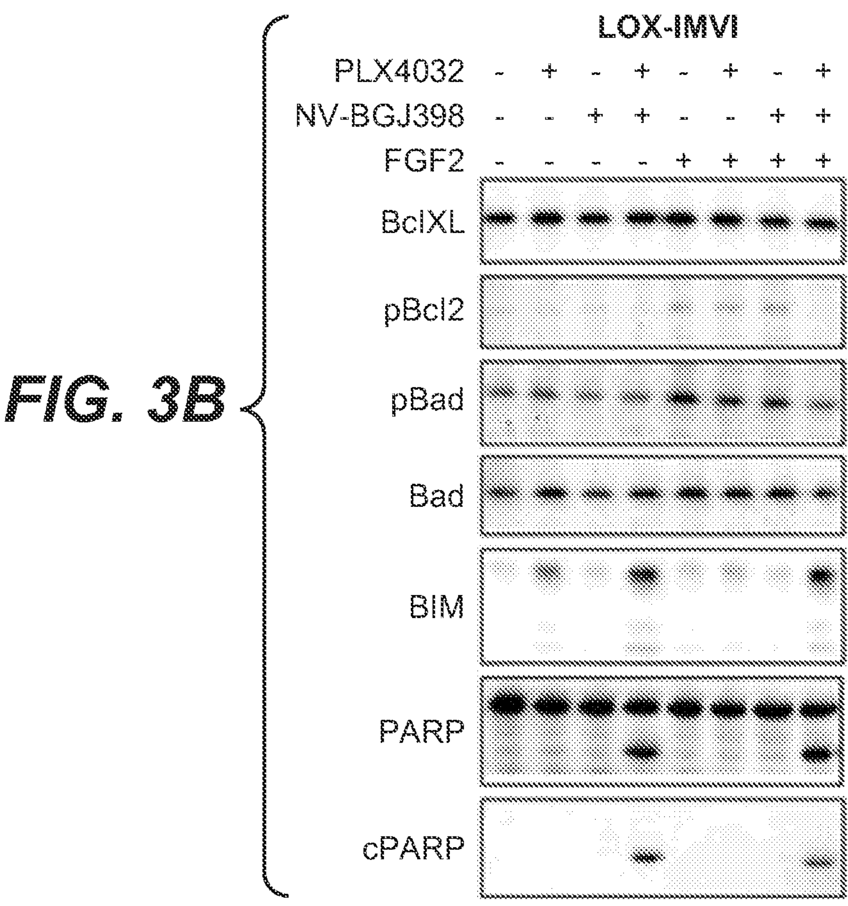


FIG. 3B

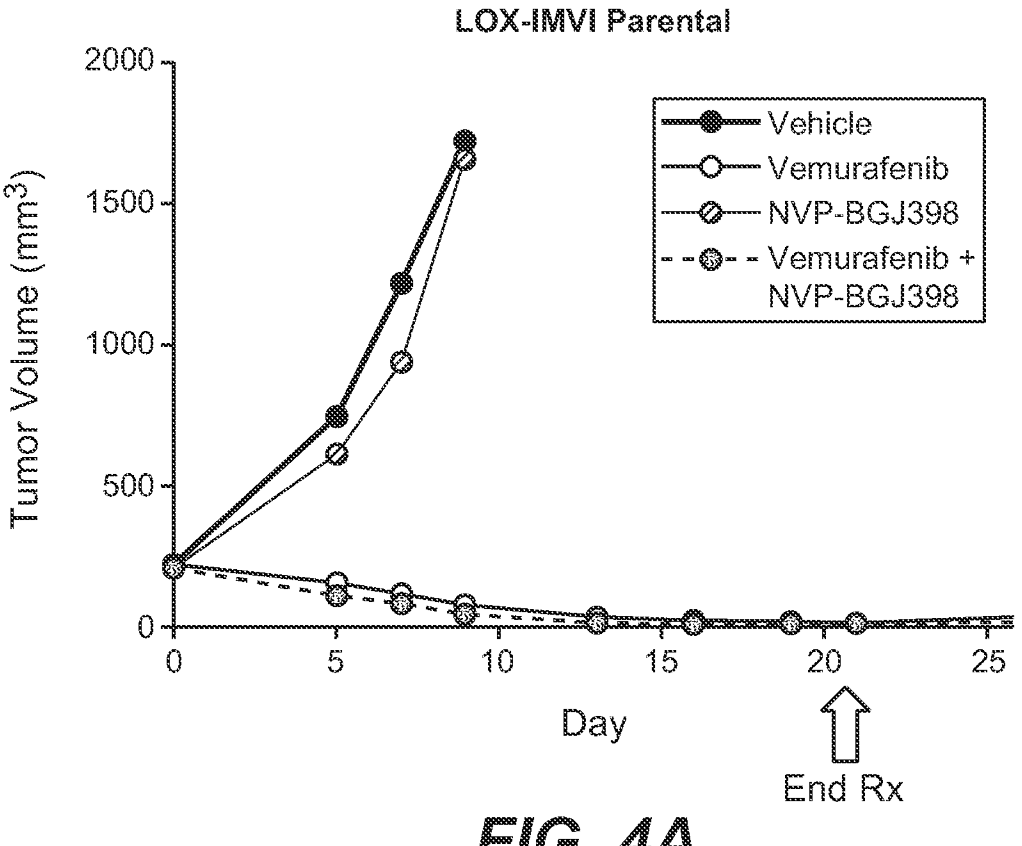


FIG. 4A

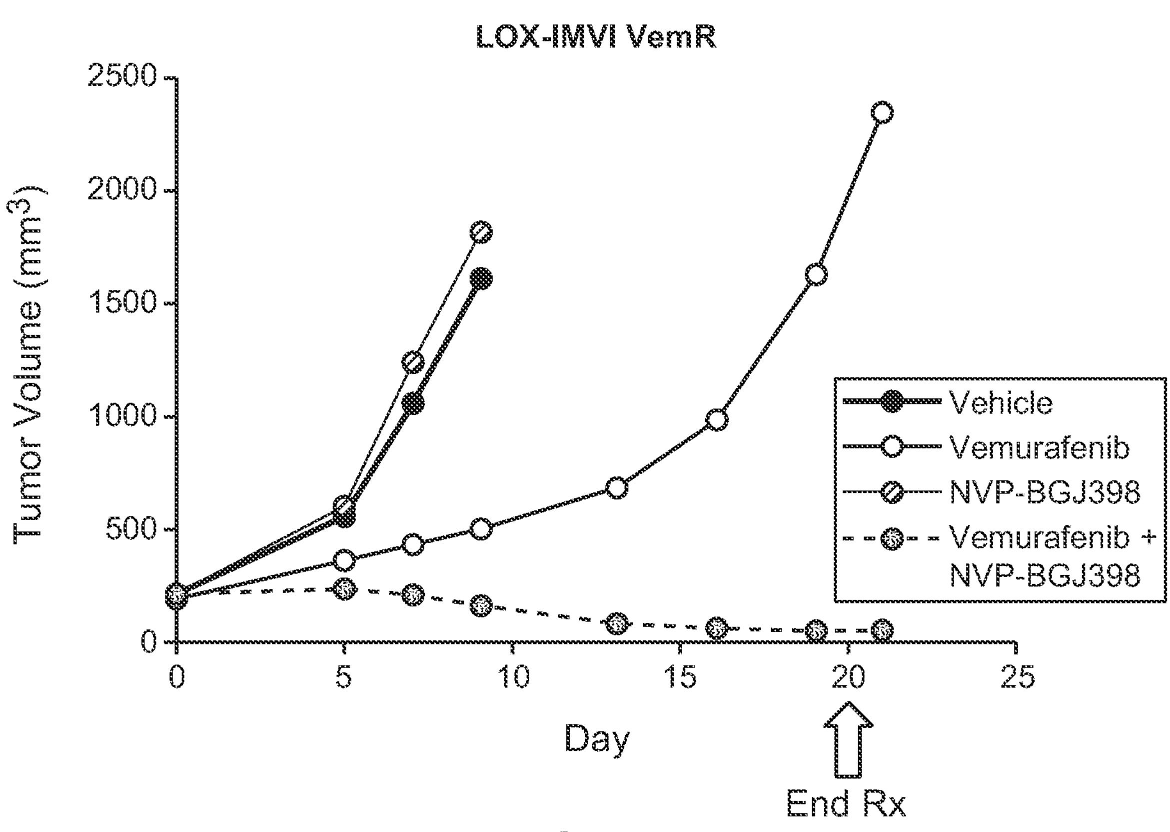


FIG. 4B

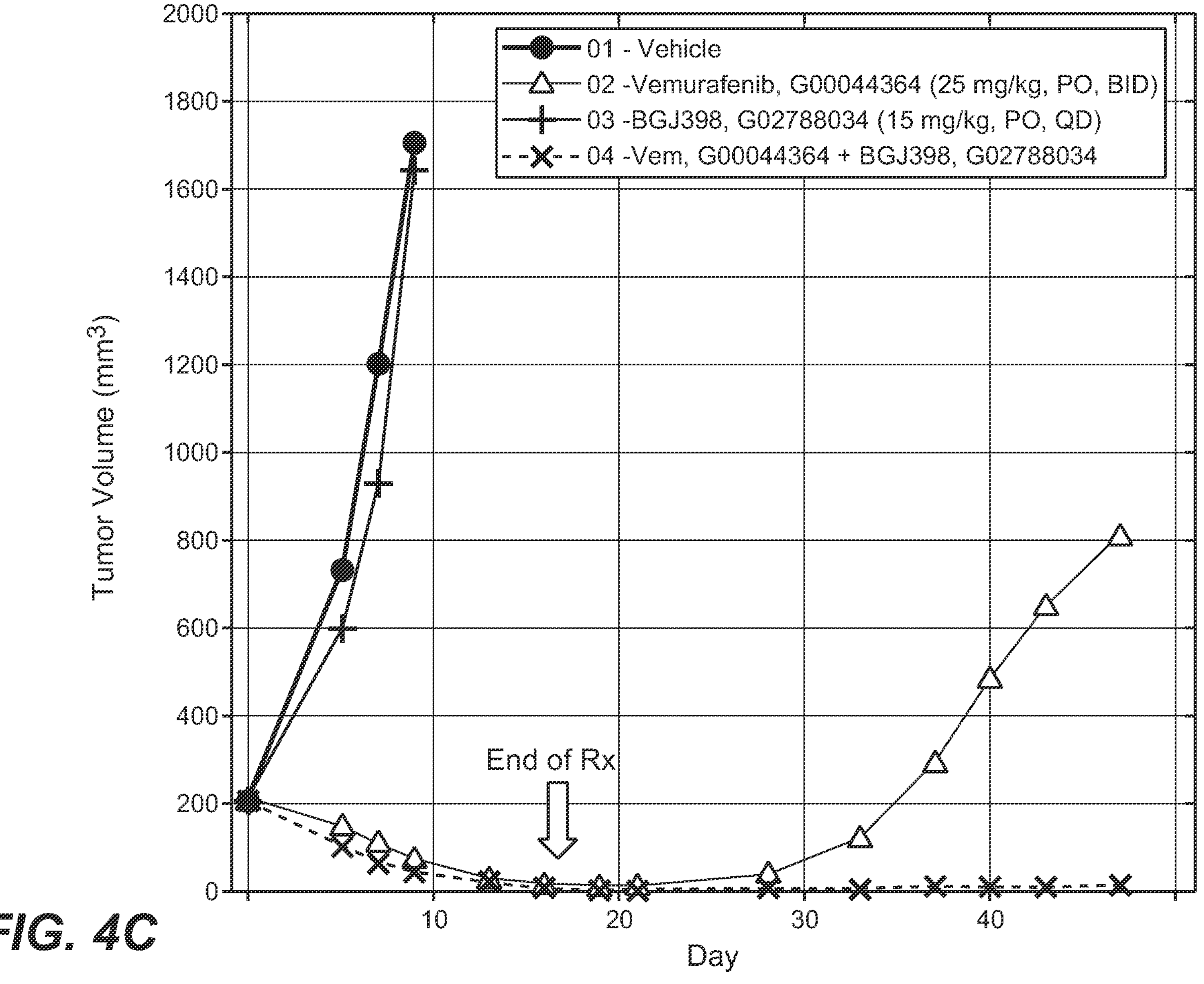


FIG. 4C

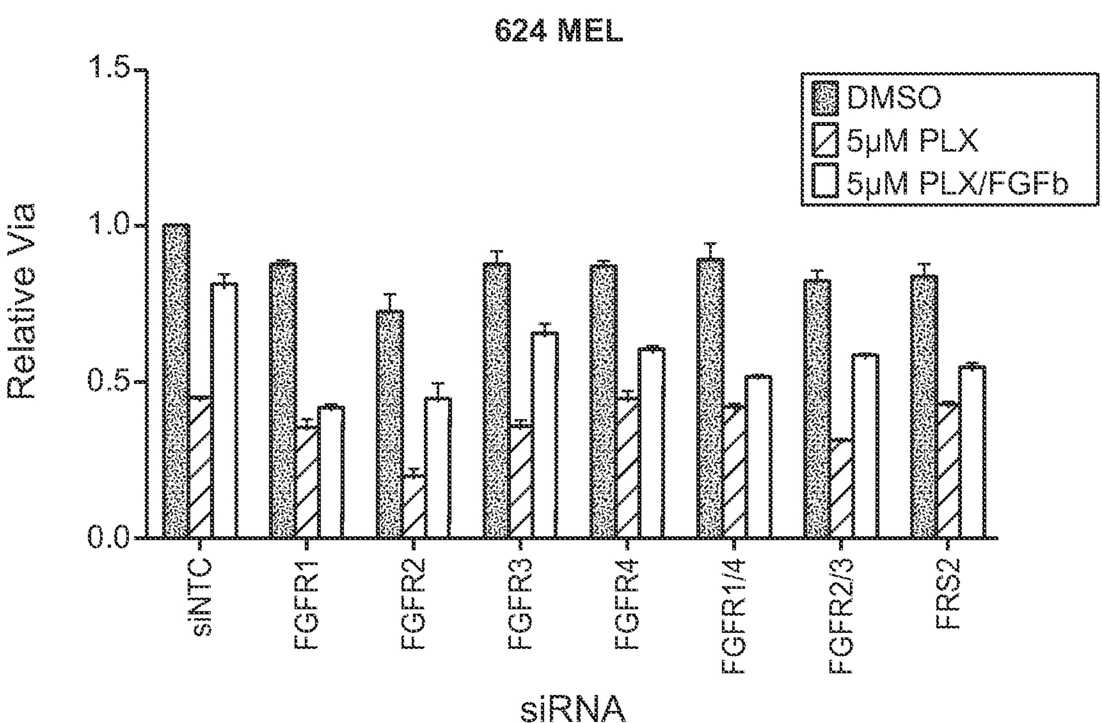


FIG. 5A

	siRNA					
	FGFR1	FGFR2	FGFR3	FGFR4	FGFR1/4	FGFR2/3
624 MEL	F	N	N	P	P	N
928 MEL	F	N	N	P	P	N
A-375	F	N	N	P	N	N
COLO 849	F	N	N	N	F	N
G-361	F	N	N	N	F	N
LOX-IMVI	F	P	N	P	F	P
UACC62	F	N	N	N	F	N

