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(54) Titre : METHODES ET COMPOSITIONS POUR L'UTILISATION D'ANTICORPS DU FACTEUR DE CROISSANCE
EN ASSOCIATION AVEC DES INHIBITEURS DE LA KINASE DE CIBLAGE NON-TYROSINE
(54) Title: METHODS AND COMPOSITIONS FOR USE OF GROWTH FACTOR ANTIBODIES IN COMBINATION WITH
NON-TYROSINE TARGETING KINASE INHIBITORS

(57) **Abrégé/Abstract:**

The disclosure relates to methods for treating cancer. More particularly, the disclosure relates to use of chimeric non-natural synthetic proteins, in combination with non-tyrosine targeting kinase inhibitors (NTKIs), in treating cancer and preventing intrinsic and/or acquired resistance to NTKIs.

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Abstract:

The disclosure relates to methods for treating cancer. More particularly, the disclosure relates to use of chimeric non-natural synthetic proteins, in combination with non-tyrosine targeting kinase inhibitors (NTKIs), in treating cancer and preventing intrinsic and/or acquired resistance to NTKIs.

**5 METHODS AND COMPOSITIONS FOR USE OF GROWTH FACTOR
ANTIBODIES IN COMBINATION WITH NON-TYROSINE TARGETING KINASE
INHIBITORS**

CROSS REFERENCE TO RELATED APPLICATIONS

10 This application claims priority to U.S. Provisional Application Serial No. 62/932,131,
filed November 7, 2019, entitled “COMPOSITIONS AND METHODS FOR USE OF ANTI-
EGF ANTIBODIES WITH MEK INHIBITORS,” and U.S. Provisional Application Serial No.
63/094,018 filed October 20, 2020, entitled “ANTI-EPIDERMAL GROWTH FACTOR
VACCINE ANTIBODIES INCREASE THE ANTITUMOR ACTIVITY OF KINASE
INHIBITORS IN NON SMALL CELL LUNG CANCER CELL LINES,” which are incorporated
15 by reference herein in their entirety for all purposes.

FIELD OF THE DISCLOSURE

The disclosure relates to methods for treating cancer. More particularly, the disclosure relates to use of chimeric non-natural synthetic proteins, in combination with non-tyrosine targeting kinase inhibitors (NTKIs), in treating cancer and preventing intrinsic and/or acquired resistance to NTKIs.

BACKGROUND OF THE DISCLOSURE

According to the World Health Organization, neoplasia (e.g., cancer) is one of the leading causes of death worldwide and was responsible for 8.8 million deaths in 2015. The frequency of cancer in the global human population is significant: accounting for nearly 1 in 6 deaths. In 2015, the most common cancer deaths occurred from the following types of cancer: lung cancer (about 1.7 million deaths), liver cancer (about 800,000 deaths), colorectal cancer (about 800,000 deaths), stomach cancer (about 800,000 deaths), and breast cancer (about 600,000 deaths).

Most chemotherapeutic agents act on a specific molecular target thought to be involved in the development of the malignant phenotype. However, a complex network of signaling pathways regulates cell proliferation and the majority of malignant cancers are facilitated by multiple genetic abnormalities in these pathways. Although treatment of cancers with standard cytotoxic chemotherapies has been optimized for efficacy, more recent approaches are based on classification into molecular subsets based on their distinct oncogene

5 driver. These molecular drivers of cancer can be attacked therapeutically with targeted agents directed against the specific oncogenes.

A promising set of targets for therapeutic intervention in the treatment of cancer includes the members of the HER-kinase axis and their downstream effectors. They frequently exhibit mutations in solid epithelial tumors of, by way of example, the prostate, lung, and breast, and are also up-regulated in glioblastoma tumors. Patients with wild type EGFR effector proteins – for example, RAS, BRAF, and PIK3CA - can be treated with tyrosine kinase inhibitors (TKIs) targeting EGFR such as Cetuximab and Erlotinib. However, there is a large cohort of cancer patients harboring mutations in EGFR effector proteins. Extant treatments are not effective for these patients because the mutations provide resistance to the TKIs targeting EGFR and enable the development of resistance to the non-tyrosine targeting kinase inhibitors (NTKIs) targeting EGFR effectors. Similarly, treatment with TKIs targeting EGFR in patients with wild type EGFR effector proteins often results in the development of resistance due to the selection for oncogenic mutations. Thus, although the extant treatments with TKIs and NTKIs exhibit strong anti-tumor properties targeting the EGFR pathway in some patients, they become less potent or entirely ineffective in patients who develop resistance. EGFR effector kinases may be referred to as 'bypass pathways', in that they have been identified as mechanisms of both intrinsic and acquired resistance to TKIs and NTKIs. Without being bound by theory, it is thought that parallel or other kinases in the EGFR signaling cascade compensate for mutated kinases, thereby inducing stronger EGFR signaling effects. Therefore, there is a need for additional therapies to overcome EGFR pathway resistance to non-tyrosine targeting kinase inhibitors in order to effectively treat EGFR or similar growth factor mediated diseases and cancers.

SUMMARY OF THE DISCLOSURE

The present disclosure is directed towards the use of chimeric non-natural synthetic proteins in combination with EGFR effector non-tyrosine targeting kinase inhibitors to prevent or minimize resistance to non-tyrosine targeting kinase inhibitors (NTKIs), thereby inhibiting cell growth. These therapies can be used to treat cancer, such as, for example, lung cancer, breast cancer, bladder cancer, prostate cancer, ovarian cancer, vulva cancer, colonic cancer, colorectal cancer, intestinal cancer, pulmonary cancer, brain cancer, esophageal cancer, and other cancers.