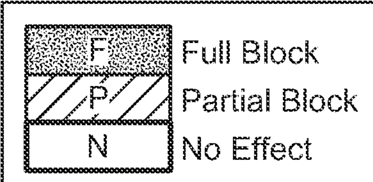


FIG. 5B

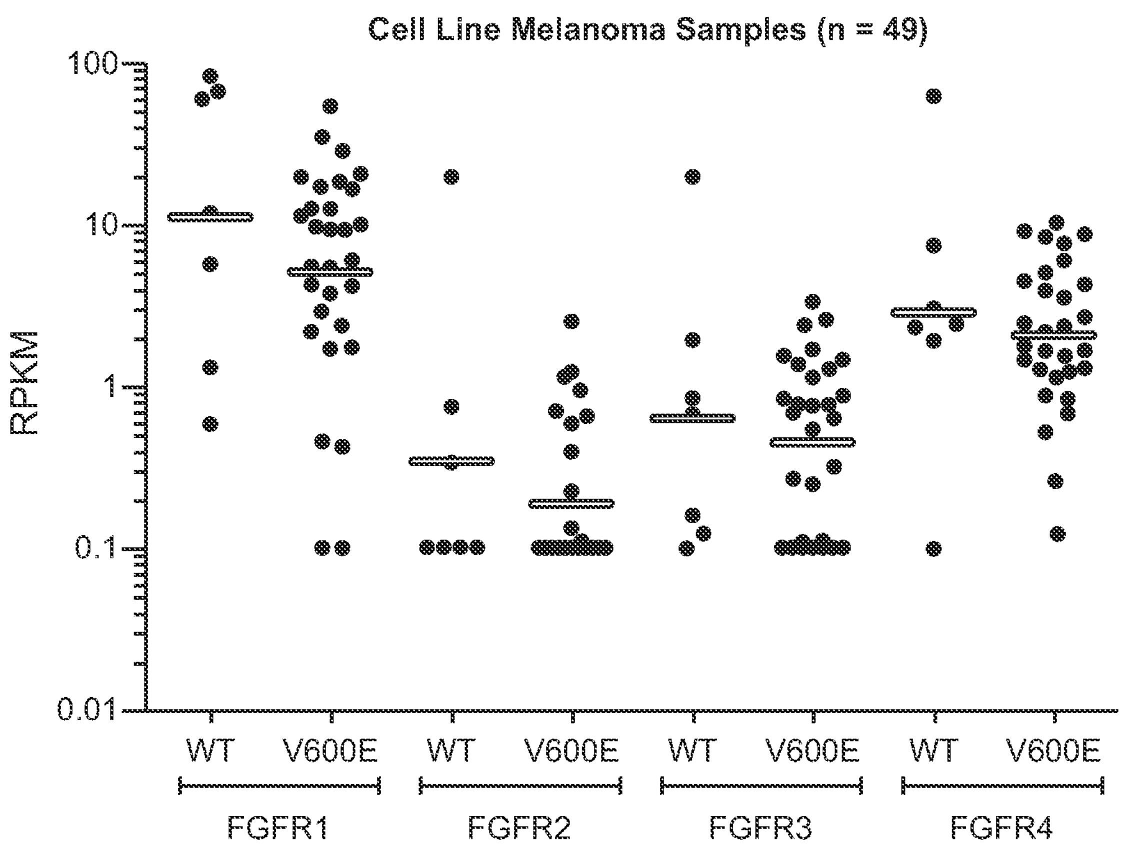


FIG. 5C

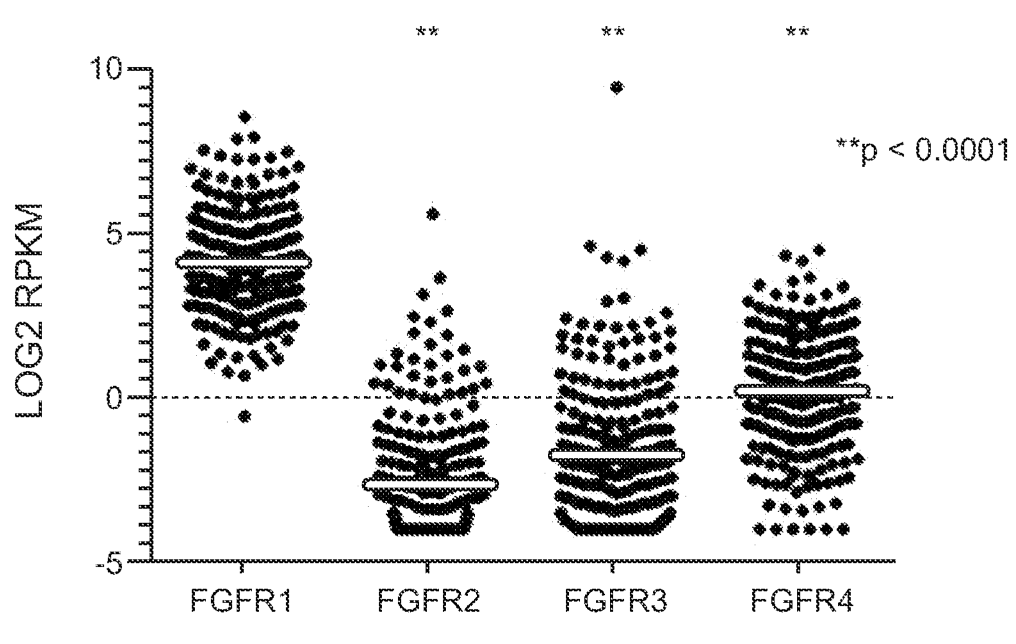


FIG. 5D

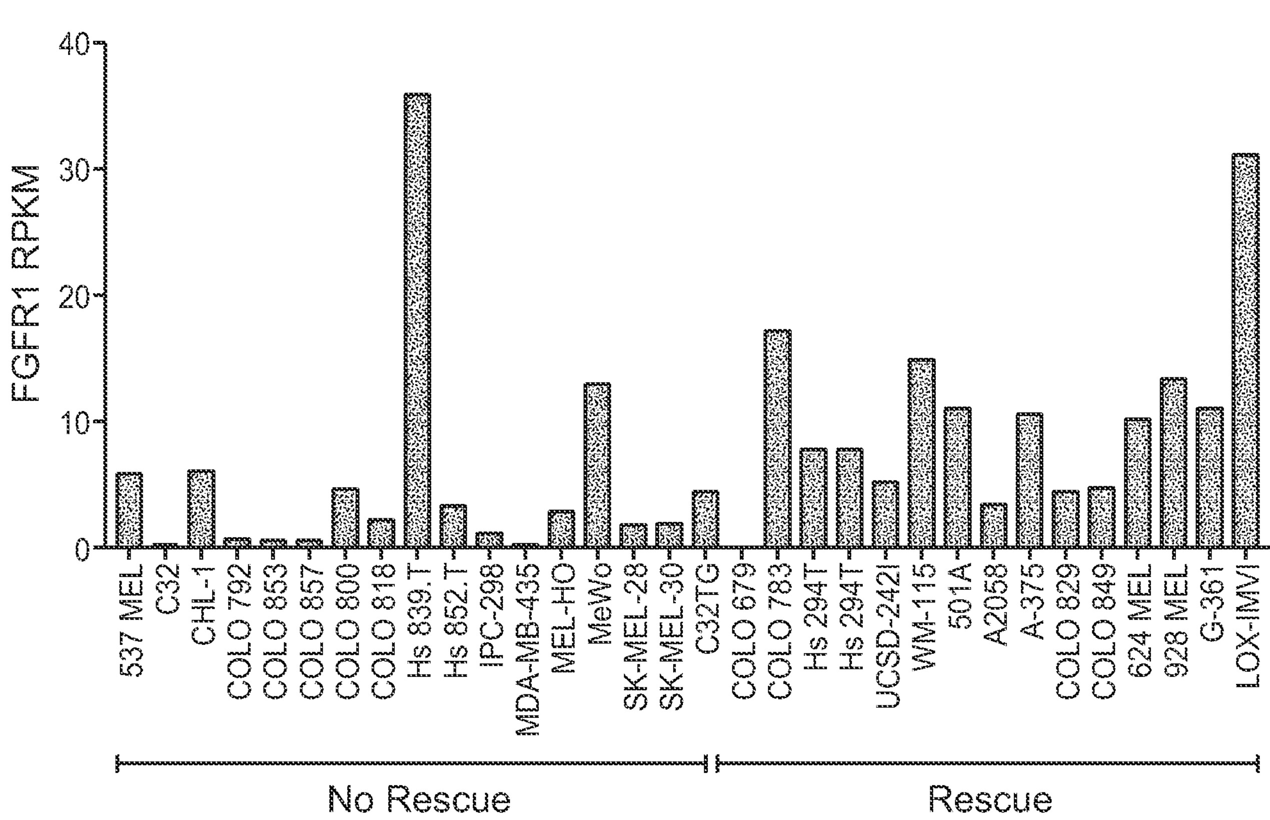


FIG. 6A

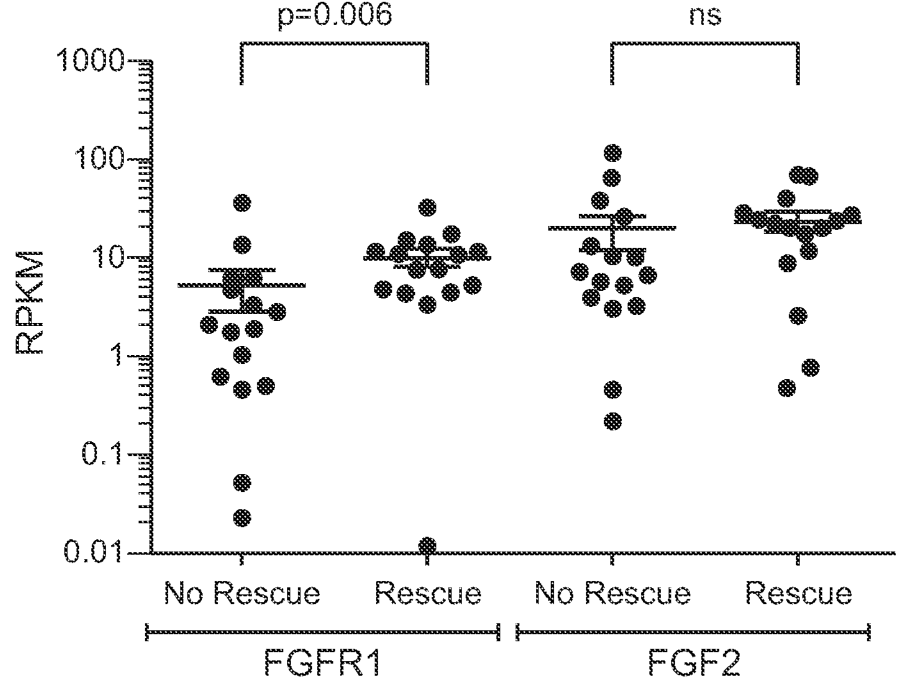


FIG. 6B

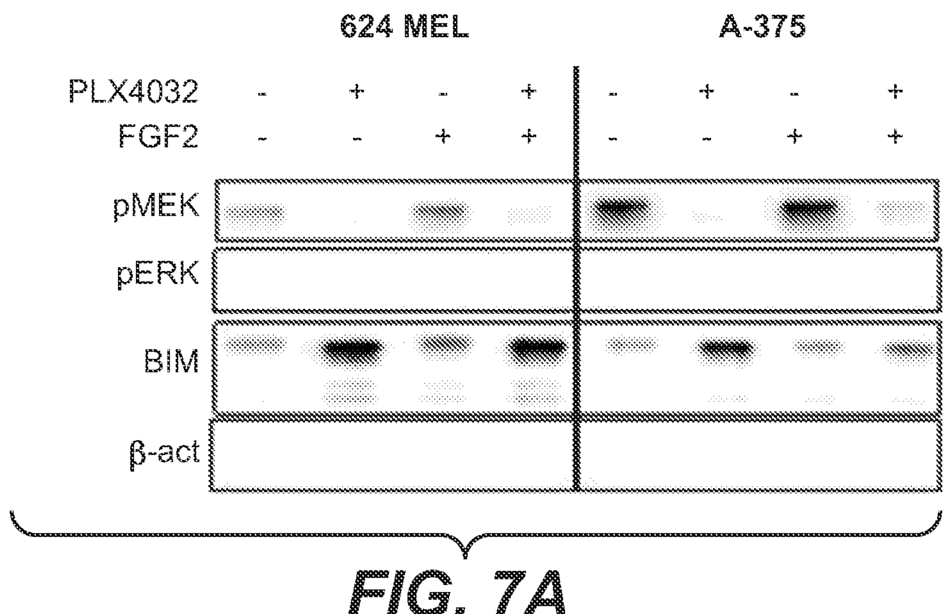


FIG. 7A

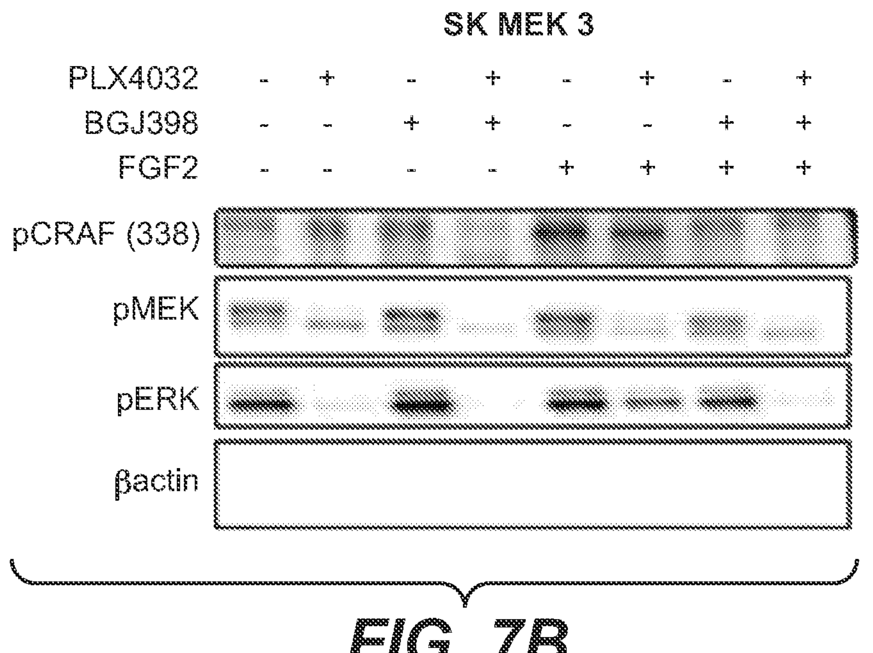


FIG. 7B

Cell line	Condition	ERK 1	ERK 2	MEK 3	MEK 4	MEK 5
A-375	Par, Con	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive
	VemR, Con	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive
	VemR, PLX	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive
COLO 829	Par, Con	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive
	VemR, Con	Resistant	Resistant	Sensitive	Sensitive	Sensitive
	VemR, PLX	Sensitive	Resistant	Sensitive	Sensitive	Sensitive
G-361	Par, Con	Sensitive	Resistant	Sensitive	Sensitive	Sensitive
	VemR, Con	Resistant	Resistant	Sensitive	Sensitive	Sensitive
	VemR, PLX	Resistant	Resistant	Sensitive	Sensitive	Sensitive
UACC-62	Par, Con	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive
	VemR, Con	Resistant	Resistant	Resistant	Resistant	Resistant
	VemR, PLX	Sensitive	Resistant	Resistant	Sensitive	Sensitive
LOX IMVI	Par, Con	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive
	VemR, Con	Resistant	Resistant	Resistant	Resistant	Resistant
	VemR, PLX	Resistant	Resistant	Resistant	Sensitive	Resistant
SK-MEL-28 928 MEL	Par, Con	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive
	VemR, Con	Resistant	Resistant	Resistant	Resistant	Resistant
	VemR, PLX	Resistant	Resistant	Resistant	Resistant	Resistant
SK-MEL-24 624 MEL	Par, Con	Sensitive	Resistant	Sensitive	Sensitive	Sensitive
	VemR, Con	Resistant	Resistant	Resistant	Resistant	Resistant
	VemR, PLX	Resistant	Resistant	Resistant	Resistant	Resistant
COLO 800 Hs695.T	Par, Con	Resistant	Resistant	Sensitive	Sensitive	Sensitive
	VemR, Con	Resistant	Resistant	Resistant	Resistant	Resistant
	VemR, PLX	Resistant	Resistant	Resistant	Resistant	Resistant
SK-MEL-3	Par, Con	Resistant	Sensitive	Sensitive	Sensitive	Resistant
	VemR, Con	Resistant	Resistant	Resistant	Resistant	Resistant
	VemR, PLX	Resistant	Resistant	Resistant	Resistant	Resistant

Inhibitor: ERK (1, 2); MEK (3, 4, 5)

1 G02442895 (ERK)
2 G02447285 (ERK)
3 GDC-0523 (MEK)
4 GDC-0973 (MEK)
5 PD-0323901 (MEK)

Sensitive: IC50 = <1μM
Resistant: IC50 >1μM

Par, Con = Parental Line in Absence of PLX4032
VemR, Con = VemR Line in Absence of PLX4032
VemR, PLX = VemR Line in Presence of 5μM PLX4032

FIG. 7C

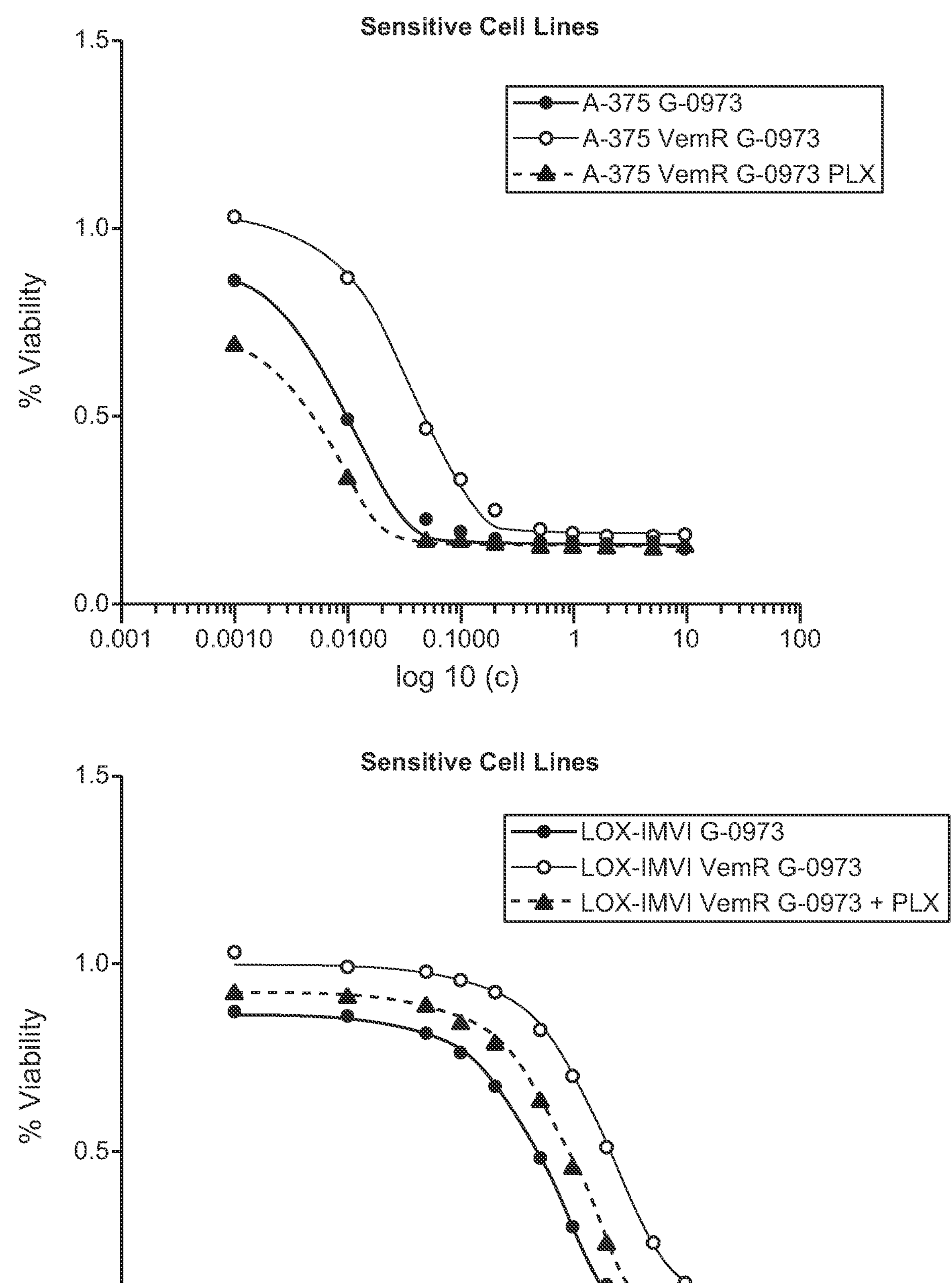


FIG. 7D-1

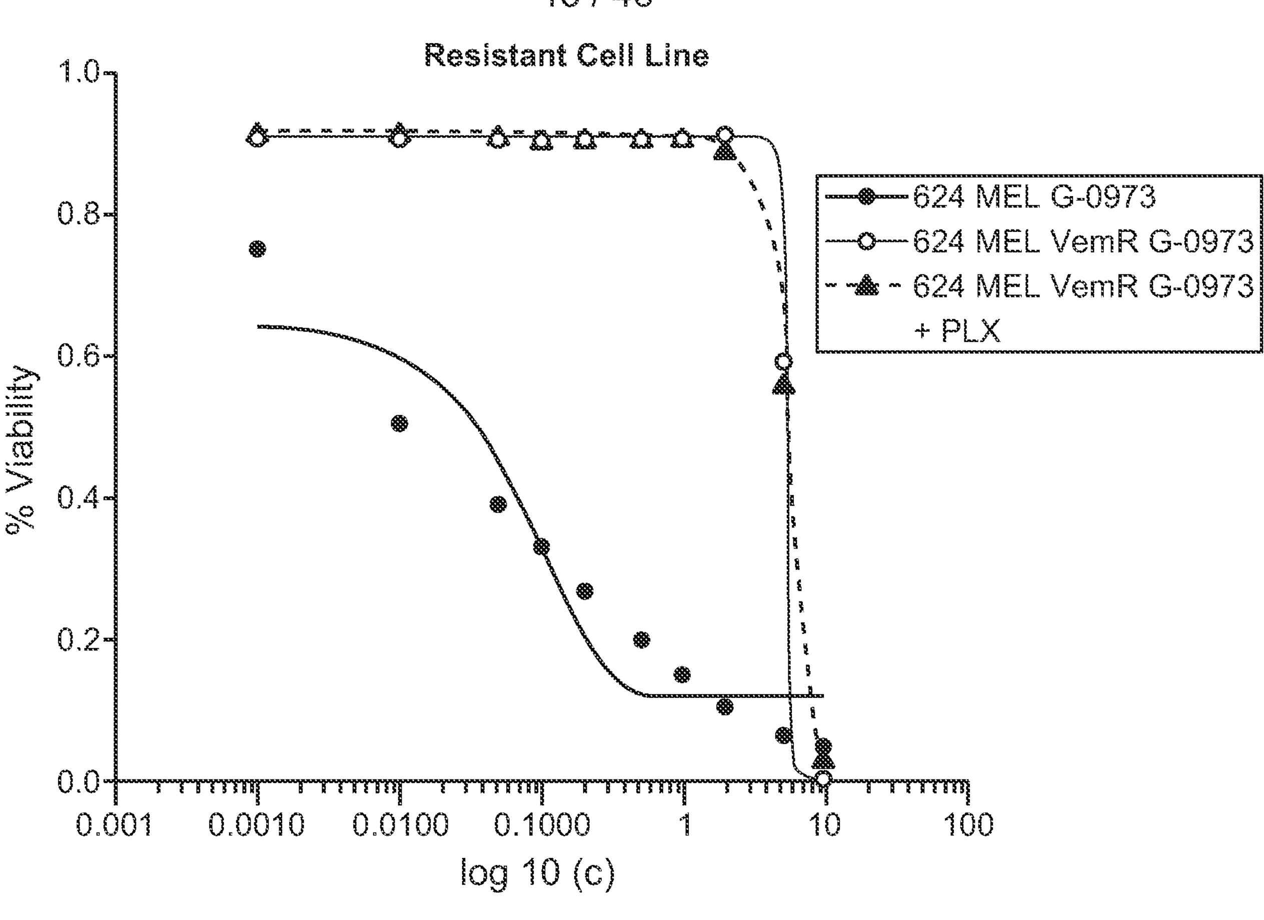


FIG. 7D-2

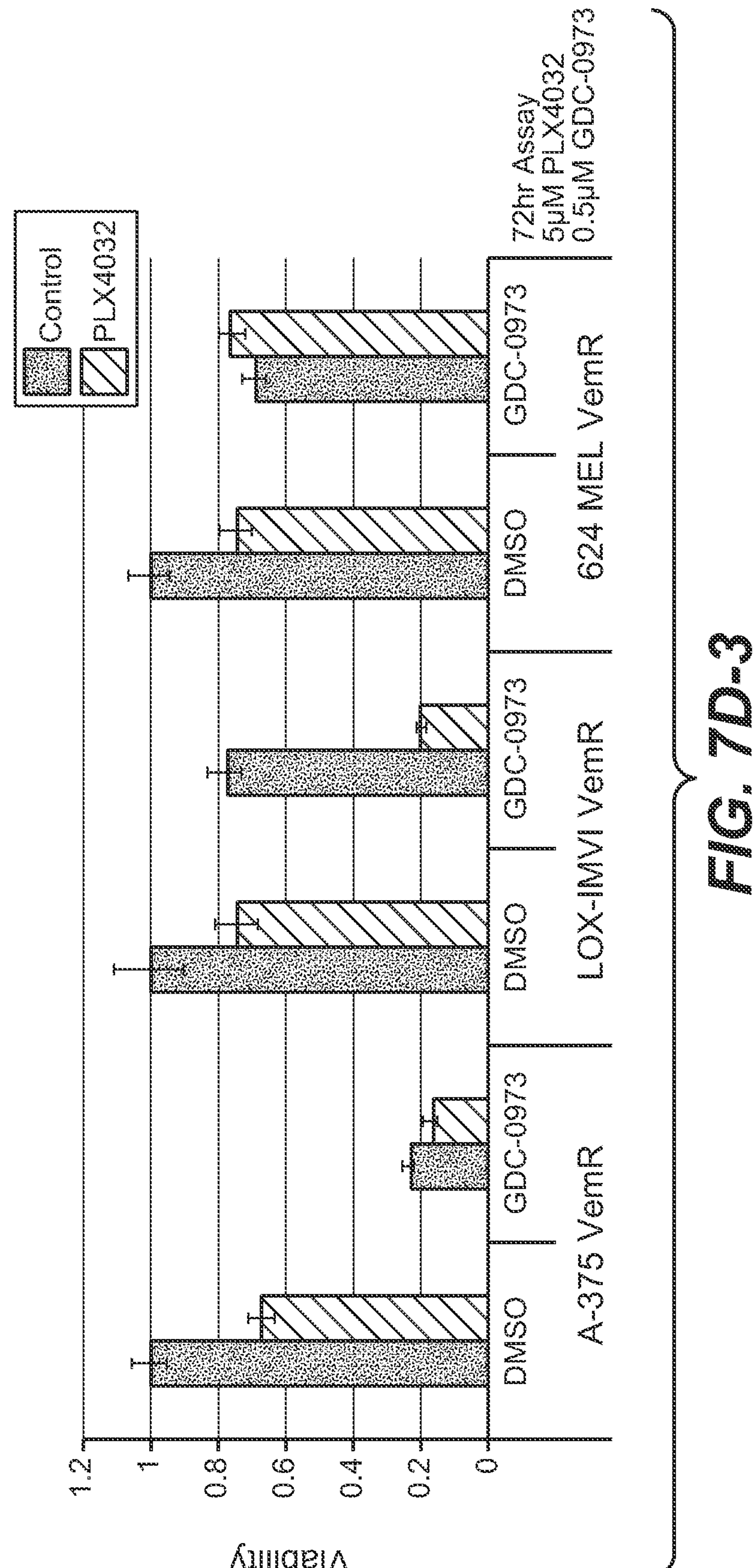


FIG. 7D-3

Inhibitor

PI3K
AKT
mTOR

G-361
Par, Con
VemR, Con
VemR, PLX

624 MEL
Par, Con
VemR, Con
VemR, PLX

UACC-62
COLO 800
Par, Con
VemR, Con
VemR, PLX

A-375
LOX IMVI
Par, Con
VemR, Con
VemR, PLX

COLO 829
Par, Con
VemR, Con
VemR, PLX

SK-MEL-28
Par, Con
VemR, Con
VemR, PLX

SK-MEL-3
Par, Con
VemR, Con
VemR, PLX

928 MEL
SK-MEL-24
Hs695.T
Par, Con
VemR, Con
VemR, PLX

Sensitive IC50 = <1μM

Resistant IC50 >1μM

FIG. 8A

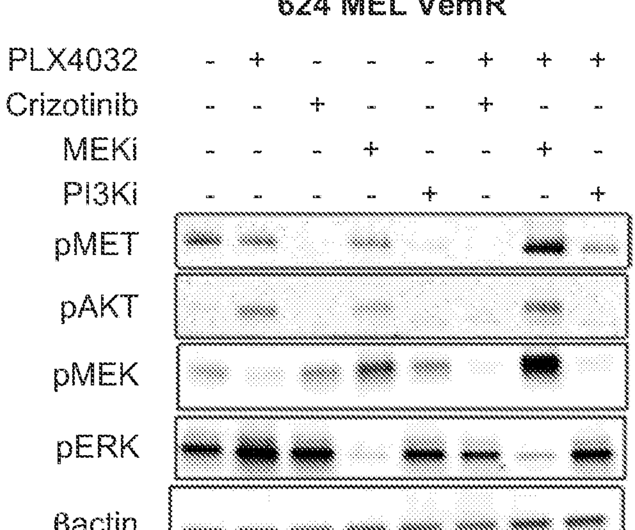


FIG. 8B

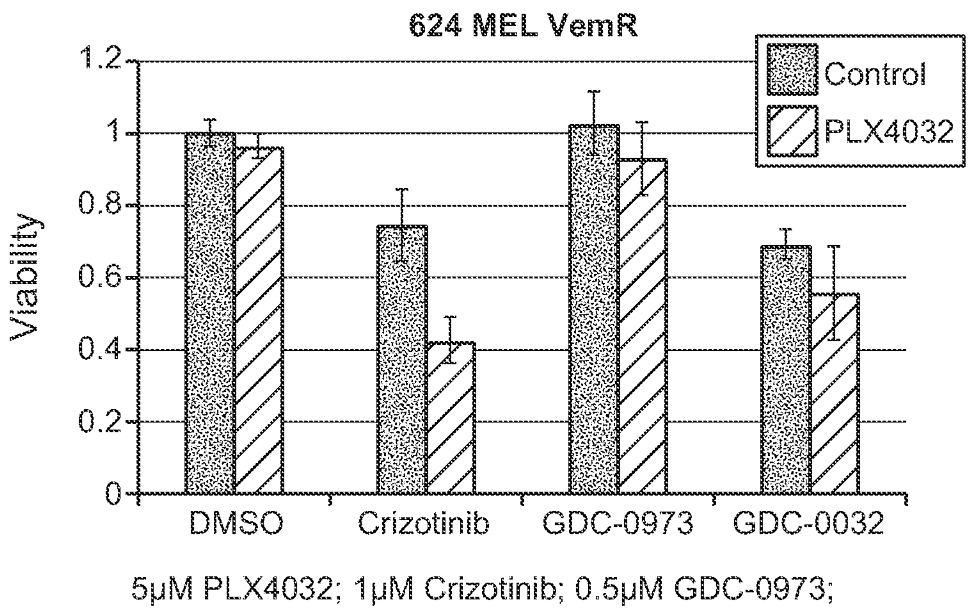


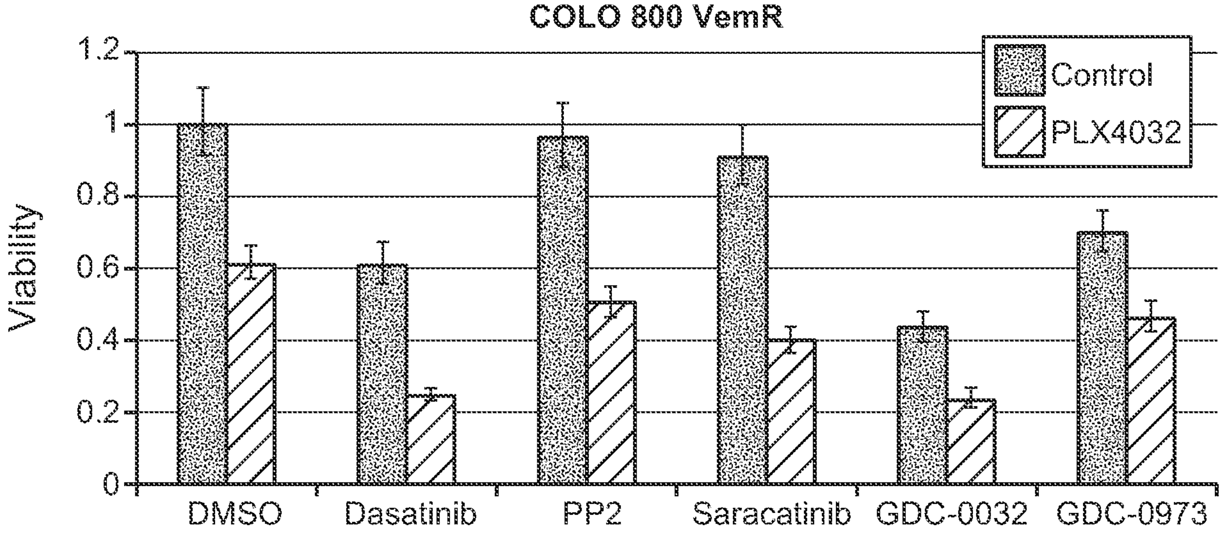
FIG. 8C

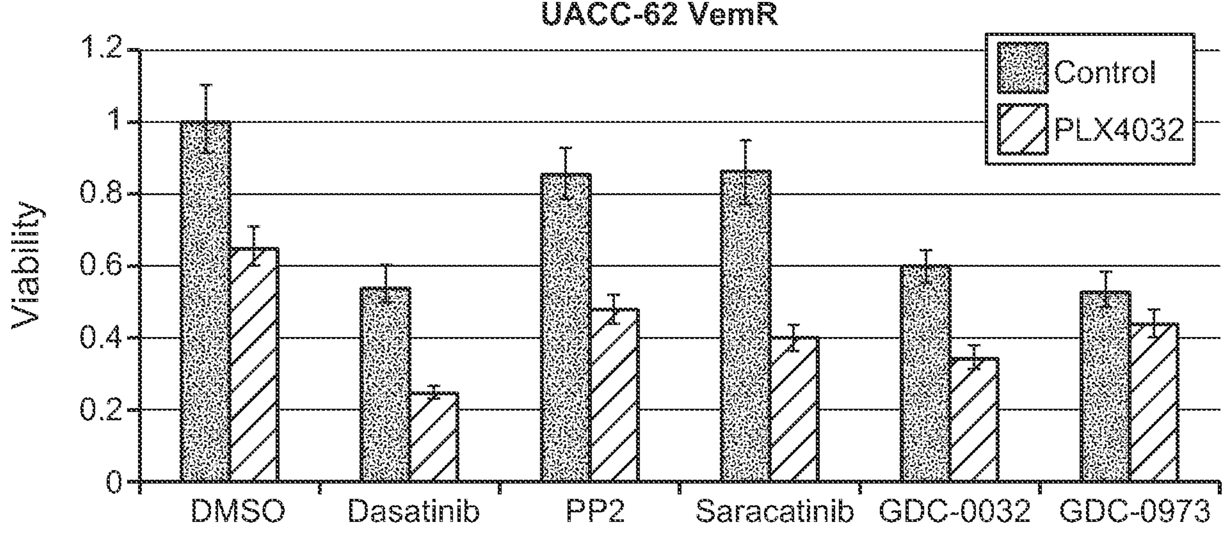
		Inhibitor			
		SRC/ABL	ABL	SRC	SRC
COLO 800 UACC-62	Par, Con	Resistant	Resistant	Resistant	Resistant
	VemR, Con	Resistant	Resistant	Resistant	Resistant
	VemR, PLX	Sensitive	Resistant	Sensitive	Sensitive
SK-MEL-28	Par, Con	Resistant	Resistant	Resistant	Resistant
	VemR, Con	Resistant	Resistant	Resistant	Resistant
	VemR, PLX	Sensitive	Resistant	Resistant	Resistant
A-375 LOX IMVI	Par, Con	Sensitive	Resistant	Resistant	Resistant
	VemR, Con	Resistant	Resistant	Resistant	Resistant
	VemR, PLX	Sensitive	Resistant	Resistant	Resistant
G-361	Par, Con	Resistant	Resistant	Resistant	Resistant
	VemR, Con	Resistant	Sensitive	Resistant	Resistant
	VemR, PLX	Resistant	Resistant	Resistant	Resistant
Other Lines	Par, Con	Resistant	Resistant	Resistant	Resistant
	VemR, Con	Resistant	Resistant	Resistant	Resistant
	VemR, PLX	Resistant	Resistant	Resistant	Resistant