5 In an illustrative embodiment, the present disclosure provides a method of treating cancer in a subject, comprising administering to the subject an active regimen of a non-tyrosine targeting kinase inhibitor (NTKI) and a non-natural synthetic polypeptide (NNSP), comprising a full length, or fragment thereof, of a growth factor or growth factor receptor, an immunogenic polypeptide, and a linker, wherein the polypeptide is separated from the
10 immunogenic polypeptide by the linker.

 In some embodiments, the method of treating cancer in a subject comprises administering to the subject an active regimen of a non-tyrosine targeting kinase inhibitor (NTKI) and an antibody directed to a growth factor or growth factor receptor.

 In other embodiments, a method of treating non-small cell lung carcinoma (NSCLC) or colorectal cancer (CRC) in a subject is disclosed, comprising administering to the subject a
15 non-tyrosine targeting kinase inhibitor (NTKI) and a non-natural synthetic polypeptide (NNSP), comprising a full length, or fragment thereof, of a growth factor or growth factor receptor, an immunogenic polypeptide, and a linker, wherein the polypeptide is separated from the immunogenic polypeptide by the linker.

20 In some embodiments, a method of treating non-small cell lung carcinoma (NSCLC) or colorectal cancer (CRC) in a subject is disclosed, comprising administering to the subject a non-tyrosine targeting kinase inhibitor (NTKI) and an antibody directed to a growth factor or growth factor receptor.

 In some embodiments, the growth factor is EGF.

25 In some embodiments, the growth factor receptor is EGFR.

 In further embodiments, the immunogenic polypeptide comprises a full length, or portion thereof, of a cholera toxin B (CT-B) protein.

 In some embodiments, the NNSP comprises SEQ ID NO: 7 or SEQ ID NO: 8, or wherein the NNSP is derived from a cell that expresses SEQ ID NO: 7 or SEQ ID NO: 8.

30 In some embodiments, the NNSP comprises SEQ ID NO: 2 or SEQ ID NO: 6, or wherein the NNSP is derived from a cell expressing SEQ ID NO: 2 or SEQ ID NO: 6.

 In some embodiments, the antibody is derived from a hybridoma cell.

5 In some embodiments, the NTKI is Trametinib, Taselisib, Alpelisib, Copanlisib, Idealisib, Duvelisib, Buparlisib, Umbralisib, PX-866, Dactolisib, CUDC-907, Voxtalisib (SAR245409, XL765) ME-401, IPI-549, SF1126, INK1117 Pictilisib, XL147 (SAR245408), GSK1059615, ZSTK474, PWT33597, IC87114, TG100-115, CAL 263, RP6503, GNE-477 AEZS-136, Encorafenib, Vemurafenib, Dabrafenib, GDC-0879, PLX-4720, Cobimetinib, 10 Binimetinib, Selumetinib, PD-325901, PD035901, CI-1040, TAK-733, Perifosine, Palomid 529 or pharmaceutically acceptable salts thereof.

In some embodiments, the EGF is human EGF or murine EGF.

In some embodiments, the linker is selected from the group consisting of SSG, GSSG (SEQ ID NO: 9), SSGGG (SEQ ID NO: 10), SGG, GGSGG (SEQ ID NO: 11), GGGGS (SEQ ID NO: 12), SSGGGSGG (SEQ ID NO: 13), SSGGGGSGGG (SEQ ID NO: 14), 15 TSGGGSG (SEQ ID NO: 15), TSGGGGSGG (SEQ ID NO: 16), SSGGSGGGSG (SEQ ID NO: 17), SSGGGSGGSSG (SEQ ID NO: 18), GGSGGTSGGGSG (SEQ ID NO: 19), SGGTSGGGGSGG (SEQ ID NO: 20), GGSGGTSGGGGSGG (SEQ ID NO: 21), SSGGGGSGGGSSG (SEQ ID NO: 22), SSGGGSGGSSGGG (SEQ ID NO: 23), and 20 SSGGGGSGGGSSGGG (SEQ ID NO: 24).

In some embodiments, the linker is GSSG (SEQ ID NO: 9) or GGSGGTSGGGGGSG (SEQ ID NO: 21).

In some embodiments, the NNSP includes a full length, or fragment thereof, of at least two different growth factors or two different growth factor receptors.

25 In some embodiments, the active regimen is administered to the subject according to a therapeutically effective amount repeated thrice, twice or once a week, once in two weeks, once in three weeks or at least once monthly, in combination with the administration of a NTKI in a continuous regimen based on an average daily dose in the range of 10 to 400 mg, wherein the method prevents acquiring resistance to the NTKI treatment.

30 In some embodiments, the average daily dose is selected from the group consisting of 10 mg, 20 mg, 30 mg, 40 mg, 50 mg, 60 mg, 70 mg, 80 mg, 90 mg, 100 mg, 110 mg, 120 mg, 130 mg, 140 mg, 150 mg, 160 mg, 170 mg, 180 mg, 190 mg, 200 mg, 210 mg, 220 mg, 230 mg, 240 mg, 250 mg, 260 mg, 270 mg, 280 mg, 290 mg, 300 mg, 310 mg, 320 mg, 330 mg, 340 mg, 350 mg, 360 mg, 370 mg, 380 mg, 390 mg, and 400 mg.

5 In some embodiments, the active regimen is administered to the subject according to a therapeutically effective amount repeated thrice, twice or once a week, once in two weeks, once in three weeks or at least once monthly, prior to administration of a NTKI in a continuous regimen based on an average daily dose in the range of 10 to 400 mg, wherein the method prevents acquiring resistance to the NTKI treatment.

10 In some embodiments, the active regimen is administered to the subject according to a therapeutically effective amount repeated thrice, twice or once a week, once in two weeks, once in three weeks or at least once monthly, after administration of a NTKI in a continuous regimen based on an average daily dose in the range of 10 to 400 mg, wherein the method prevents acquiring resistance to the NTKI treatment.

15 In some embodiments, the NNSP produces pathway immunization that results in inhibition of an EGF/EGFR pathway.

 In some embodiments, the antibody produces pathway immunization that results in inhibition of an EGF/EGFR pathway.

 In some embodiments, the subject expresses mutated forms of HRAS, NRAS, KRAS,
20 PIK3CA, BRAF, and/or MEK proteins.

 In some embodiments, the NTKI targets HRAS, NRAS, KRAS, PIK3CA, BRAF, and/or MEK proteins.

 In some embodiments, the subject has acquired resistance to NTKI treatment and the method overcomes resistance to NTKI treatment.

25 In some embodiments, the cancer is associated with a mutant form of HRAS, NRAS, KRAS, PIK3CA, BRAF, and/or MEK proteins.

 In some embodiments, the method of treating cancer in a subject further comprises obtaining a tissue sample from the subject; identifying one or more mutations in the tissue sample from the subject; and conducting the administering steps if the one or more mutations
30 are identified as being in one or more growth factor receptor effector proteins.

 In some embodiments the one or more effector proteins is HRAS, NRAS, KRAS, PIK3CA, BRAF, and/or MEK proteins.

5 In some embodiments the one or more mutations is selected from the following:
KRAS G12S, KRAS G12C, KRAS G13D, KRAS G12D, EML4-ALK (V3; E6:A20), BRAF
V600E, BRAF G596R, PIK3CA E545K, and NRAS Q61K.

In some embodiments the EGF is human EGF or murine EGF.

In some embodiments the antibody is administered at about 0.01-0.1 µg/kg to about
10 95 mg/kg body weight; or from about 0.1-1.0 µg/kg to about 90 mg/kg body weight; or from
about 0.5-5.0 µg/kg to about 80 mg/kg body weight; or from about 1.0-10.0 µg/kg to about
60 mg/kg body weight; or from about 5.0-20.0 µg/kg to about 50 mg/kg body weight; or from
about 20-50 µg/kg to about 25 mg/kg body weight; or from about 50-100 µg/kg to about 20
mg/kg body weight. In other embodiments this dose may be about 1, 5, 10, 25, 50, 75, 100,
15 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000,
1050, 1100, 1150, 1200, 1250, 1300, 1350, 1400, 1450, 1500, 1600, 1700, 1800, 1900, 2000,
2500, 3000, 3500, 4000, 4500, or 5000 µg/kg body weight.

Definitions

By “ameliorate” is meant decrease, suppress, attenuate, diminish, arrest, or stabilize
20 the development or progression of a disease.

By “disease” is meant any condition or disorder that damages or interferes with the
normal function of a cell, tissue, or organ.

By “BVN22E nucleic acid molecule,” also known as “IN01,” is meant a
polynucleotide encoding a BVN22E polypeptide. An exemplary BVN22E nucleic acid
25 molecule is reproduced below (SEQ ID NO: 1):

>BVN22E

aataccgaaaaacgattgccctctgtctcatgaagcgtattgtctgcacgacggcgtgtgtat
gtacattgaagccctggacaaatatgcatgtaactgtgtcgtgggctacgtgggggagcgat
gtcagtttcgagacctgcgttggtgggatgcgcgcggctcgagcggtaataccgaaaaacgat
30 tgcctctgtctcatgaagcgtattgtctgcacgacggcgtgtgtatgtacattgaagccct
ggacaaatatgcatgtaactgtgtcgtgggctacgtgggggagcgatgtcagtttcgagacc
tgcttggtgggatgcgcgcggcgggtctggaggtagtggcggcgggtggagggtcgggt
acccgcagaaacatcacgacctgtgcgcgcgagtaccacaacacccagatccacacctgaa
cgacaagatcttctcgtacaccgagagccctggccgataagcgtgaaatggccatcatcacct
35 tcaagaacggtgcgaccttccaggtggagggtcccgggtagccagcacatcgattcacagaag
aaggccatcgagcgtatgaaggacacctgcgtatcgctacctgaccgaagccaagggtgga
aaagctgtgcgtctggaacaacaagacgcgcgcacgccatcgccgccatcagcatggccaat

5

By “BVN22E polypeptide,” also known as “IN01,” is meant a polypeptide or fragment thereof having at least about 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% amino acid identity (excluding the following amino acid changes: T2S, E3D, N4S, D5E, E11D, A12G, V38I, F44Y, R48K, D51E, and A52L) to the amino acid sequence below (SEQ ID NO: 2):

10

```
>BVN22E
NTENDCPLSHEAYCLHDGVCMYIEALDKYACNCVVGVGERCQFRDLRWWWDARGSSGNTEND
CPLSHEAYCLHDGVCMYIEALDKYACNCVVGVGERCQFRDLRWWWDARGSGGGTSGGGGGSG
TPQNTITDLCAEYHNTQIHTLNDKIFSYTESLADKREMAIITFKNGATFQVEVPGSQHIDSQK
15 KAIERMKDTLRIAYLTEAKVEKLCVWNNKTPHAIAAISMAN
```