Sensitive IC50 = <1µM

Resistant IC50 >1µM

FIG. 9A





1μM Dasatinib; 1μMPP2; 1μM Saracatinib; 5μM GDC-0032; 0.5μM GDC-0973, 72hr Assay

FIG. 9B

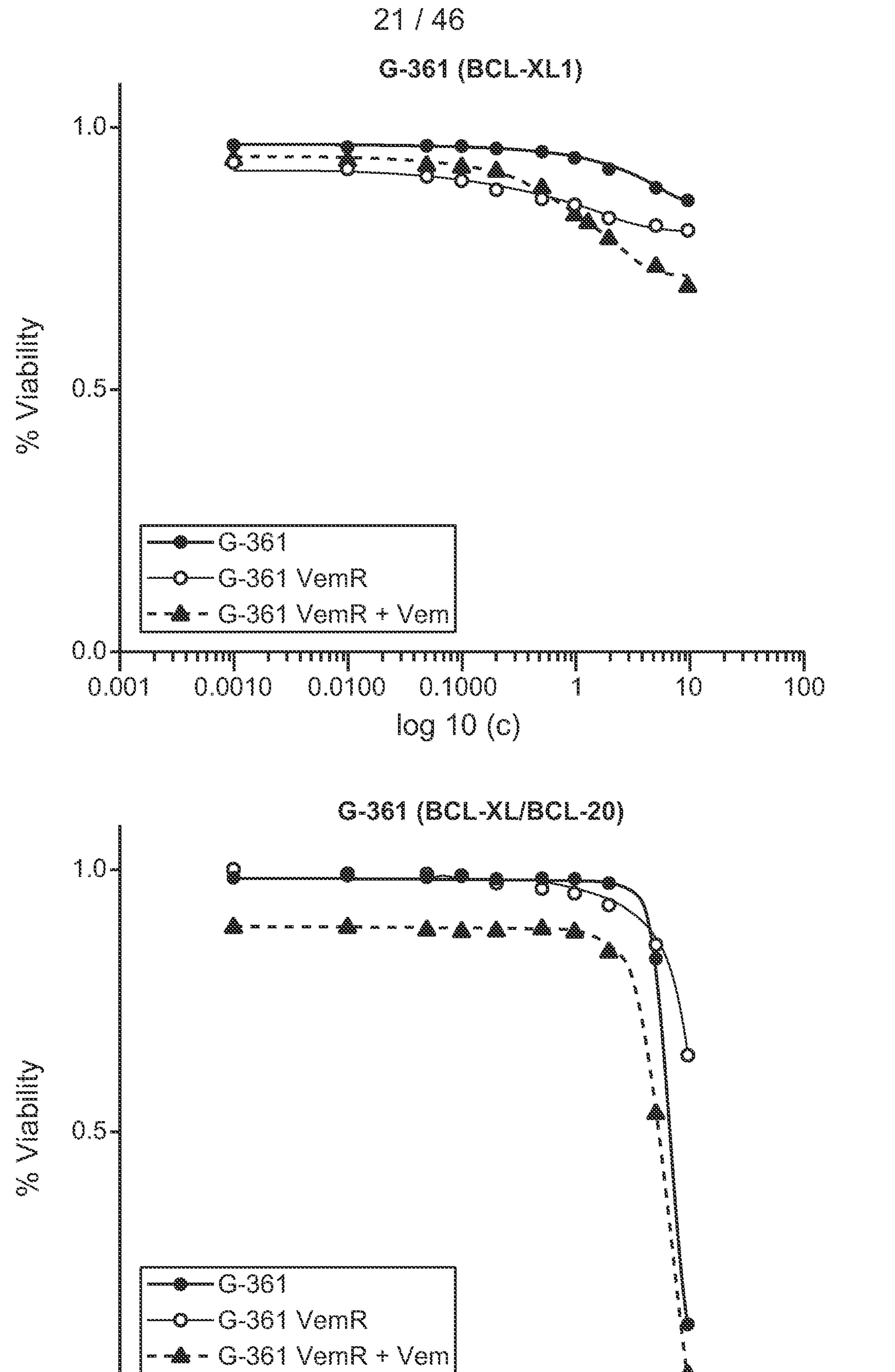
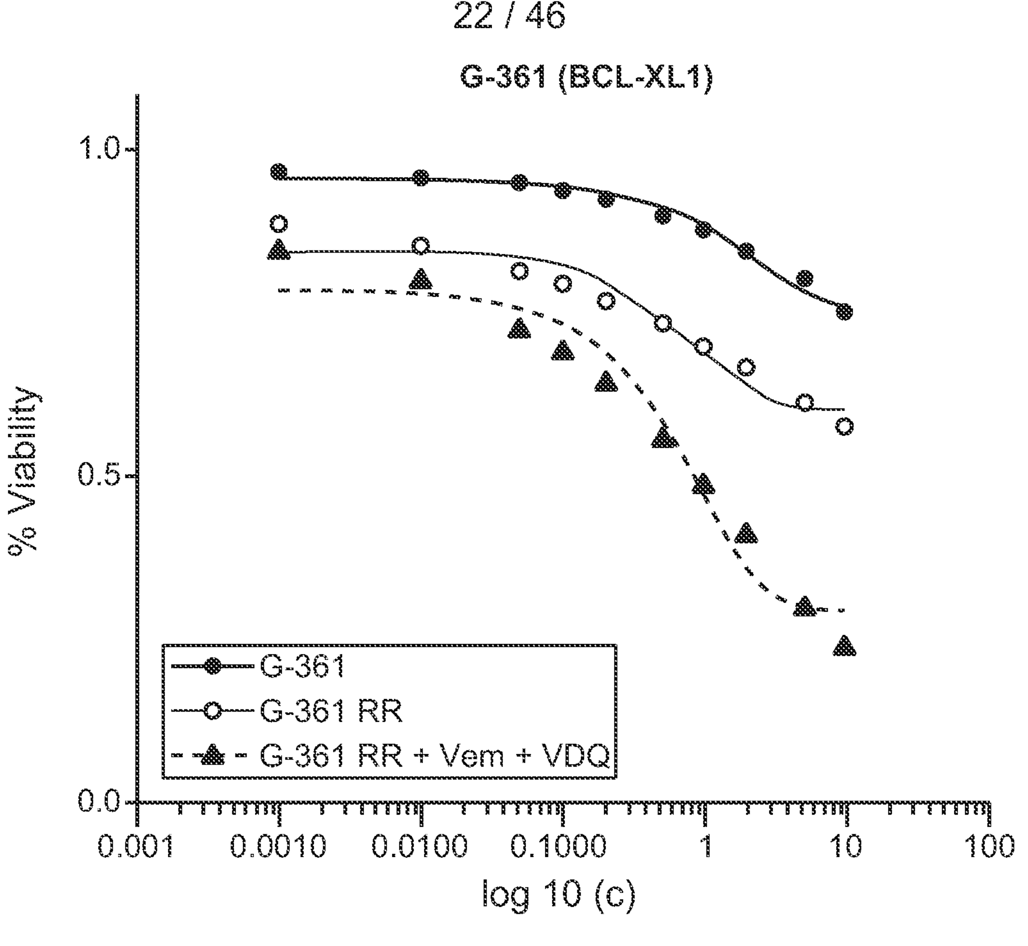