By “Epidermal Growth Factor Receptor (EGFR) nucleic acid molecule” is meant a polynucleotide encoding an EGFR polypeptide. An exemplary EGFR nucleic acid molecule is provided at NCBI Accession No. NM_005228.4, and reproduced below (SEQ ID NO: 3):

20

```
>NM_005228.4
gtccgggcagccccggcgccagcgcggccgcagcagccctccgccccccgcacgggtgtgag
cgcccgacgcggccgagggcgccggagtcctcgagctagccccggcgccgcgcgcgcacca
gaccggacgacagggccacctcgctcgccgctccgccccgagtcctccgctcgccgccaacgcc
acaaccaccgcgcacggccccctgactccgtccagttatgatcgaggagagccgggagcgag
25 ctcttcgggggagcagcgatgacgacctccgggacggccggggcagcgctcctggcgctgc
tggtctgcgctctgcccggcgagtcgggctctggaggaaaagaaagtttgccaaggcacga
gtaacaagctcacgcagttgggcaacttttgaagatcattttctcagccctccagaggatgt
tcaataactgtgaggtggctccttgggaattttggaaattacctatgtgcagagggaattatg
atctttccttcttaagaccatccaggaggtggctgggttatgtcctcattgccctcaaca
30 cagtgaggagcaatttcctttggaaaacctgcagatcatcagaggaaatatgtactacgaaa
attcctatgccttagcagtccttatctaactatgatgcaataaaaaccggactgaaggagc
tgcccatgagaaatttacaggaaatcctgcattggcgccgtgcggttcagcaacaacctg
cctgtgcaacgtggagagcatccagtgggcgggacatagtcagcagtgactttctcagca
acatgtcgatggacttccagaaccacctgggcagctgccaaaagtgtgatccaagctgtc
35 ccaatgggagctgctgggggtgcaggagaggagaactgccagaaactgaccaaatacatct
gtgcccgagcagtgctccggggcgtgcccgtggcaagtcctcccgagtgactgctgccacaacc
agtgtgctgcaggctgcacaggccccgggagagcgactgcctggtctgcgcgcaaatcc
gagacgaagccacgtgcaaggacacctgccccccactcatgctctacaacccccaccacgt
accagatggatgtgaacccccgaggggcaatacagcttttggtgccacctgcgtgaagaagt
40 gtccccgtaattatgtgggtgacagatcacggctcgtgcgctccgagcctgtggggccgaca
gctatgagatggaggaagacggcgctccgcaagtgtgaagaagtgcgaagggccttggccga
aagtgtgtaacggaataggatattgggtgaatttaagactcactctccataaatgctacga
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catttaggggtgactccttcacacatactcctcctctggatccacaggaaactggatatcc
45 tgaaaaccgtaaaggaaatcacagggttttttgctgattcaggcttggcctgaaaacagga
cggacctccatgccttttgagaacctagaaatcatagcgggcaggaccaagcaacatgggtc
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agataagtgatggagatgtgataatttcaggaaacaaaaatttgctgctatgcaatacaaa
taaaactggaaaaaactgtttggggacctccgggtcagaaaaacaaaattataagcaacagag
```

5 gtgaaaacagctgcaaggccacaggccaggtctgccatgccttgtgctcccccgagggt
 gctggggcccgaggagccaggactgcgtctcttgccggaatgtcagccgaggcagggaat
 gcgtggacaagtgcacaccttctggaggggtgagccaaggagtttgtggagaactctgagt
 gcatacagtgccacccagagtgccctgcctcaggccatgaacatcacctgcacaggacggg
 gaccagacaactgtatccagtggtgcccaactacattgacggcccccaactgcgtcaagacct
 10 gcccggcaggagtcattgggagaaaaacaacacacctgggtctggaagtacgcagacgcccggcc
 atgtgtgccacctgtgccatccaaactgcacctacggatgcactgggcccagggtcttgaag
 gctgtccaacgaatgggacctaaagatcccgctccatcgccactgggatgggtgggggacctcc
 tcttgcctgctgggtgggtggccctggggatcgccctcttcattgcgaaggcgccacatcgcttc
 ggaagcgacgctgcggaggctgctgcaggagagggagcttgtggagcctcttacacca
 15 gtggagaagctcccaaccaagctctcttgaggatcttgaaggaaactgaattcaaaaaga
 tcaaatgtgctgggctccgggtgcgttcggcacgggtgtataagggactctggatcccagaag
 gtgagaaaagttaaaattcccgctcgctatcaagggaattaagagaagcaacatctccgaaag
 ccaacaaggaaatcctcgatgaagcctacgtgatggccagcgtggacaaccccccaagtg
 gccgctgctgggcatctgcctcacctccacgggtgcagctcatcacgcagctcatgcct
 20 tcggctgcctcctggactatgtccgggaacacaaagacaaatattggctcccagtaacctgc
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 aagtgcctatcaagtggatggcattggaatcaatttttacacagaatctatacccaccaga
 25 gtgatgtctggagctacgggggtgactggttgggagttgatgacctttggatccaagccat
 atgaaggaaatccctgccagcgagatctcctccatcctggagaaaggagaacgcctccctc
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 cagatagtcgccccaaagttccgtgagttgatcatcgaaattctccaaaatggccccgagacc
 ccagcgctaccttgtcattcaggggggatgaaagaatgcatttgccaagtccctacagact
 30 ccaacttctacctgtgcctgatggatgaagaagacatggacgacgtgggtggatgccgacg
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 tgagctctctgagtgcacccagcaacaattccacgggtggcttgcattgatagaaatgggc
 tgcaaagctgtcccatcaagggaagacagcttcttgccagcgatacagctcagacccccacag
 gcgccttgactgaggacagcatagacgacaccttccctccagtgccctgaatacataaacc
 35 agtcogttcccaaaaaggcccgctggctctgtgcagaatcctgtctatcacaatcagcctc
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 accccgagtatctcaacactgtccagccacctgtgtcaacagcacattcgacagccctg
 ccaactgggcccagaaaaggcagccaccaaataggcctggacaacccctgactaccagcagg
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 40 cagaatacctaagggtcgcgccacaaagcagtgaaatttattggagcatgaccacggagga
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 45 cagaaatttatctttcaagagggtatatttgaaaaaaggtatatgtgaggattt
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5 accgctttttgttctcgcaaaaacgtatctctcctaatttgaggctcagatgaaatgcatcag
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10 agcacttacagctcttgccacacacagggcatttttacaggtgccaatgacagtagcattat
gagtagtgtggaattcaggttagtaaatatgaaactagggtttgaaattgataatgctttc
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cctgtaacctgactgggttaacagcagtcctttgttaaacagtgttttaaacctctcctagtc
15 aatatccaccccatccaatttatcaaggaagaaatgggttcagaaaaatattttcagcctac
agttatgttccagtcacacacacatacaaaaatgttcccttttgcttttaaaagtaatttttga
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tactagcctgggtatgggtatgaaagatacaaaagataaaaataaaacatagtccttgattcta
20 agaaattcacaaatttagcaaagggaatggactcatagatgctaaccttaaaacaacgtga
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tcttgctgaggagacctggaaggaggcctcaccaggaggatgaccaggtctcagtcagc
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gcctccactgctaaagtccacataagggtgaggtcagtcaccctaaacaacctgctccct
25 ctaagccaggggatgagcttggagcatccacaagttccctaaaagttgcagccccaggg
gggattttgagctatcatctctgcacatgcttagtgagaagactacacaacattttctaag
aatctgagattttatattgtcagtttaaccactttcattattcattcacctcaggacatgc
agaaatattttcagtcagaactgggaaacagaaggacctacattctgctgtcacttatgtg
tcaagaagcagatgatcgatgaggcaggtcagttgtaagtgagtcacattgtagcattaa
30 attctagtatttttgtagtttgaaacagtaacttaataaaaagagcaaaaagctaaaaaaaa
aaaaaaaa

By “Epidermal Growth Factor Receptor (EGFR) polypeptide” is meant a polypeptide
or fragment thereof having at least about 85% amino acid identity to NCBI Accession No.
NP_005219.2 and having Epidermal Growth Factor (EGF) binding activity, as reproduced
35 below (SEQ ID NO: 4):

>NP_005219.2
MRPSGTAGAALLALLAALCPASRALEEKKVCQGTSNKLTQLGTFEDHFLSLQRMFNCEV
VLGNLEITYVQRNYDLSFLKTIQEVAGYVLIALNTVERIPLLENLQIIRGNMYEYENSYALA
VLSNYDANKTGLKELPMRNLQEILHGAVRFSNNPALCNVESIQWRDIVSSDFLSNMSMDF
40 QNHLGSCQKCDPSCPNGSCWGAGEENCQKLTKIICAQQCSGRRCRGKSPSDCCHNQCAAGC
TGPRESCLVCRKFRDEATCKDTCPLMLYNPTTYQMDVNPEGKYSFGATCVKKCPRNYV
VTDHGSCVRACGADSYEMEEDGVRKCKKCEGPCRKVCNGIGIGEFKDSLSINATNIKHFK
NCTSLSGDLHILPVAFRGDSFTHTPLDPQELDILKTVKEITGFLLIQAWPENRTDLHAF
ENLEIRGRTRKHGQFSLAVVSLNITSLGLRSLKEISDGDVVISGNKNLCYANTINWKKL
45 FGTSGQKTKIISNRGENSCKATGQVCHALCSPEGCWGPEPRDCVSCRNVSRGRECVDKCN
LLEGEPRFVENSECIQCHPECLPQAMNITCTGRGPDNCIQCAHYIDGPHCVKTCPAGVM
GENNTLVWKYADAGHVCHLCHPNCTYGCTGPGLEGCPNTPGPKIPSIATGMVGALLLLLVV
ALGIGLFMRRRHIVRKRTLRLRLQERELVEPLTPSGEAPNQALLRILKETEFKKIKVLGS
GAFGTVYKGLWIPEGEKVKIPVAIKELREATSPKANKEILDEAYVMASVDNPHVCRLGI
50 CLTSTVQLITQLMPEGCLLDYVREHKDNIGSQYLLNWCVQIAKGMNYLEDRLVHRDLAA
RNVLVKTPQHVKITDFGLAKLLGAEEKEYHAEGGKVPIKWMALESIHLRIYTHQSDVWSY

5 GVTVWELMTFGSKPYDGIPASEISSILEKGERLPQPPICTIDVYMIMVKCWMIDADSRPK
 FRELIIEFSKMARDPQRYLVIQGDERMHLPSPTDSNFYRALMDEEDMDDVVDADLEYLIPO
 QGFFSSPSTSRTPLLSSLSATSNNSTVACIDRNGLOSCPIKEDSFLORYSSDPTGALTED
 SIDDTFLPVPEYINQSVPKRPAGSVQNPVYHNQPLNPAPSRDPHYQDPHSTAVGNPEYLN
 TVQPTCVNSTFDSPAHWAKGSHQISLDNPDYQQDFFPKAKFNGIFKGSTAENAEYLRV
 10 APQSSEFIGA