FIG. 9C-1



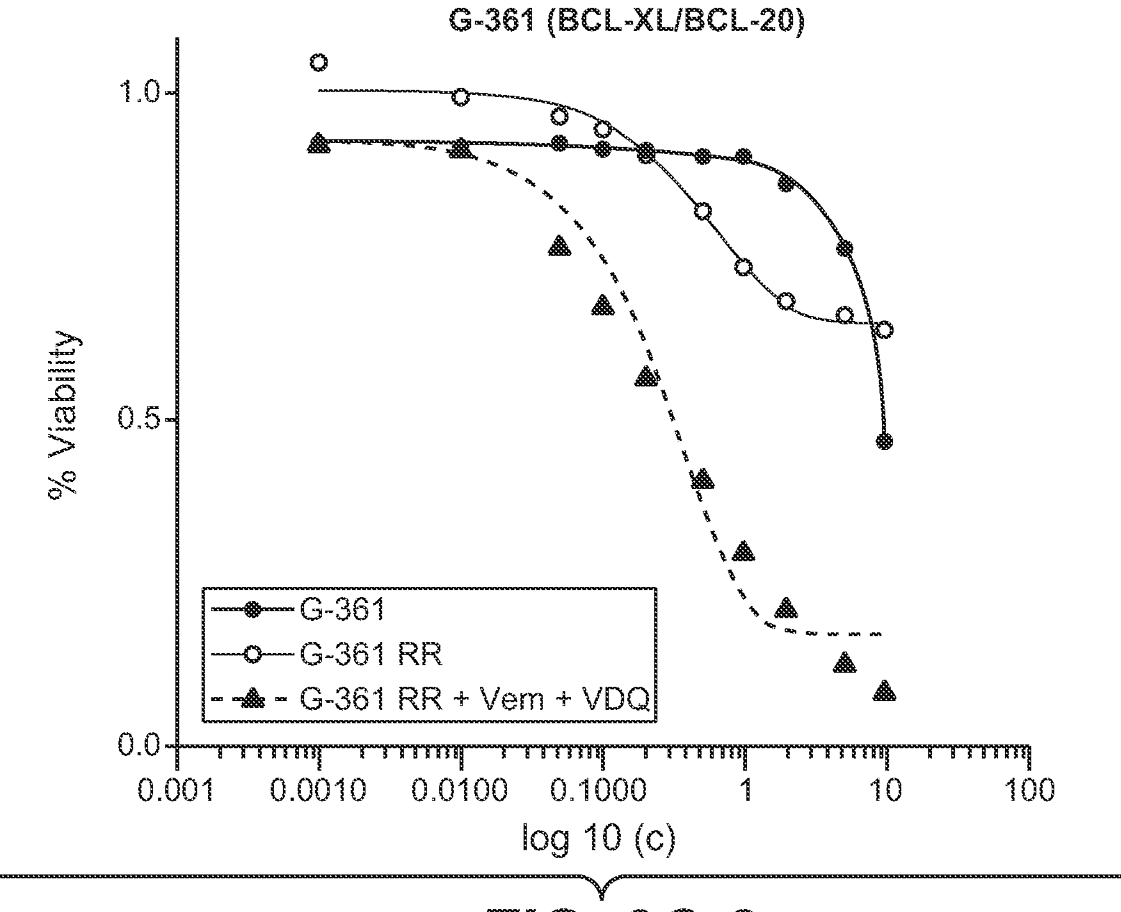


FIG. 9C-2

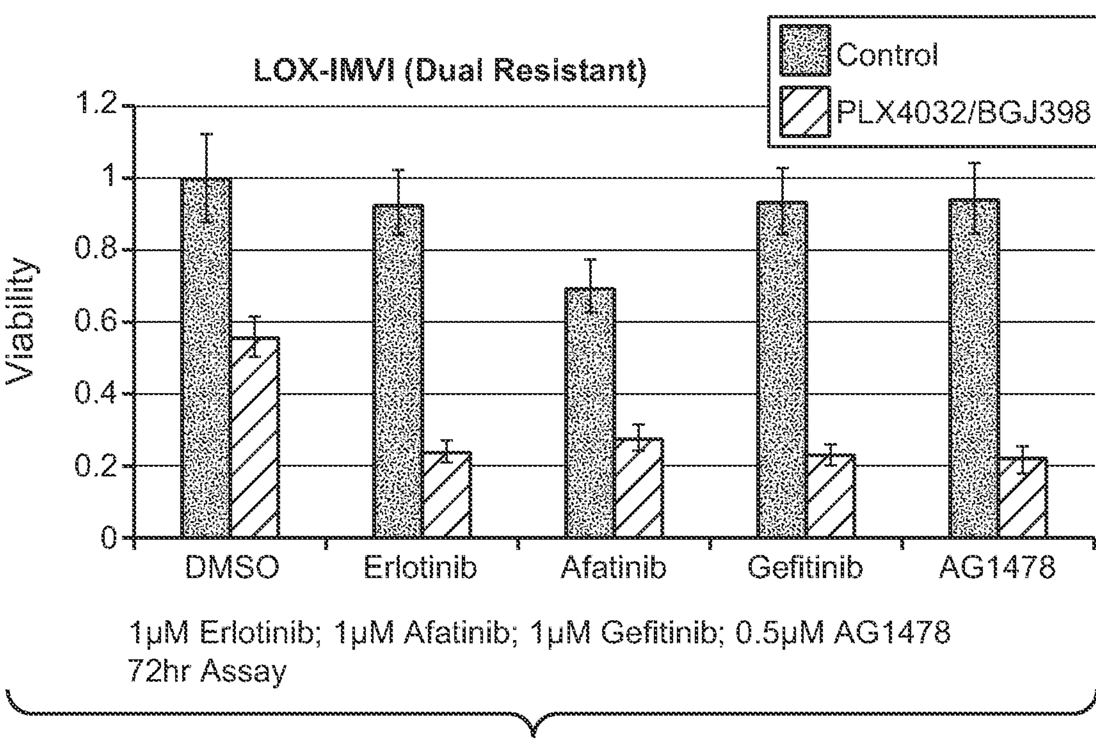


FIG. 10A

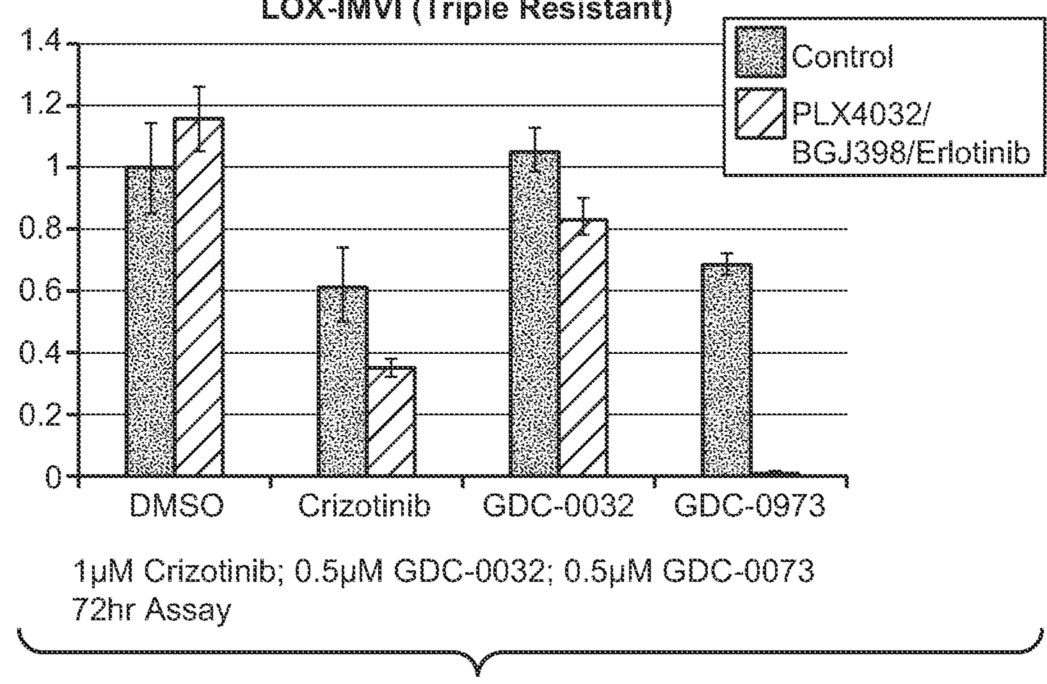


FIG. 10B

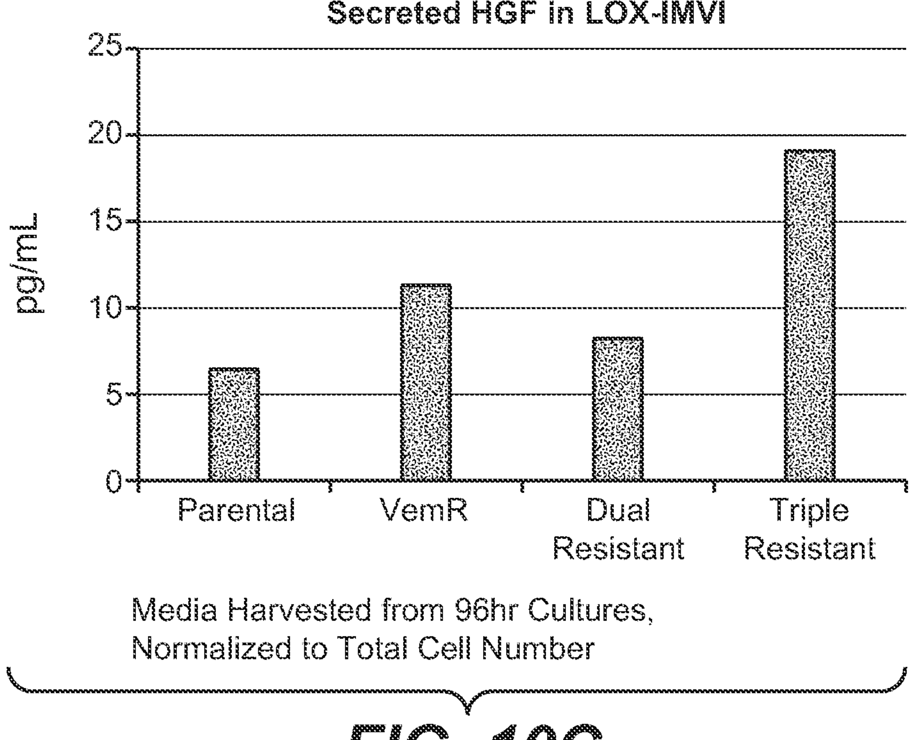


FIG. 10C

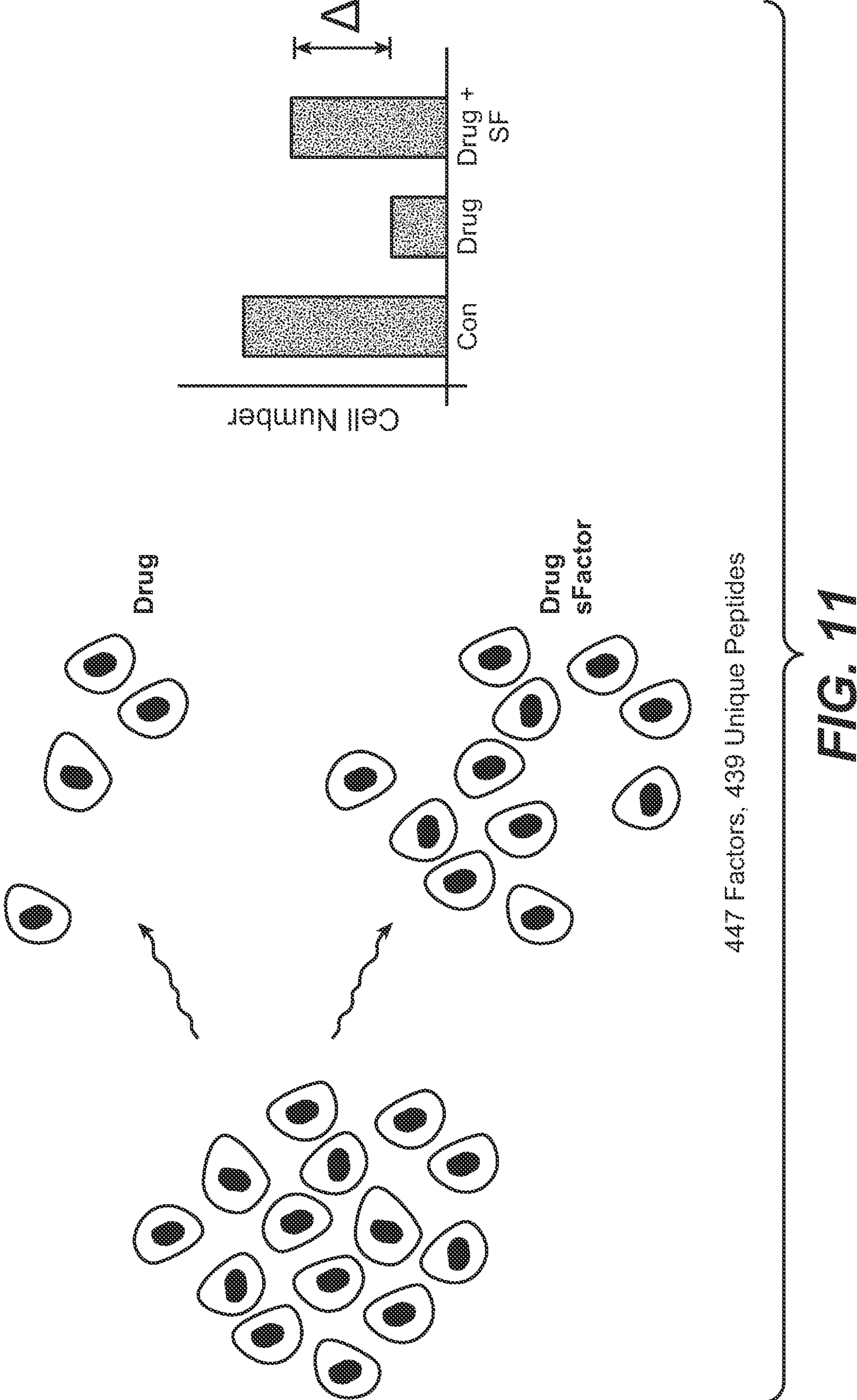
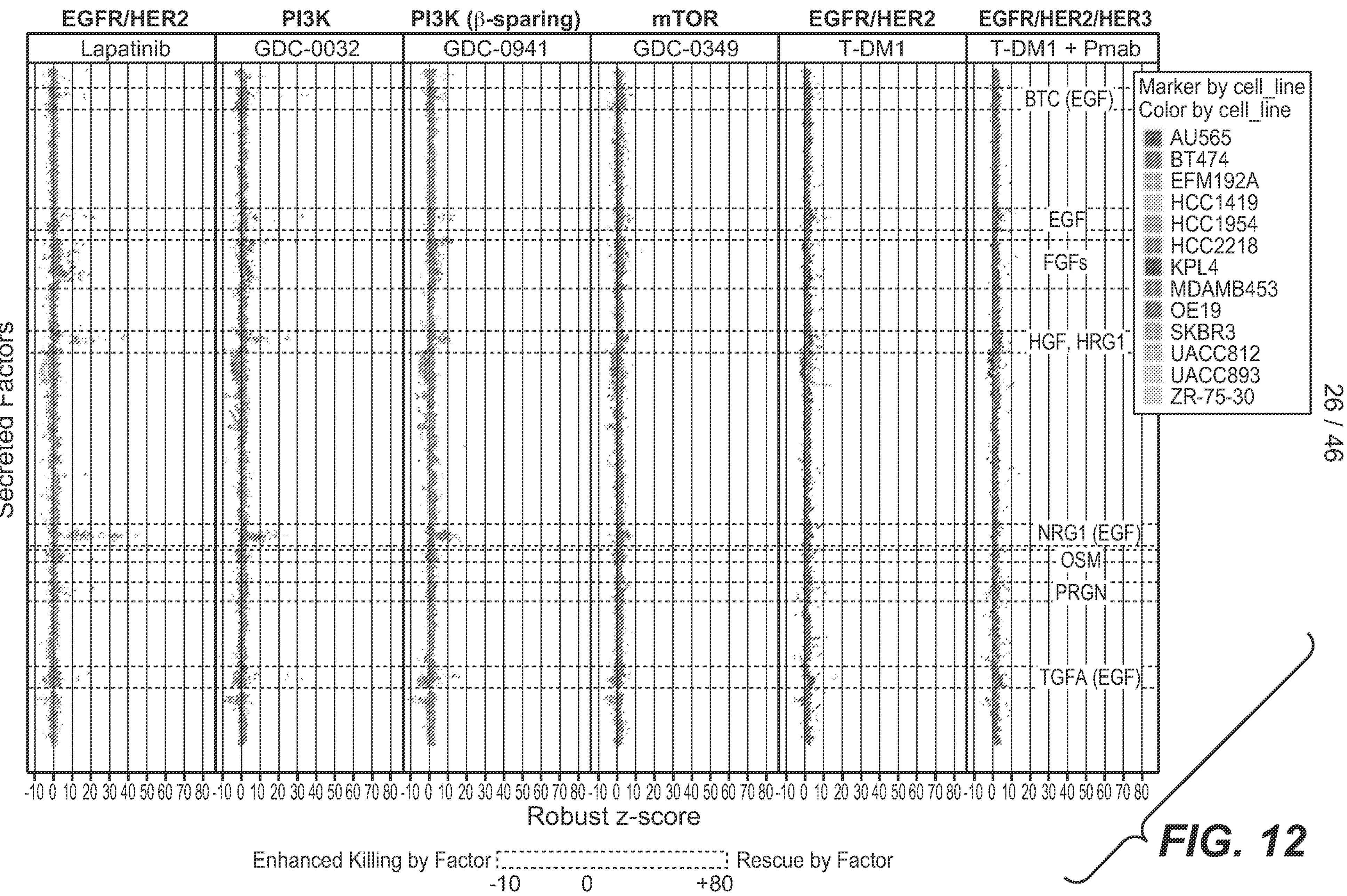


FIG. 11


EGFR/HER2
Lapatinib
PI3K
GDC-0032
PI3K (β-sparing)
GDC-0941
mTOR
GDC-0349
EGFR/HER2
T-DM1
EGFR/HER2/HER3
T-DM1 + Pmab
BTC (EGF)
EGF
FGFs
HGF, HRG1
NRG1 (EGF)
OSM
PRGN
TGFA (EGF)
Marker by cell_line
Color by cell_line
AU565
BT474
EFM192A
HCC1419
HCC1954
HCC2218
KPL4
MDAMB453
OE19
SKBR3
UACC812
UACC893
ZR-75-30
Secreted Factors
-10 0 10 20 30 40 50 60 70 80
Robust z-score
Enhanced Killing by Factor
Rescue by Factor
-10
0
+80
FIG. 12

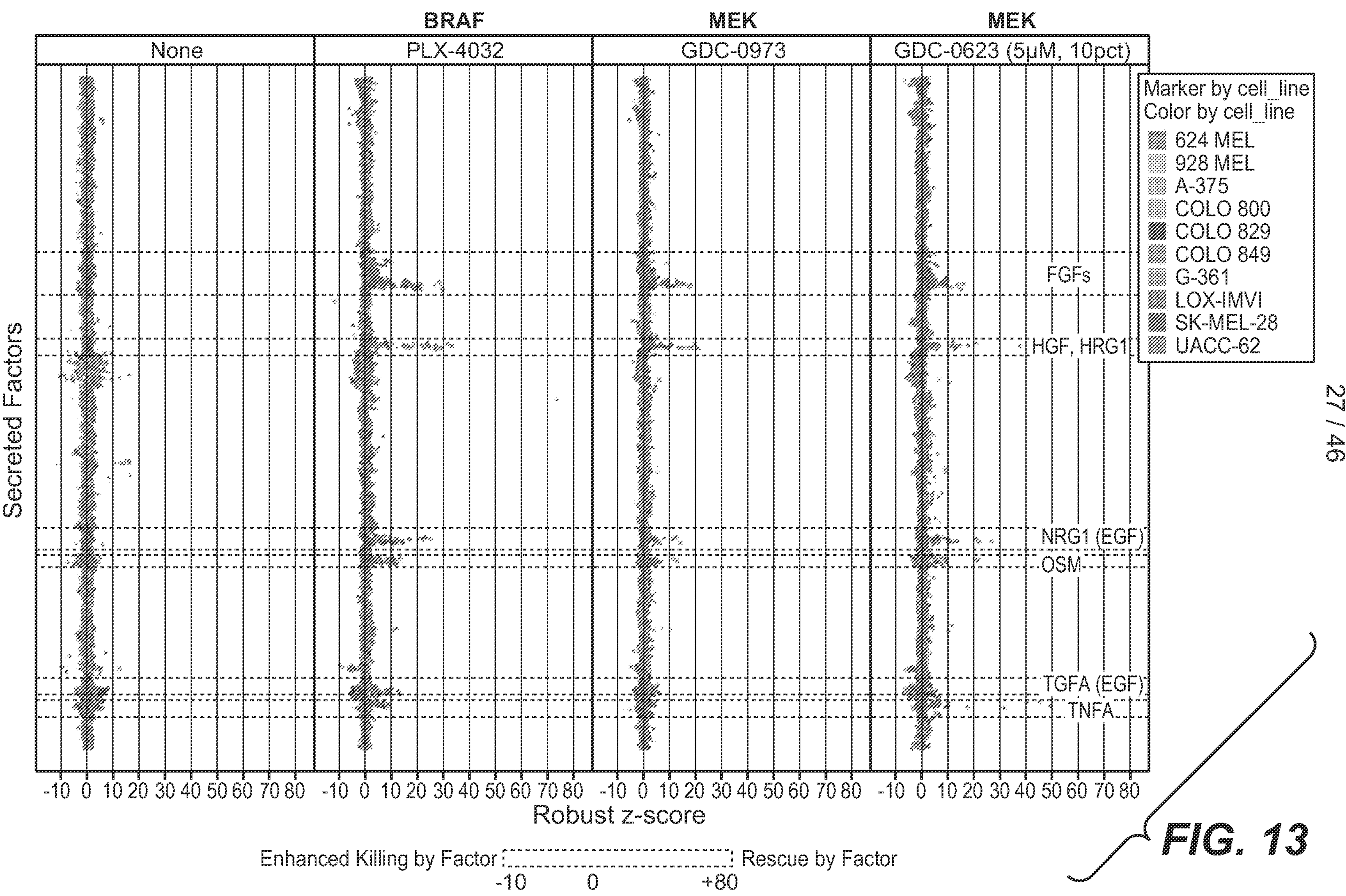


FIG. 13