By “Epidermal Growth Factor (EGF) nucleic acid molecule” is meant a polynucleotide encoding an EGF polypeptide. An exemplary EGF nucleic acid molecule is provided at NCBI Accession No. NM_001963.5, and reproduced below (SEQ ID NO: 5):

>NM_001963.5
 15 aaaaagagaaactgttgggagaggaatcgatatccatattttcttctttcagccccaatc
 caaggggtgtagctggaactttccatcagttcttccctttctttttctctcttaagccttt
 gccttgctctgtcacagtgaaagtcagccagagcaggggtgttaaactctgtgaaatttgt
 cataaggggtgtcaggtattttcttactggcttccaaagaaacatagataaagaaatctttc
 ctgtggcttcccttggcagggctgcattcagaaggctctctcagttgaagaaagagcttggg
 20 ggacaacagcacaaacaggagagtaaaagatgccccaggggtgaggcctccgctcaggcag
 ccgcatctgggggtcaatcatactcaacttgcctggggccatgctccagcaaaatcaagctg
 ttttctttttgaaagttcaaaactcatcaagattatgctgctcactcttatcattctgttgc
 cagtagttttcaaaatttagttttgttagtctctcagcaccgcagcactggagctgtcctg
 aaggtactctcgcaggaaatgggaattctacttgtgtgggtcctgcaccttcttaattt
 25 tctcccatggaaatagttatcttttaggattgacacagaaggaaaccaattatgagcaattgg
 tgggtggatgctggtgtctcagtgatcatggatttttcattataatgagaaaagaatctatt
 ggggtggatttagaaaagacaacttttgcaaagagtttttctgaatgggtcaaggcaagaga
 gagtatgtaatatagagaaaaatgttttctggaatggcaataaattggataaatgaagaag
 ttatttgggtcaaatcaacaggaaggaatcattacagtaacagatatgaaaggaaataatt
 30 cccacattctttttaagtgttttaaaatatcctgcaaatgtagcagttgatccagtagaaa
 ggtttatatttttgggtcttcagaggtggctggaagccttttatagagcagatctcgatgggtg
 tgggagtgaaggctctgttggagacatcagagaaaataacagctgtgtcattgggatgtgc
 ttgataagcggctgttttggattcagtacaacagagaagggaagcaattctcttatttgc
 cctgtgattatgatggaggttctgtccacatttagtaaacatccaacacagcataatttgc
 35 ttgcaatgtcccttttttgggtgaccgtatcttctattcaacatggaaaatgaagacaattt
 ggatagccaacaaacacactggaaaggacatgggttagaattaacctccattcatcatttg
 taccacttgggtgaactgaaagttagtgcatccacttgcacaaccaaggcagaagatgaca
 cttgggagcctgagcagaaactttgcaaattgaggaaaggaaactgcagcagcactgtgt
 gtgggcaagacctccagtcacacttgtgcatgtgtgcagagggatacgccttaagtcgag
 40 accggaagtactgtgaagatgttaatgaatgtgtcttttgggaatcatggctgtactcttg
 ggtgtaaaaaacacccctgggacctattactgcacgtgacctgtaggatttgttctgtctc
 ctgatgggaaacgatgtcatcaacttgtttcctgtccacgcaatgtgtctgaatgcagcc
 atgactgtgttctgacatcagaaggctcccttatgtttctgtcctgaaggctcagtgcttg
 agagagatgggaaaacatgttagcgggtgttccctcaccgcgataatgggtggatgttagccagc
 45 tctgcgttctcttagcctcagttatcctgggaatgtgattgctttcctgggtatgacctac
 aactggatgaaaaaagctgtgcagcttcaggaccacaaccatttttgcgtgtttgccatt
 ctcaagatatctgacacatgcattttgatggaacagactatggaactctgctcagccagc
 agatgggaatgggtttatgccttagatcatgacctgtggaaaataagatatactttgccc
 atacagccctgaagtggatagagagagctaatatggatgggttccagcagagaaaggctta
 50 ttgaggaaggagtagatgtgccagaaggctcttgcgtgtggactggattggccgtagattct
 attggacagacagaggggaaatctctgattggaaggagtgatttaaatgggaaacgttcca
 aaataatcactaaggagaaacatctctcaaccacgaggaattgctgttcatccaatggcca