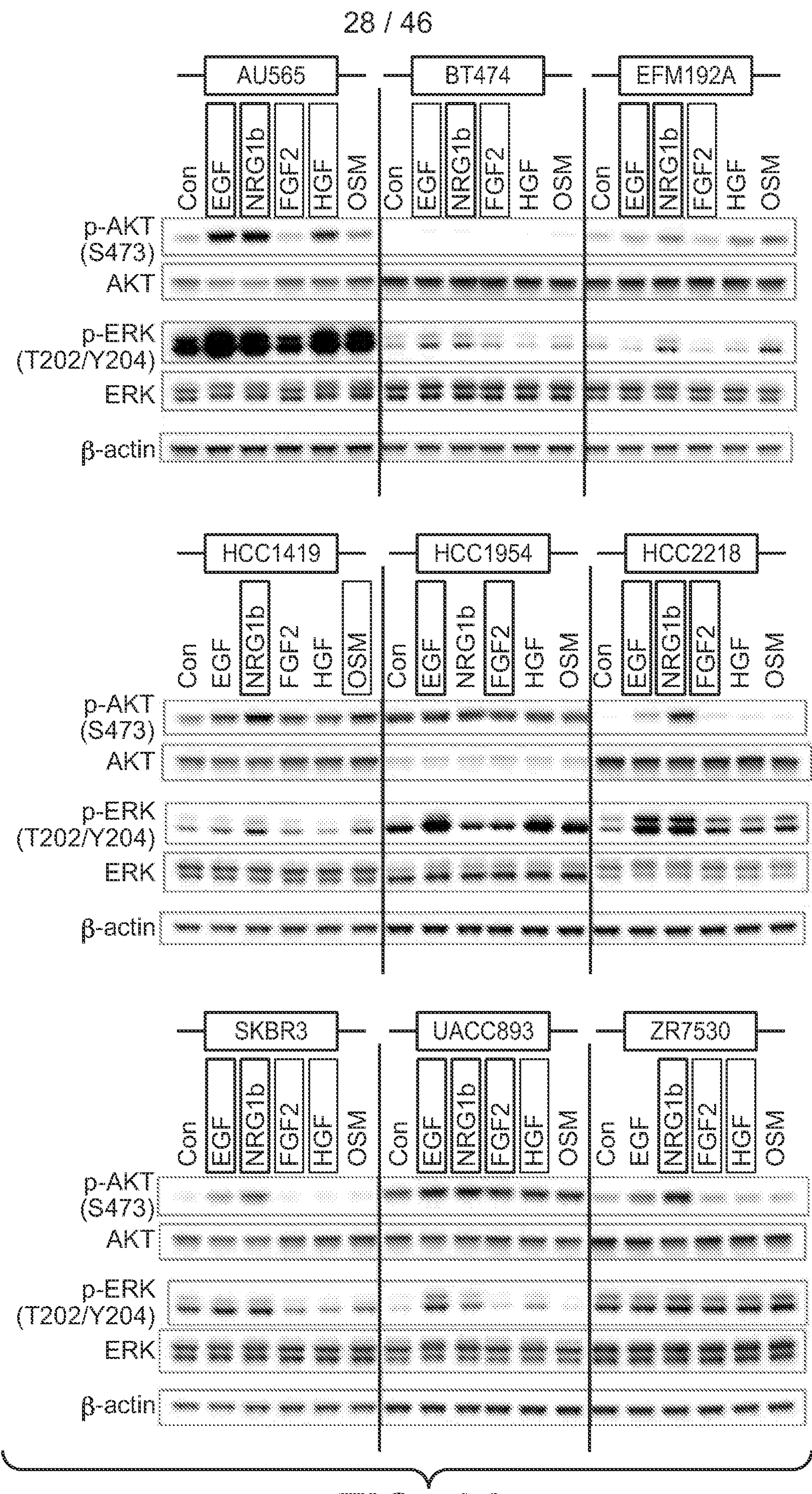


FIG. 14

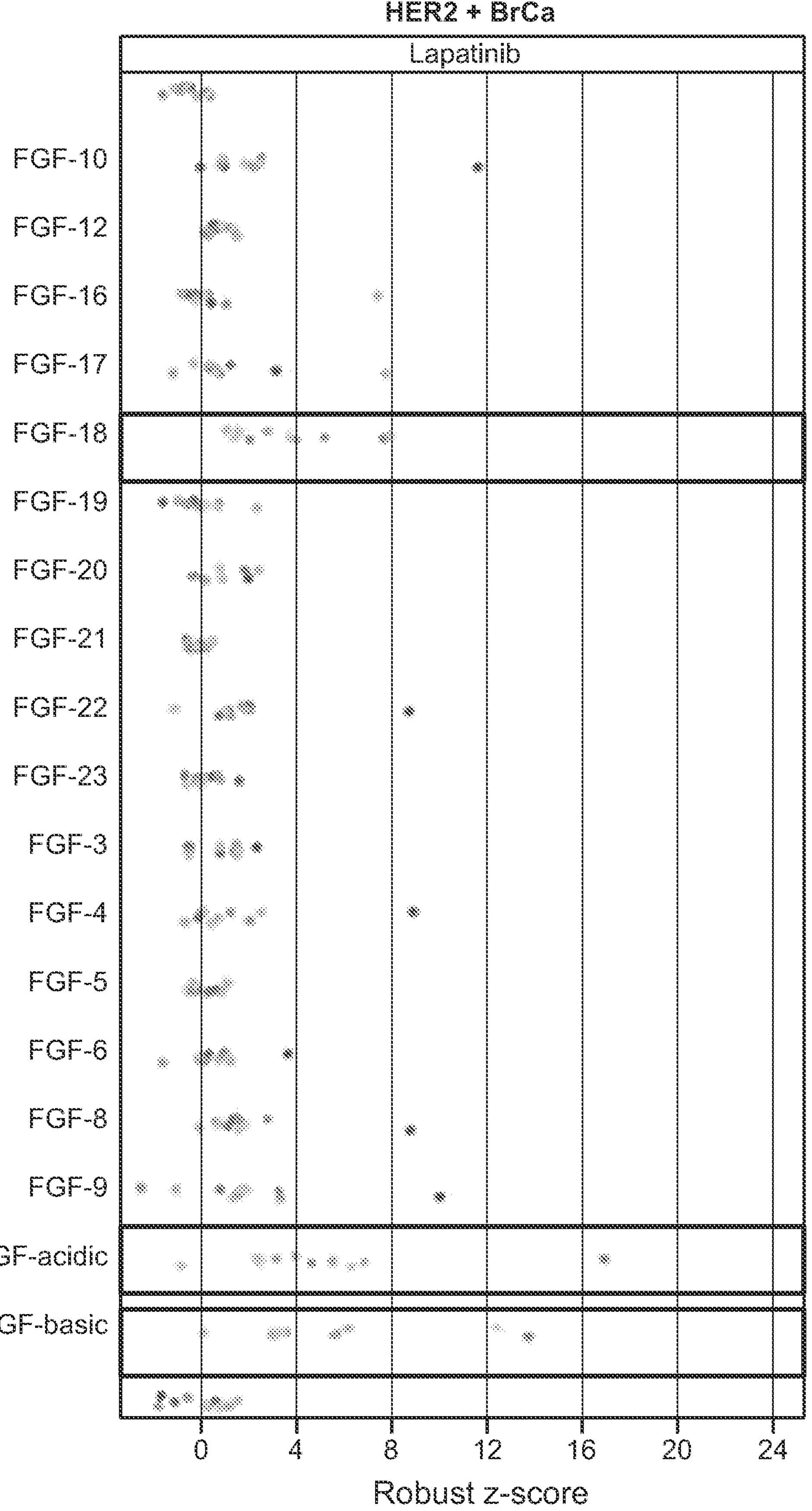


FIG. 15A

FIG. 15B

Subfamily	Ligand	Alternate Name	FGF Receptors						
			R1b	R1c	R2b	R2c	R3b	R3c	R4
FGF-1	FGF-1	FGF-acidic	■	■	■	■	■	■	■
	FGF-2	FGF-basic	■	■		■		■	■
FGF-4	FGF-4	K-FGF, int-1		■		■		■	■
	FGF-5	HBGF-5		■		■			
	FGF-6	HBGF-6		■		■			■
FGF-7	FGF-3	int-2	■		■				
	FGF-7	EGF			■				
	FGF-10	KGF-2	■		■				
	FGF-22		■		■				
FGF-8	FGF-8a	AIGF							
	FGF-8b	AIGF		■		■		■	■
	FGF-8e	AIGF						■	■
	FGF-8f	AIGF				■		■	■
	FGF-17			■		■		■	■
	FGF-18					■		■	■
FGF-9	FGF-9	GAF				■	■	■	■
	FGF-16					■	■	■	■
	FGF-20			■	■	■	■	■	■
FGF-11	FGF-11	FHF-3							
	FGF-12	FHF-1							
	FGF-13	FHF-2							
	FGF-14	FHF-4							
FGF-19	FGF-19*	FGF-15(ndata)		■		■		■	■
	FGF-21*			■		■		■	■
	FGF-23*			■		■		■	■

FGF1, FGF2, FGF9, FGF18

FIG. 15C

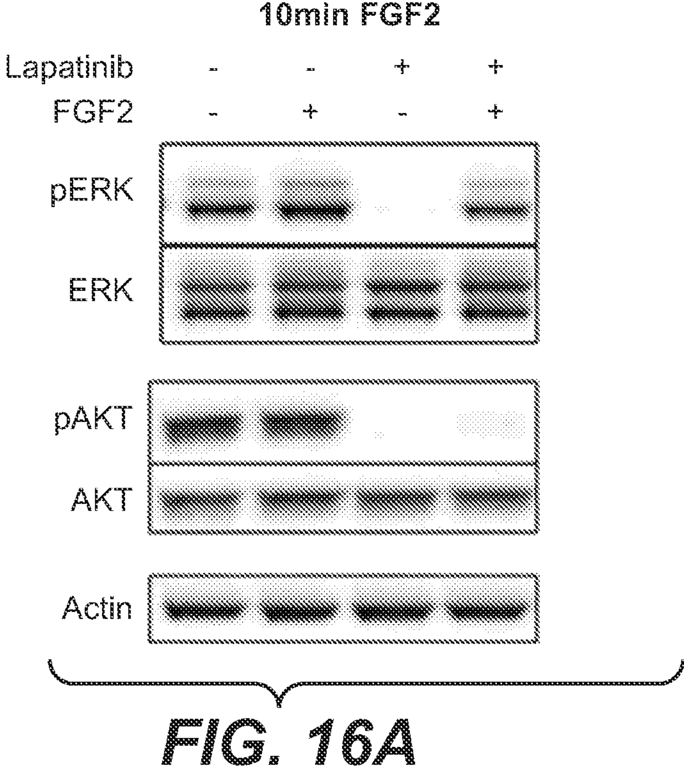


FIG. 16A

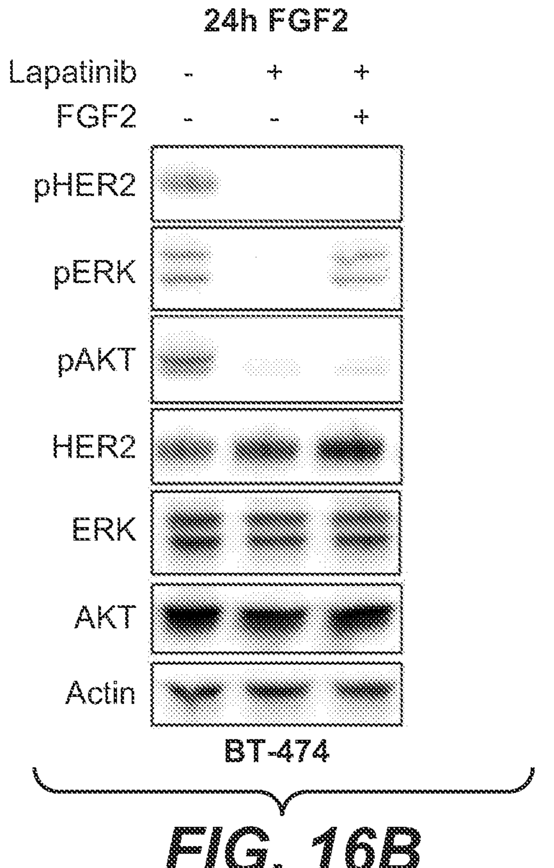


FIG. 16B

4h PLX, 10min FGF

FGF

Con Con 1 2 3 4 5 6 7 8 9 10 12 16 17 18 19 20 21 22 Con

PLX-4032: - + + + + + + + + + + + + + + + + + -

624 MEL: p-MEK, p-ERK

928 MEL: p-MEK, p-ERK

LOX-IMVI: p-MEK, p-ERK

50kDa, 40kDa

FIG. 17A

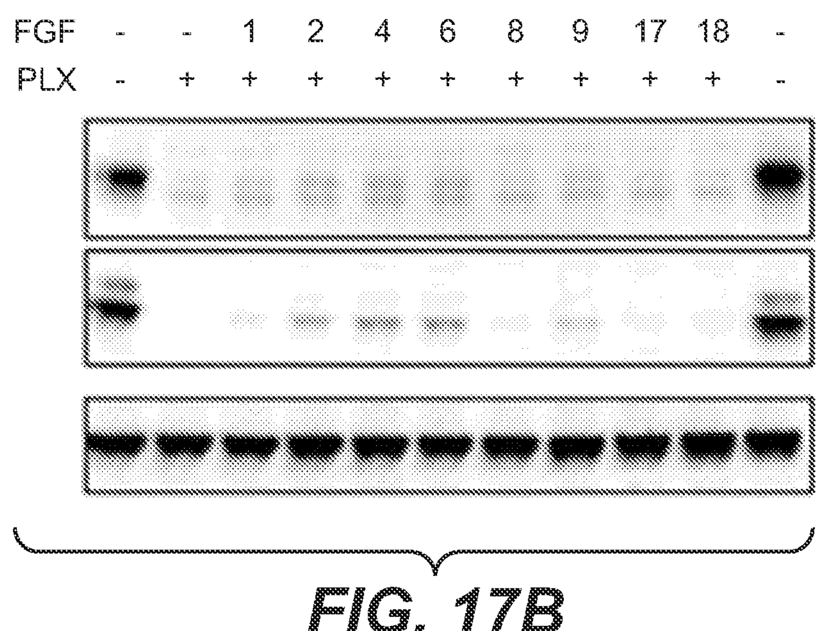


FIG. 17B

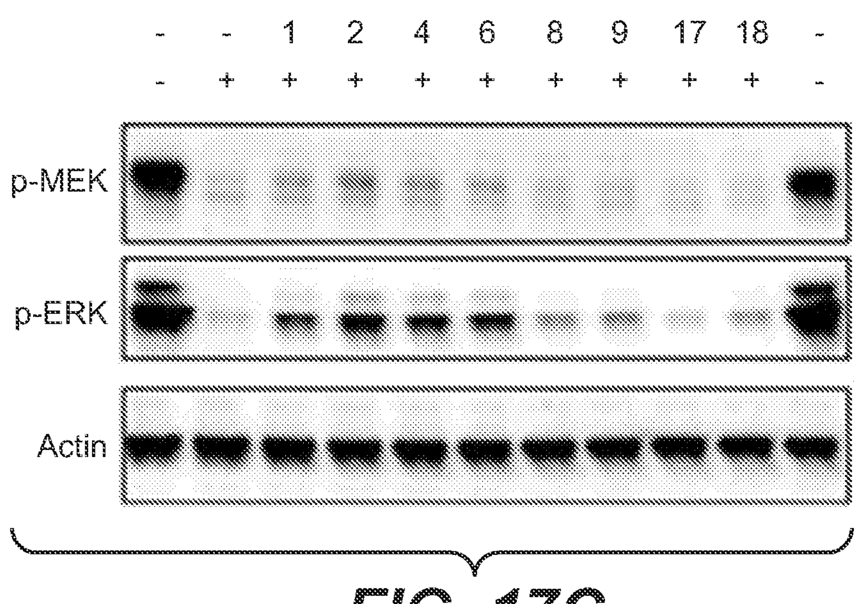


FIG. 17C

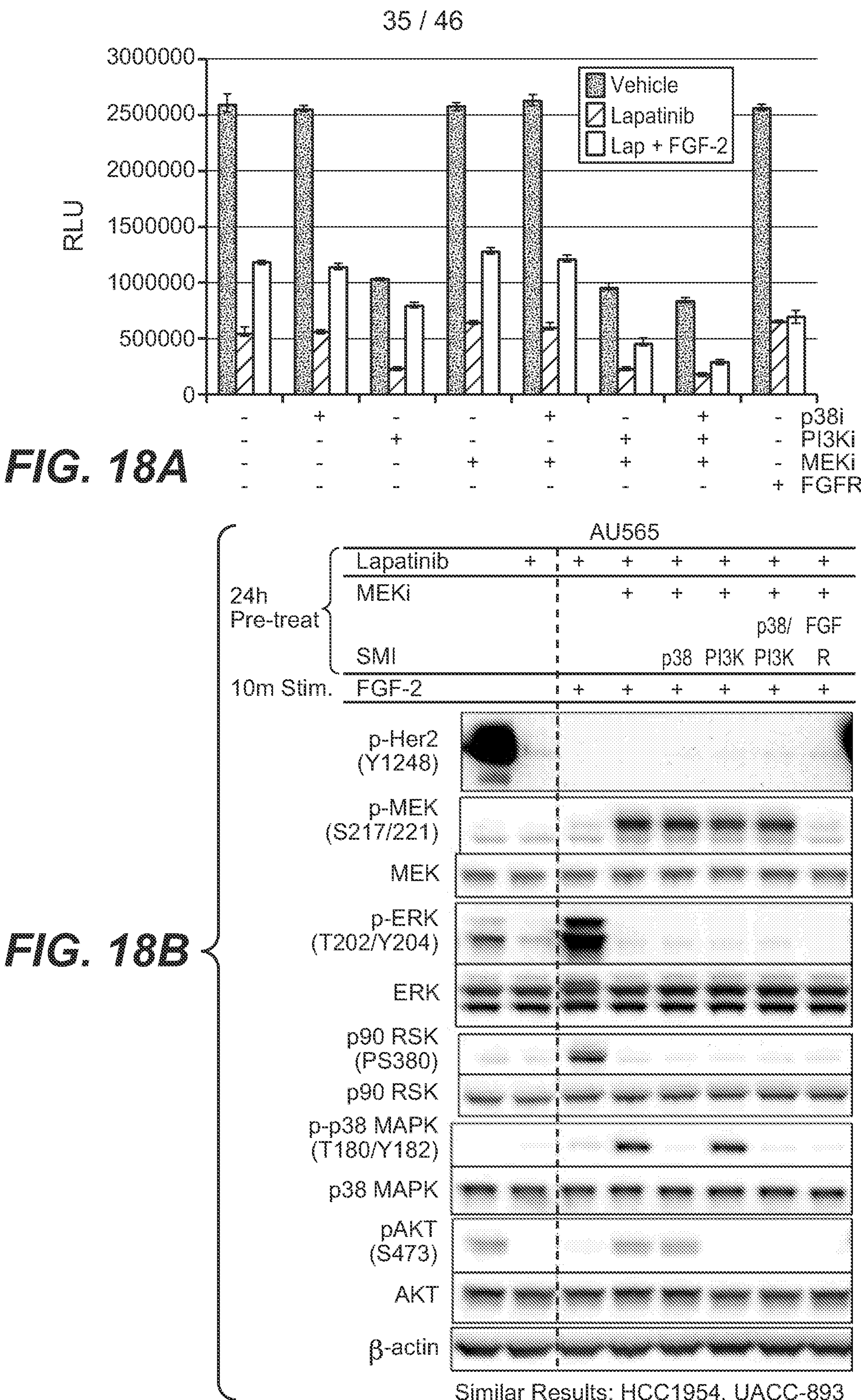


FIG. 18A

FIG. 18B

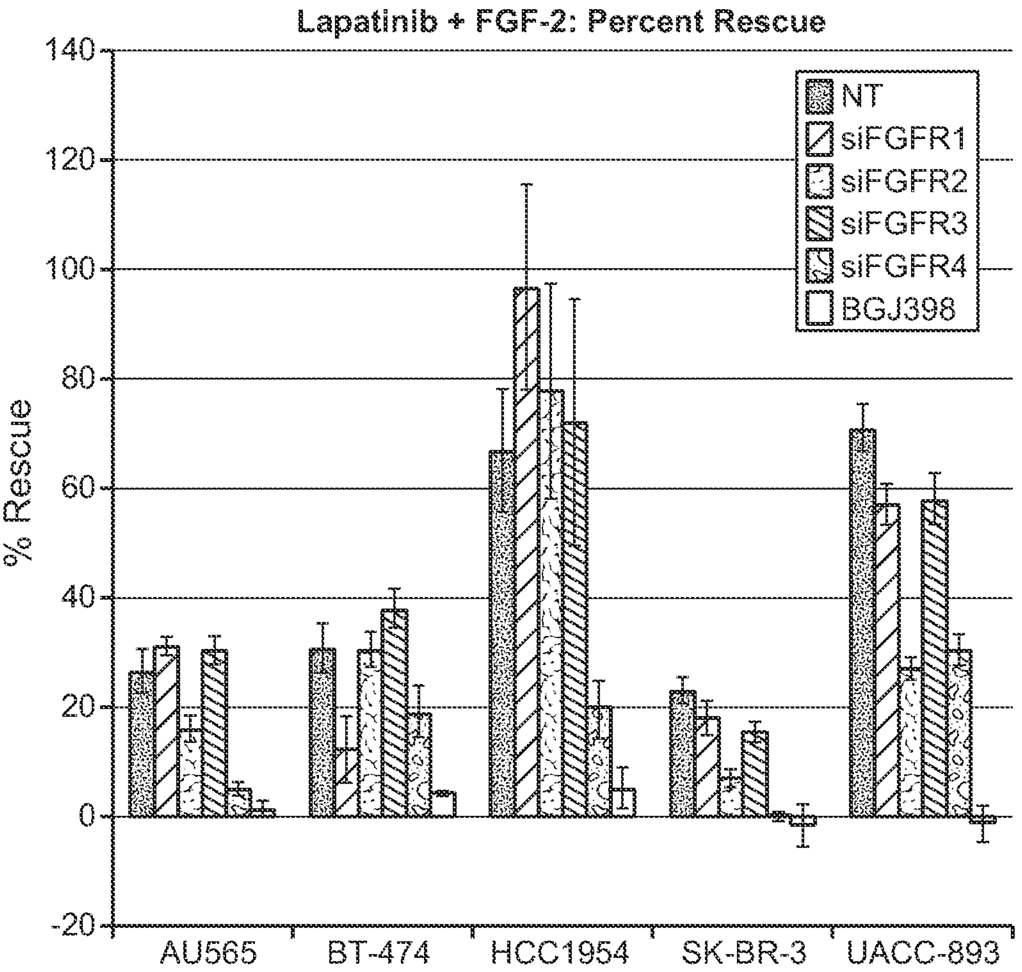


FIG. 19A

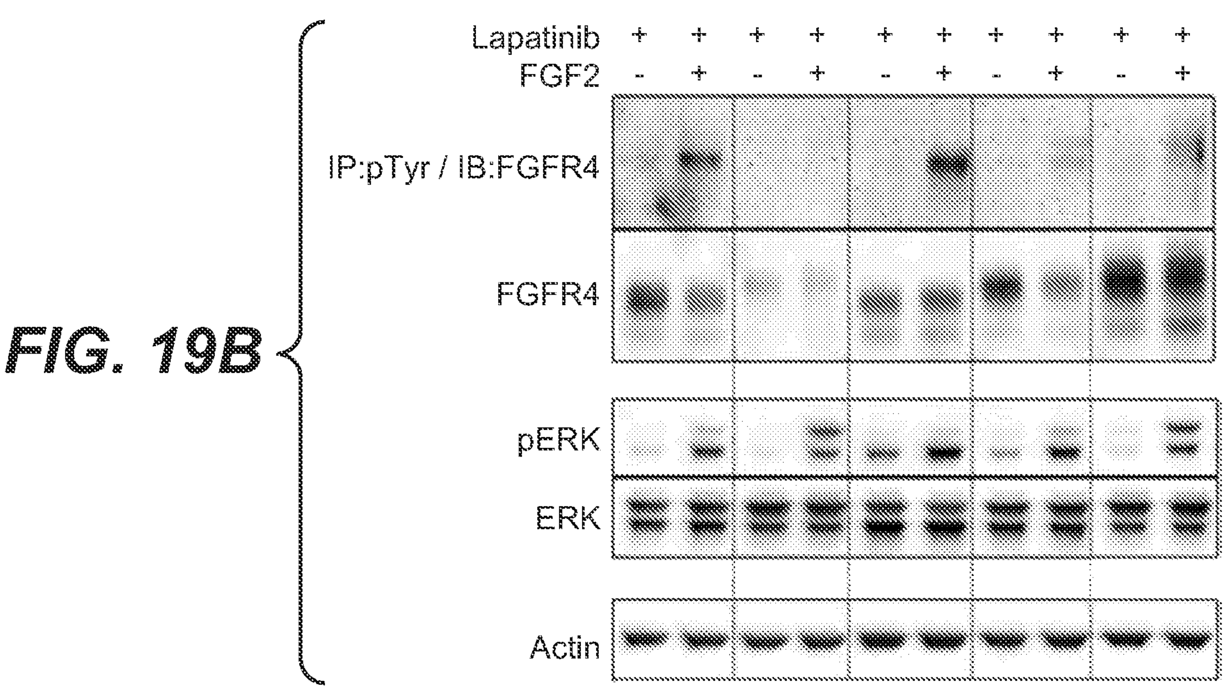


FIG. 19B

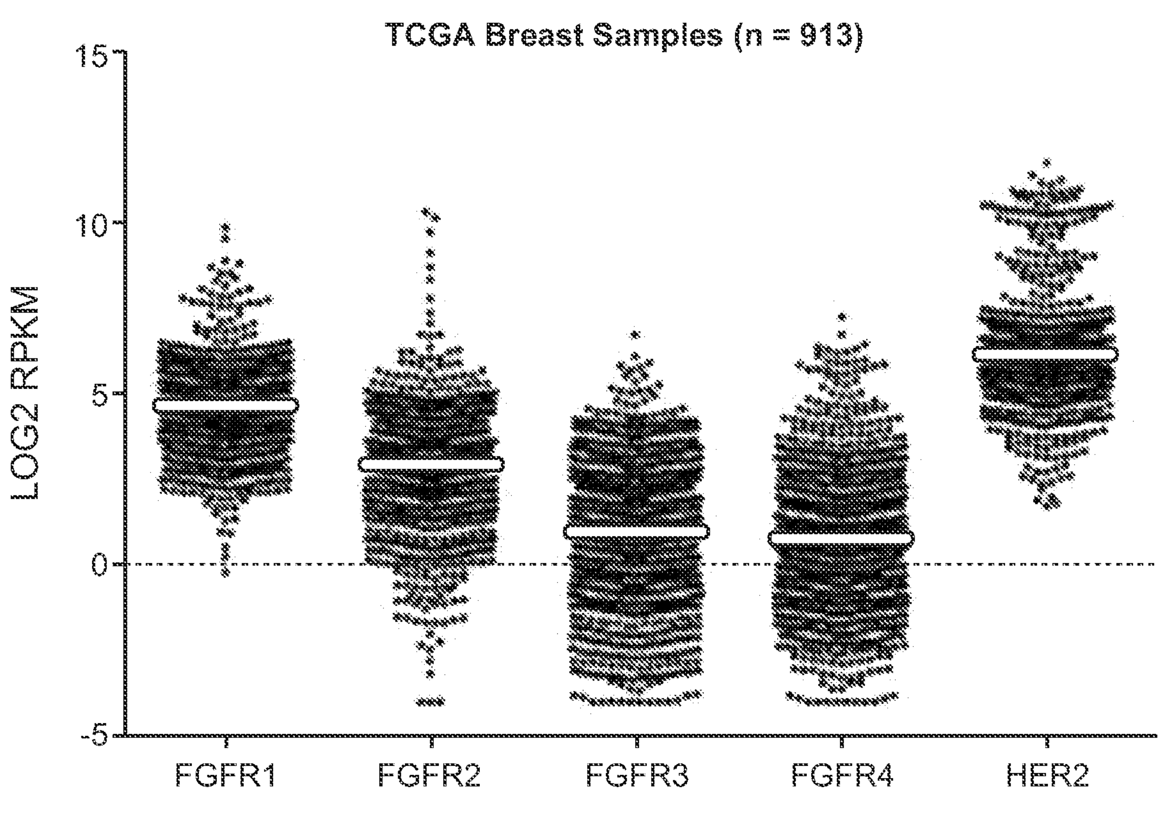


FIG. 19C

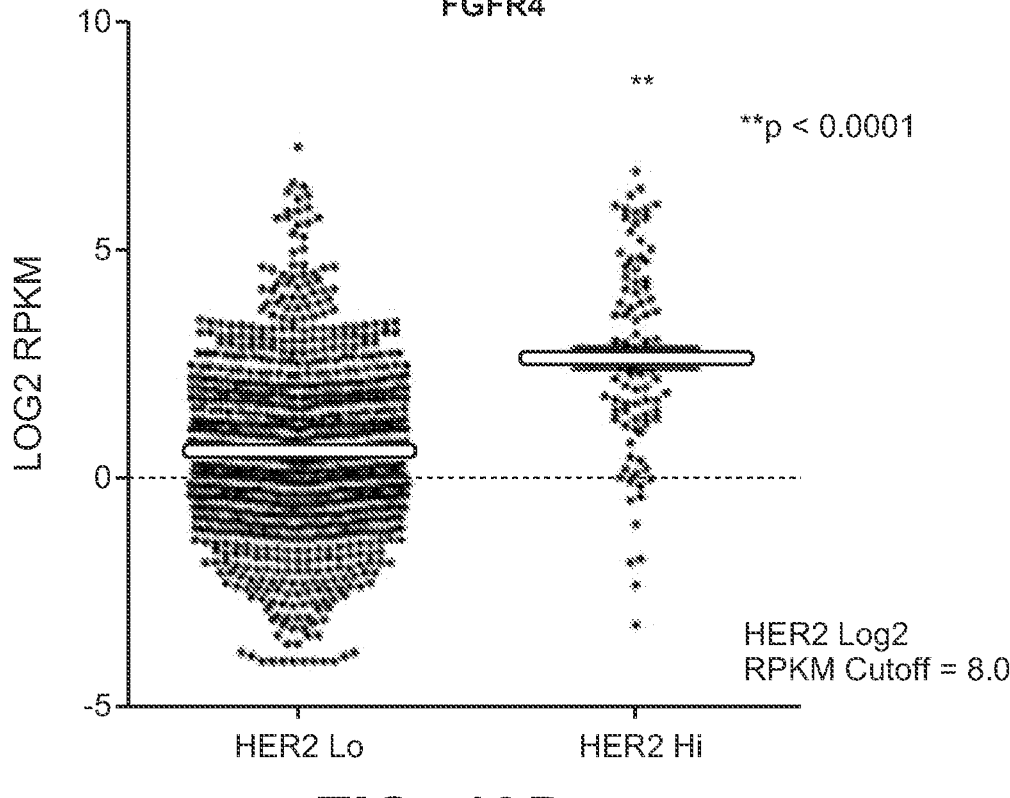


FIG. 19D

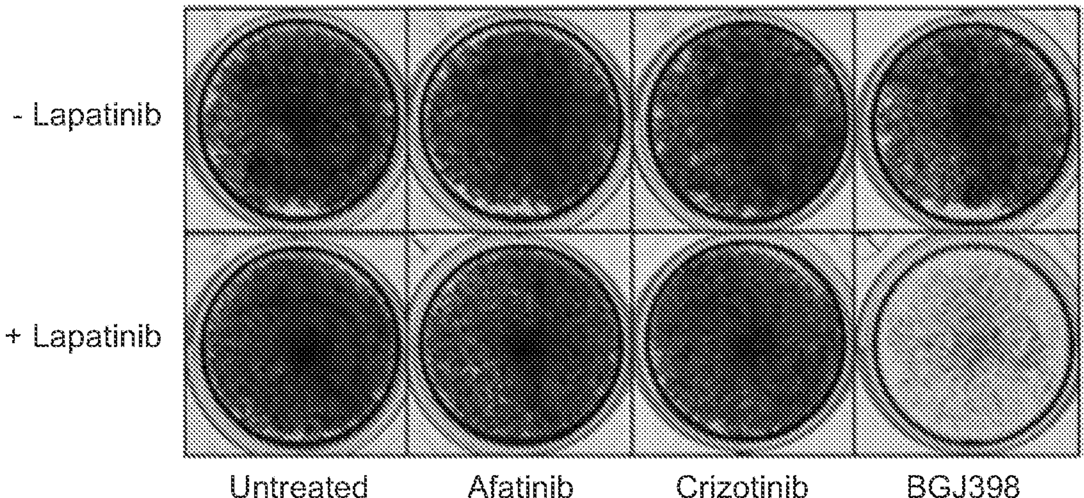


FIG. 20A

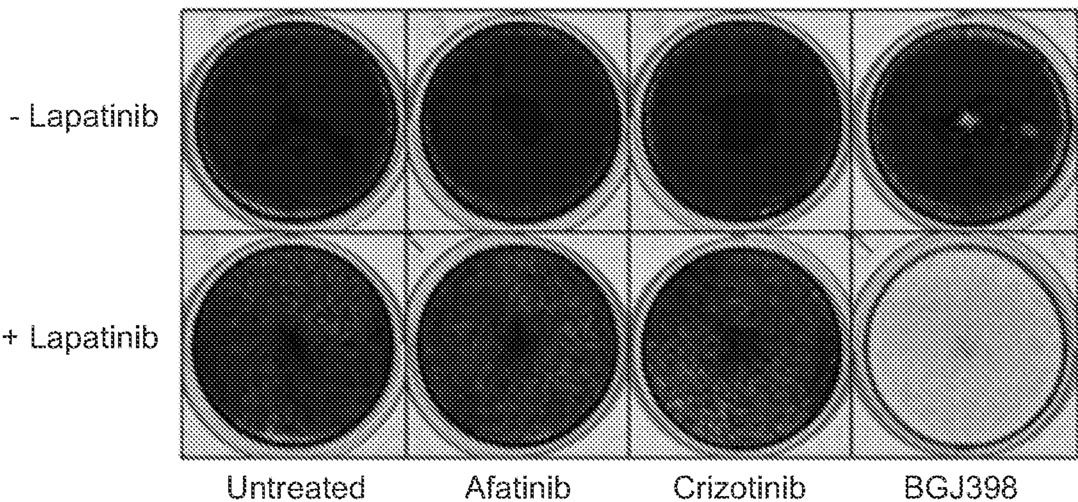


FIG. 20B

Lapatinin (EGFR/HER2)

CTG Norm. DMSO

1.5
1.0
0.5
0.0

-4 -3 -2 -1 0 1 2

Lapatinib

AU565
BT474
EFM192A
HCC1419
HCC1569
HCC1954
HCC2218
MDAMB453
SKBR3
UACC893
ZR7530

FIG. 20C-1

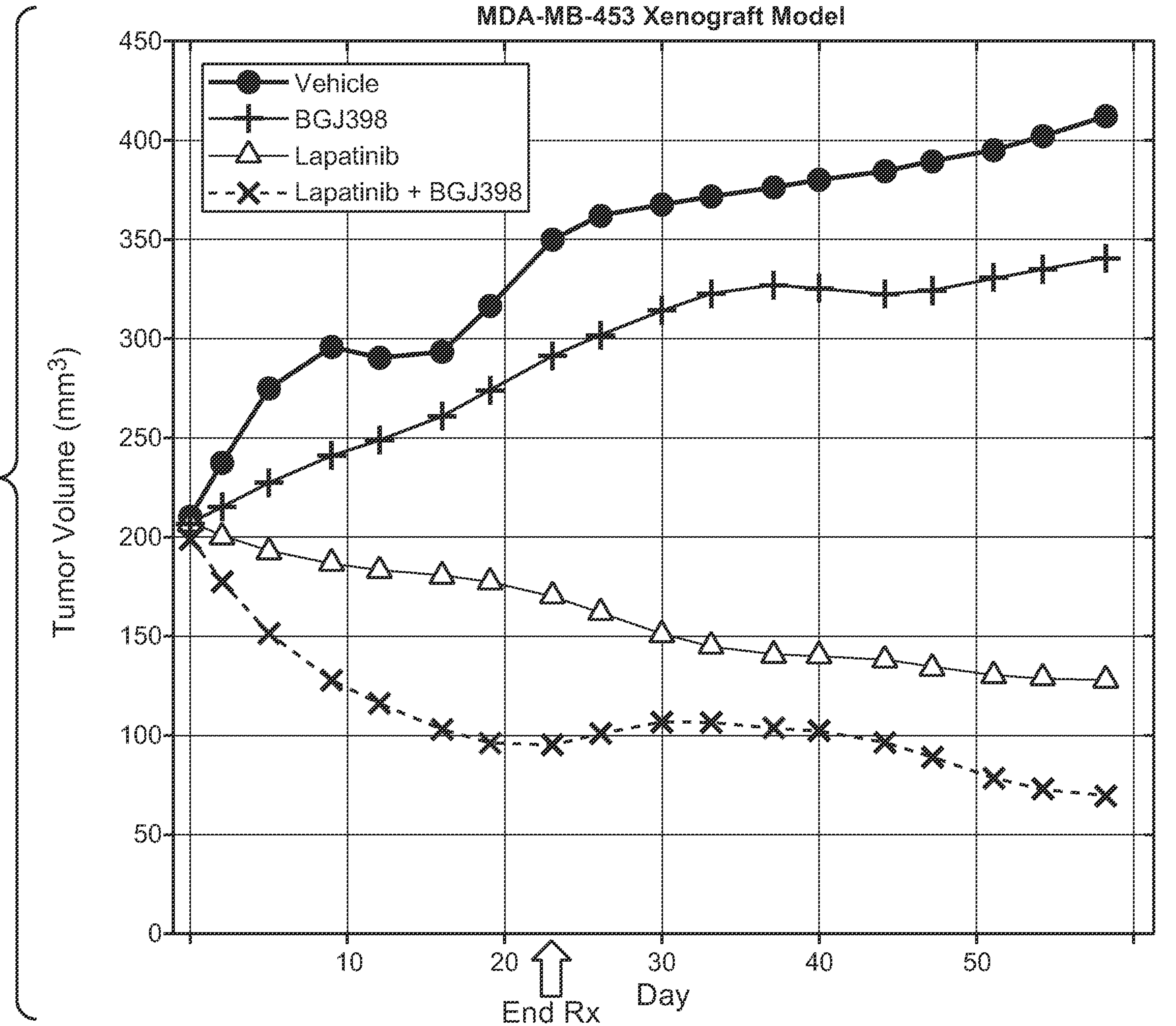


FIG. 20C-2

GDC-0973

PLX:
0μM
5μM

1.2
1
0.8
0.6
0.4
0.2
0

DMSO
GDC-0973

624 MEL VemR
A-375 VemR
COLO 800 VemR
COLO 829 VemR
G-361 VemR
LOX-IMVI VemR
SK-MEL-28 VemR
UACC-62 VemR

Sensitive to ERK/MEK Inhibitors

FIG. 21A-1

G02447286

PLX:
0μM
5μM

1.2 1.8 1.6 1.4 1.2 1 0.8 0.6 0.4 0.2 0

DMSO G02447286 — 624 MEL VemR
DMSO G02447286 — A-375 VemR
DMSO G02447286 — COLO 800 VemR
DMSO G02447286 — COLO 829 VemR
DMSO G02447286 — G-361 VemR
DMSO G02447286 — LOX-IMVI VemR
DMSO G02447286 — SK-MEL-28 VemR
DMSO G02447286 — UACC-62 VemR