5 agagattattctggactgatacagggattaatccacgaattgaaagttcttccctccaag
 gccttggccgtcttggttatagccagctctgatctaattctggcccagtggaataacgattg
 acttcttaactgacaagttgtactgggtgcgatgccaaagcagtcctgtgattgaaatggcca
 atctggatgggttcaaaaacgccgaagacttacccagaatgatgtagggtcacccatttgcctg
 tagcagtggtttgaggattatgtgtgggttctcagattgggctatgccatcagtaatgagag
 10 taaacaagaggactggcaaagatagagtaacgtctccaaggcagcatgctgaagccctcat
 cactgggttggtgttcatccattggcaaaaaccaggagcagatccctgcttataatcaaaacg
 gaggtgtgaacatatttgcaaaaagaggcttggaactgcttgggtgttcgtgtcgtgaag
 gtttttatgaaagcctcagatgggaaaacgtgtctgggtctggatgggtcatcagctgttgg
 caggtgggtgaagttgatctaaagaaccaagtaacaccatttgacatcttgtccaagacta
 15 gagtgtcagaagataacattacagaatctcaacacatgctagtgggtgaaatcatgggtgt
 cagatcaagatgactgtgctcctgtgtgggatgcagcatgtatgctcgggtgtatttcagagg
 gagaggatgccacatgtcagtggtttgaaaggatttgcctggggatggaaaactatgttctg
 atatagatgaatgtgagatgggtgtcccagtggtgccccctgctcctccaagtgcac
 acaccgaagggtggttatgtctgccggtgctcagaaggctaccaaggagatgggattcact
 20 gtcttgatattgatgagtgccaactgggggagcacagctgtggagagaatgccagctgca
 caaatacagaggggaggtatacctgcattgtgtgctggacgcctgtctgaaccaggactga
 tttgcctgactctactccacccctcactcagggaagatgaccaccactattccgtaa
 gaaatagtgaactctgaatgtccctgtccacagatgggtactgcctccatgatgggtgtgt
 gcatgtatatattgaagcatitggacaagtatgcattgcaactgtgttgggttacatcgggg
 25 agcgtatgtcagtaaccgagacctgaagtgggtgggaactgcgccacgctggccacgggcagc
 agcagaagggtcatcgtgggtggctgtctgcgtgggtgggtgcttgcattgctgctcctcctga
 gcctgtggggggccactactacaggactcagaagctgctatcgaaaaacccaaagaatc
 cttatgaggagtcgagcagagatgtgaggagtcgcaggcctgctgacactgaggatggga
 tgtcctccttgcctcaaccttgggtttgtgggttataaaaagaacaccaagacctcaagaatg
 30 ggggtcaaccagtggtgtgaggatggccaggcagcagatgggtcaatgcaaccaactt
 catggaggcaggagccccagttatgtggaatgggcacagagcaaggctgctggattccag
 tatccagtgataagggctcctgtcccaggtaatggagcgaagctttcatatgccctcct
 atgggacacagaccttgaaggggggtgtcgagaagccccattctctcctatcagctaacc
 cattatggcaacaaagggccctggacccacacaccaaataaggagctgactcagtgaaaac
 35 tggaaattaaaaggaaagtcaagaagaatgaactatgtcgatgcacagtatcttttcttctc
 aaaagtagagcaaaaactataggttttgggttccacaattctctacgactaatcacctactca
 atgcctggagacagatacgtagtgtgtcttttgtttgctcttttaagcagtcctcactgca
 gtcttattttccaagtaagagtactgggagaatcactaggtaacttattagaaacccaaat
 tgggacaacagtgcttttgtaaatgtgttgccttcagcagtcatacaaatagattttttg
 40 tttttgttgttctgcagccccagaagaaattaggggttaaagcagacagtcacactggt
 ttgggtcagttacaaagtaatttctttgatctggacagaacatttatatcagtttcatgaa
 atgattggaatattacaataaccgttaagatacagtgtaggcattttaactcctcattggcg
 tgggtccatgctgatgattttgcaaaatgagttgtgatgaatcaatgaaaaatgtaattta
 gaaactgatttcttcagaattagatggcttatttttttaaaatatttgaatgaaaacattt
 45 tatttttaaaaatattacacaggaggcttcggagtttcttagtcattactgtccttttccc
 ctacagaattttccctccttgggtgtgattgcacagaatttgtatgtattttcagttacaag
 attgtgaagtaaaattgcctgattttgttttcatatatagacaaacgatgaatttcttotaatta
 ttttaataaaaatcaccaaaaacataaacatttttattgtatgcctgatttaagtagttaatt
 atagtctaaggcagtaactagagttgaaccaaataatgatttgtcaagcttgctgatgtttct
 50 gtttttctgttttttttttttttttccggagagaggataggatctcactctgttatccaggct
 ggagtggtgcaatggcacaatcatagctcagtgccagcctcaaactcctggggtcaagcaat
 cctcctgcctcagcctcccagagtaactaggaccacaggccacaggccaccatgcctggcta
 aggttttttattttttattttttgttagacatggggatcacacaatgttgcccagggtggtct
 tgaactcctggcctcaagcaaggctcgtgctggtaatttttgcaaaatgaattgtgattgac
 55 tttcagcctcccaacgtattagattataggcattagccatgggtgccagccttgtaactt

5 ttaaaaaaattttttaatctacaactctgtagattaaaatttcacatggtgttctaatta
aatatttttcttgcagccaagatatgttactacagataacacaacctgatatggtaact
ttaaatttttgggggctttgaatcattcagtttatgcattaactagtccctttgtttatct
ttcattttctcaaccccttgtactttgggtgataccagacatcagaataaaaagaaattgaa
gtacctgttttcaaattggatactttataggaattttggtaaagatttgggtgatgggagga
10 tgacttgagggtttgtggatattagtttaattattcagtatgataacctcaccagctaattt

By "Epidermal Growth Factor (EGF) polypeptide" is meant a polypeptide or fragment thereof having at least about 85% amino acid identity to NCBI Accession No. NP_001954.2 and corresponding to a pre-pro-protein form of EGF that is processed to produce a 53 amino acid EGF molecule (shown in bold) and having EGFR binding activity (an hEGF Targeted
15 Signaling Pathway (TSP) domain), as reproduced below (SEQ ID NO: 6):