Sensitive to ERK/MEK Inhibitors

FIG. 21A-2

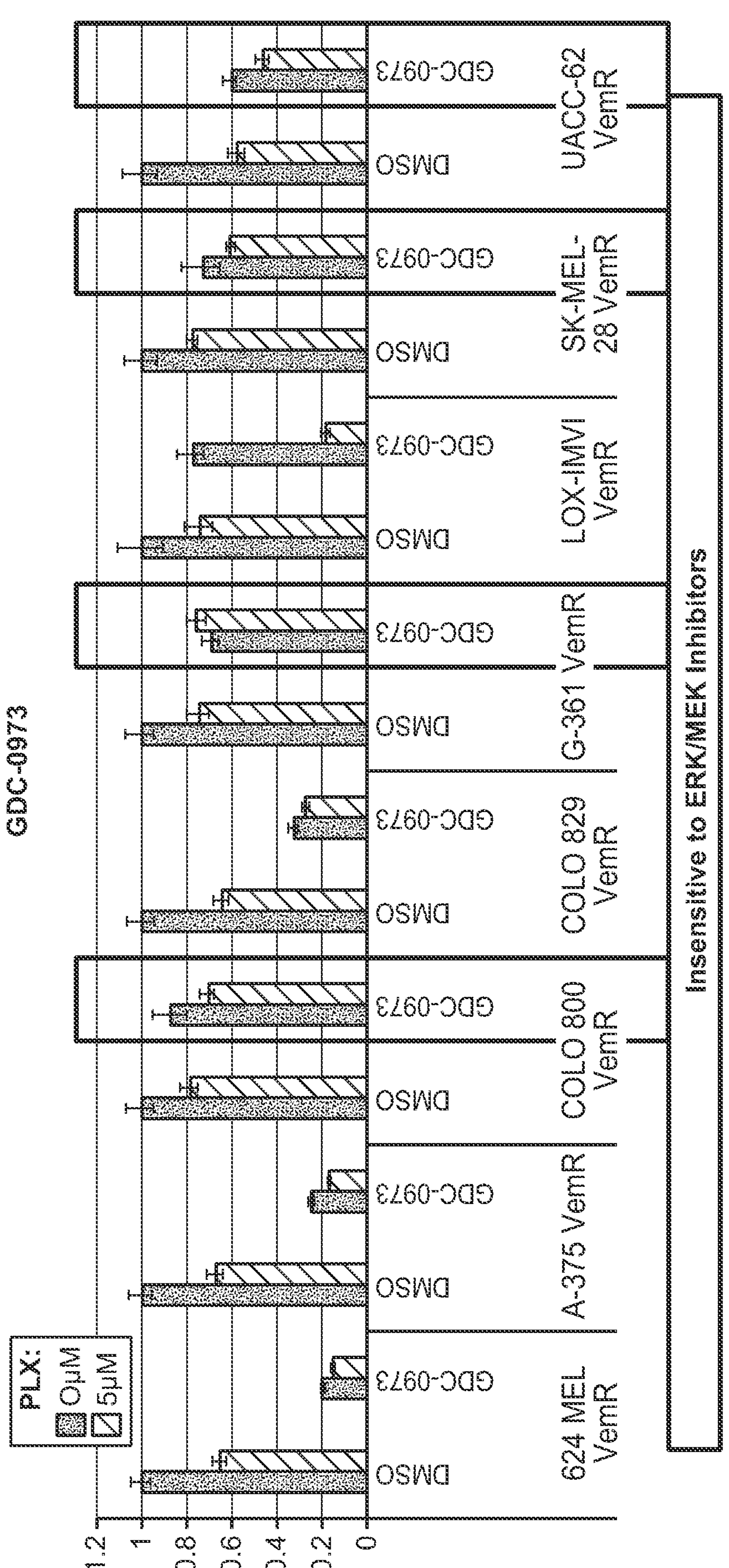


FIG. 21B-1

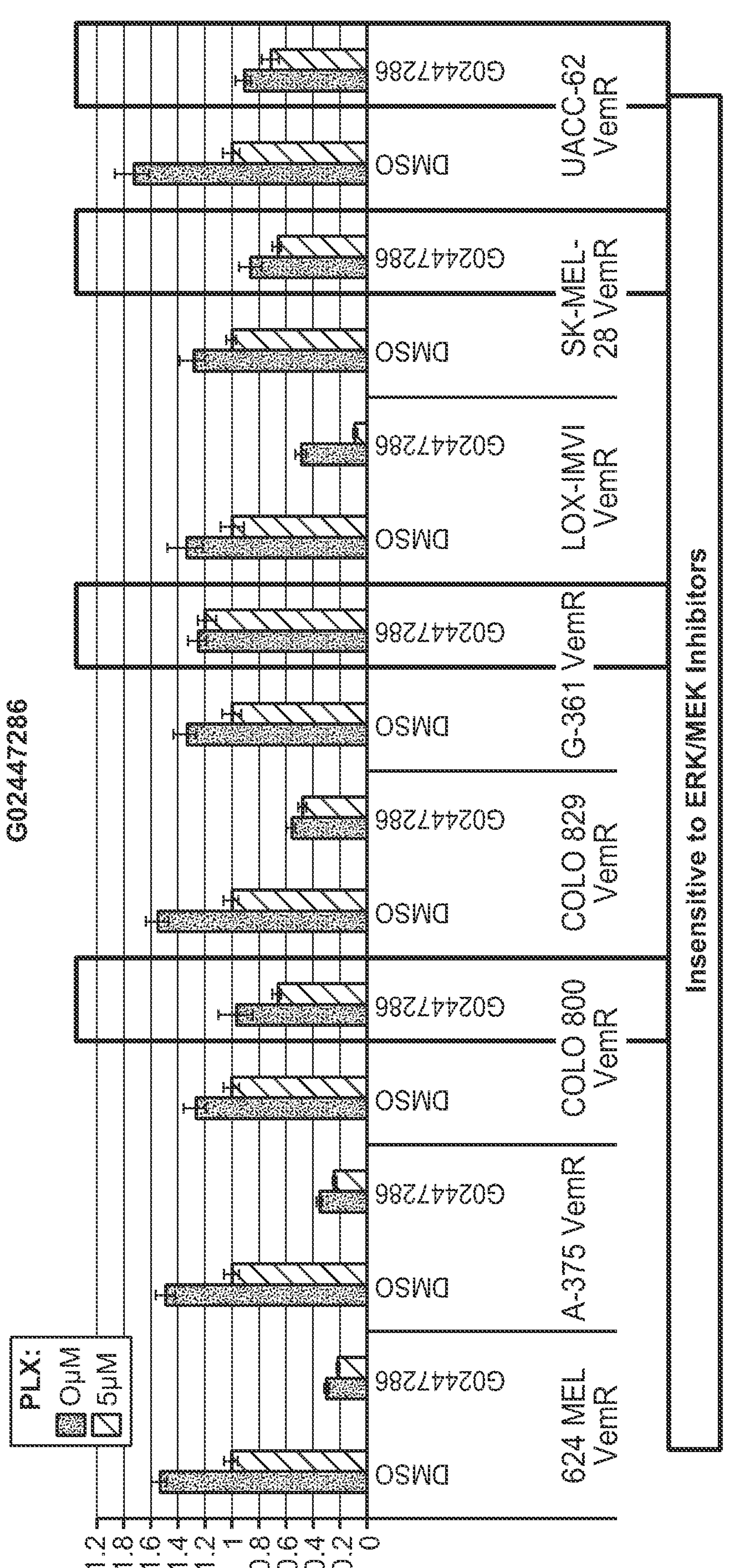
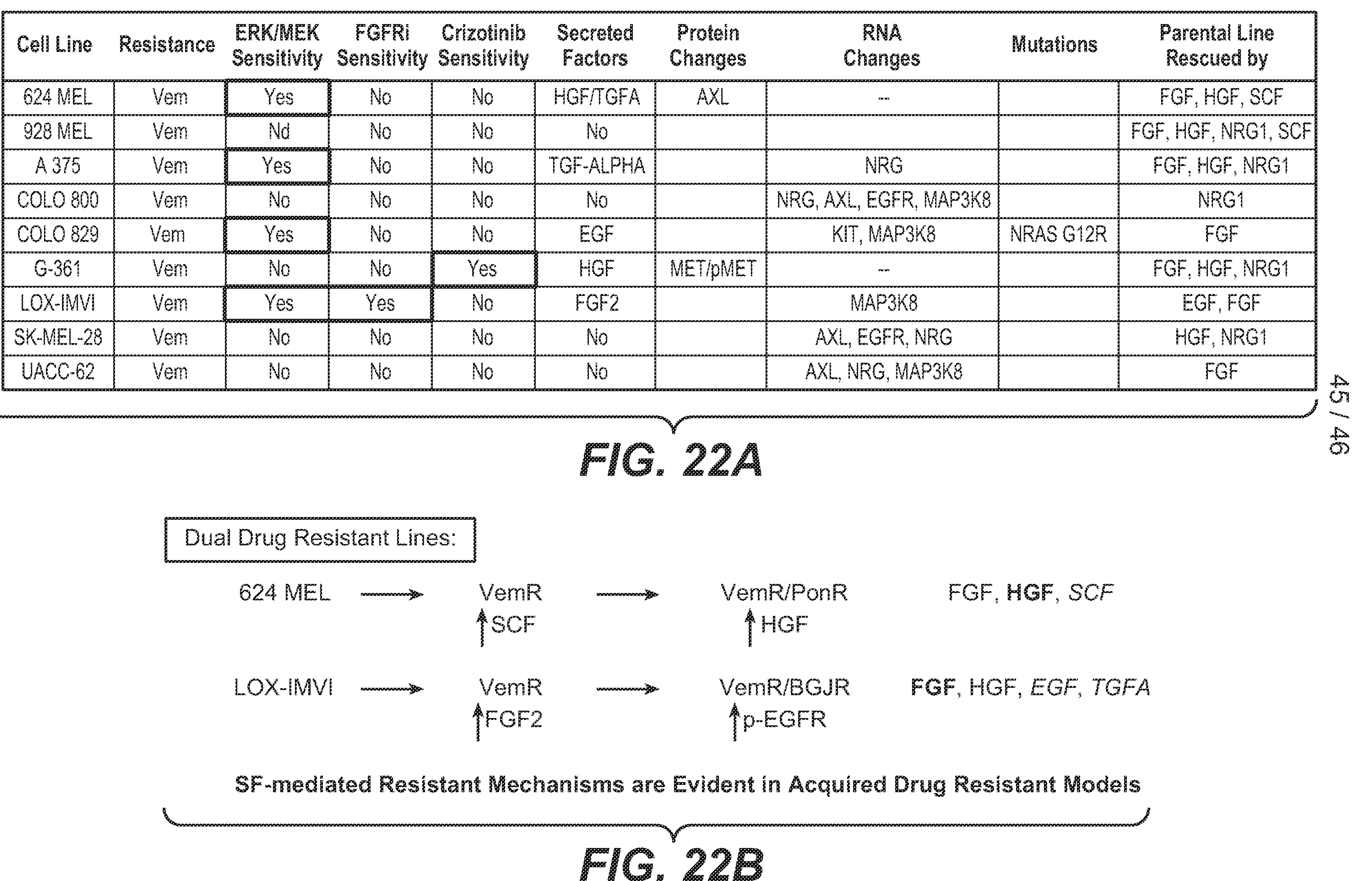


FIG. 21B-2



Cell Line	Resistance	ERK/MEK Sensitivity	FGFRi Sensitivity	Crizotinib Sensitivity	Secreted Factors	Protein Changes	RNA Changes	Mutations	Parental Line Rescued by
624 MEL	Vem	Yes	No	No	HGF/TGFA	AXL	--		FGF, HGF, SCF
928 MEL	Vem	Nd	No	No	No				FGF, HGF, NRG1, SCF
A 375	Vem	Yes	No	No	TGF-ALPHA		NRG		FGF, HGF, NRG1
COLO 800	Vem	No	No	No	No		NRG, AXL, EGFR, MAP3K8		NRG1
COLO 829	Vem	Yes	No	No	EGF		KIT, MAP3K8	NRAS G12R	FGF
G-361	Vem	No	No	Yes	HGF	MET/pMET	--		FGF, HGF, NRG1
LOX-IMVI	Vem	Yes	Yes	No	FGF2		MAP3K8		EGF, FGF
SK-MEL-28	Vem	No	No	No	No		AXL, EGFR, NRG		HGF, NRG1
UACC-62	Vem	No	No	No	No		AXL, NRG, MAP3K8		FGF

FIG. 22A

FIG. 22B

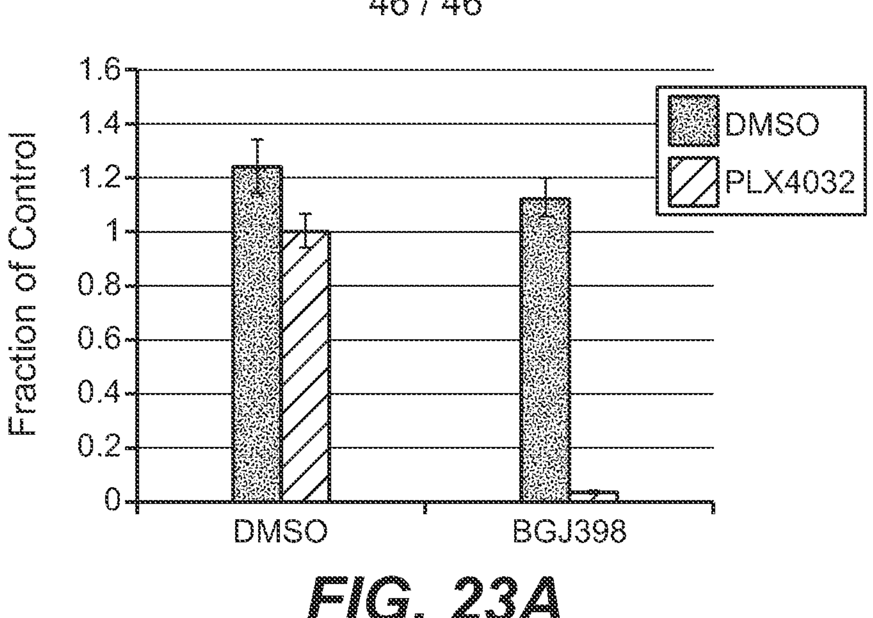


FIG. 23A

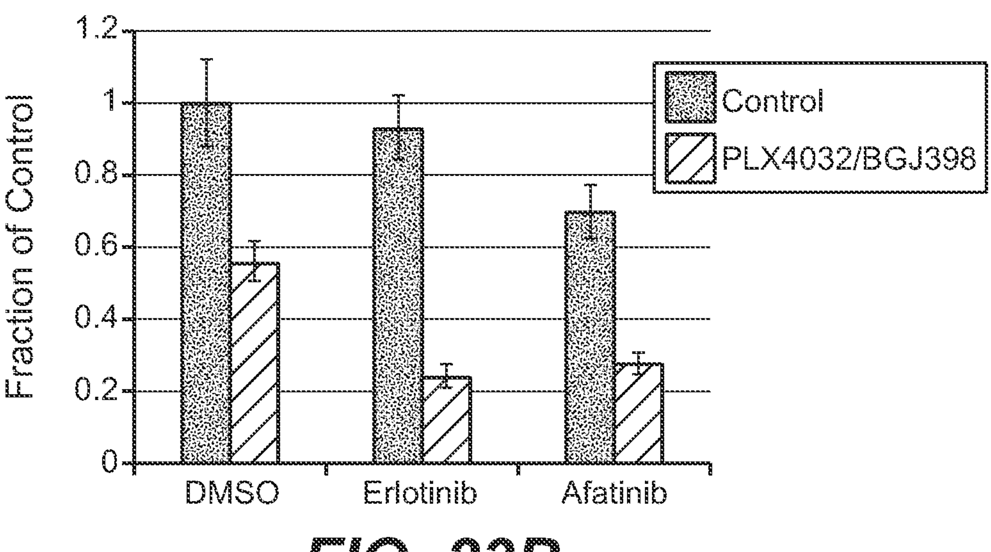


FIG. 23B

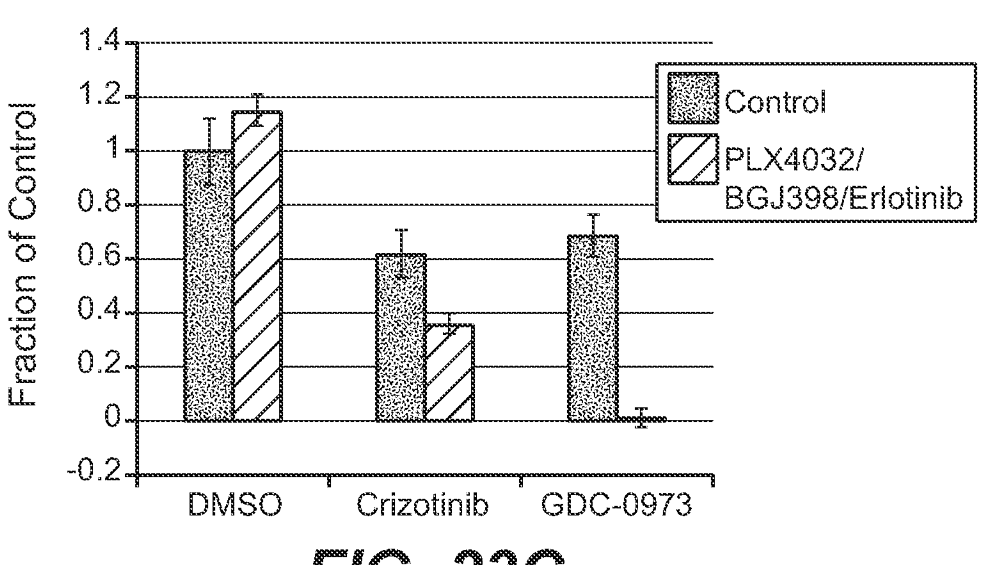


FIG. 23C

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2015/035547

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K45/06 A61K31/437 A61K31/519 A61P35/00
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	M. A. HELD ET AL: "Genotype-Selective Combination Therapies for Melanoma Identified by High-Throughput Drug Screening", CANCER DISCOVERY, vol. 3, no. 1, 1 January 2013 (2013-01-01), pages 52-67, XP055207100, ISSN: 2159-8274, DOI: 10.1158/2159-8290.CD-12-0408 p. 56, left col.; Fig. 3C, 3F -----	1-22

-/--

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

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

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
11 August 2015	18/08/2015

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Scheithe, Rupert

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INTERNATIONAL SEARCH REPORT

International application No
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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	THOMAS METZNER ET AL: "Fibroblast Growth Factor Receptors as Therapeutic Targets in Human Melanoma: Synergism with BRAF Inhibition", JOURNAL OF INVESTIGATIVE DERMATOLOGY, vol. 131, no. 10, 14 July 2011 (2011-07-14), pages 2087-2095, XP055141652, ISSN: 0022-202X, DOI: 10.1038/jid.2011.177 abstract; p. 2088, chapter "FGFR inhibitors enhance the effect of anti-melanoma drugs"; p. 2090, right col., second paragraph; Fig. 4, 6; p. 2092, left col., l. 20 to right col., l. 14 -----	1-18, 20-22
X	V. YADAV ET AL: "Reactivation of Mitogen-activated Protein Kinase (MAPK) Pathway by FGF Receptor 3 (FGFR3)/Ras Mediates Resistance to Vemurafenib in Human B-RAF V600E Mutant Melanoma", JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 287, no. 33, 22 June 2012 (2012-06-22), pages 28087-28098, XP055141868, ISSN: 0021-9258, DOI: 10.1074/jbc.M112.377218 whole document, particularly abstract; chapter "FGFR3 Activity Contributes to Resistance of A375-R1 Cells to Vemurafenib"; Fig. 4C -----	1-18, 20-22
T	G. ZHAO ET AL: "A Novel, Selective Inhibitor of Fibroblast Growth Factor Receptors That Shows a Potent Broad Spectrum of Antitumor Activity in Several Tumor Xenograft Models", MOLECULAR CANCER THERAPEUTICS, vol. 10, no. 11, 7 September 2011 (2011-09-07), pages 2200-2210, XP055160880, ISSN: 1535-7163, DOI: 10.1158/1535-7163.MCT-11-0306 p. 2203, left col., last complete paragraph -----	
A	SHARMA A ET AL: "Vemurafenib: Targeted inhibition of mutated BRAF for treatment of advanced melanoma and its potential in other malignancies", DRUGS, ADIS INTERNATIONAL LTD, NZ, vol. 72, no. 17, 1 January 2012 (2012-01-01), pages 2207-2222, XP008165507, ISSN: 0012-6667, DOI: 10.2165/11640870-000000000-00000 the whole document -----	1-22

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