>NP_001954.2
MLLTLLIILLPVVSKFSFVSLAPQHWSCPEGTLAGNGNSTCVGPAPFLIFSHGNSIFRID
TEGTNYEQLVVDAGVSVIMDFHYNEKRIYWVDLERQLLQRVFLNGSRQERVNCNIEKNVSG
MAINWINEEVIWSNQOEGIIITVTDMKGNNSHILLSALKYPANVAVDFVERFI FWSSEVAG
20 SLYRADLDGVGVKALLETSEKITAVSLDVLDRKLEFWIQYNREGSNSLICSCDYDGGSVHI
SKHPTQHNLFAMSLFGDRI FYSTWKMKTIWIANKHTGKDMVRINLHSS FVPLGELKVVHP
LAQPKAEDDTWEPEQKLCKLRKGNCSSTVCGQDLQSHLCMCAEGYALS RDRKYCEDVNEC
AFWNHGCTLGCKNTPGSYYCTCPVGFVLLPDGKRCHQLVSCPRNVSECSHDCVLTSEGPL
CFCPEGSVLERDGTCSGCSSPDNGGCSQLCVPLSPVSWECDCFPGYDLQLDEKSCAASG
25 PQPFLLEFANSQDIRHMHFDGTDYGTLLSQQMGMVYALDHDPVENKIYFAHTALKWIERAN
MDGSQRERLIEEGVDVPEGLAVDWIGRRFYWTD RGKSLIGRSDLNGKRSKIITKENISQP
RGLAVHMPMAKRLFWTDTGINPRIESSSLQGLGRLVIASSDLIWP SGITIDELTDKLYWCD
AKQSVIEMANLDGSKRRRLTQNDVGHPFAVAVFEDYVWFSDWAMP SVMRVNKRTGKDRVR
LQGSMLKPSSLVVVHPLAKPGADPCLYQNGGCEHICKKRLGTAWCSCREGFMKASDGKTC
30 LALDGHQLLAGGEVDLKNQVTPLDILSKTRVSEDNITESQHMLVAEIMVSDQDDCAPVGC
SMYARCISEGEDATCQCLKGFAGDGKLCSDIDECCEMGVPVCPPEASSKCINTEGGYVCRCS
EGYQGDGIHCLDIDECQLGEHSCGENASCTNTEGGYTMCAGRLSEPGLICPDSTPPPHL
REDDHHYSVRNSDSECLSHDGYCLHDGVC MYIEALDKYACNCVVG YIGERCQYRDLKWW
ELRHAGHGQQQKVIIVAVCVVVLVMLLLLSLWGAHYRTQKLLSKNPKNPYEESSRDVRS
35 **RRPADTEDGMSSCPQPFVVIKEHQDLKNGGQPVAGEDGQAADGSMQFTSWRQEPQLCGM**
GTEQGCWIPVSSDKGSCFQVMERSFHMPSYGTQTLEGGVEKPHSLLSANPLWQQRALDPP
HQMELTQ

By "fragment" is meant a portion of a polypeptide or nucleic acid molecule. This portion contains, preferably, at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% of
40 the entire length of the reference nucleic acid molecule or polypeptide. A fragment may contain 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100, 200, 300, 400, 500, 600, 700, 800, 900, or 1000 nucleotides or amino acids.

By "marker" is meant any protein or polynucleotide having an alteration in expression level or activity that is associated with a disease or disorder.

5 By “neoplasia” is meant a disease or disorder characterized by excess proliferation or reduced apoptosis. Illustrative neoplasms for which the invention can be used include, but are not limited to pancreatic cancer, leukemias (e.g., acute leukemia, acute lymphocytic leukemia, acute myelocytic leukemia, acute myeloblastic leukemia, acute promyelocytic leukemia, acute myelomonocytic leukemia, acute monocytic leukemia, acute
 10 erythroleukemia, chronic leukemia, chronic myelocytic leukemia, chronic lymphocytic leukemia), polycythemia vera, lymphoma (Hodgkin's disease, non-Hodgkin's disease), Waldenstrom's macroglobulinemia, heavy chain disease, and solid tumors such as sarcomas and carcinomas (e.g., fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma,
 15 lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma,
 20 hepatoma, nile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilm's tumor, cervical cancer, uterine cancer, testicular cancer, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, glioblastoma multiforme, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, schwannoma, meningioma,
 25 melanoma, neuroblastoma, and retinoblastoma).

By “protein” or “polypeptide” or “peptide” is meant any chain of more than two natural or unnatural amino acids, regardless of post-translational modification (e.g., glycosylation or phosphorylation), constituting all or part of a naturally-occurring or non-naturally occurring polypeptide or peptide, as is described herein.

30 By “subject” is meant a mammal, including, but not limited to, a human or non-human mammal, such as a bovine, equine, canine, ovine, or feline.

By “EGFR effector” is meant any protein including kinases, downstream of EGFR, as disclosed herein and as is commonly known in the art.

35 By “NTKI” or “non-tyrosine targeting kinase inhibitor” is meant any compound that specifically targets kinases without affecting tyrosine phosphorylation. In contrast to

- 5 Tyrosine Kinase Inhibitors (TKIs), “NTKIs” refers to kinase inhibitors of kinases that are not Receptor Tyrosine Kinases (e.g EGFR). Rather NTKIs target kinases downstream of receptors. In some embodiments these kinases are downstream of EGFR.

Ranges can be expressed herein as from “about” one particular value, and/or to “about” another particular value. When such a range is expressed, another aspect includes
10 from the one particular value and/or to the other particular value. Similarly, when values are expressed as approximations, by use of the antecedent “about,” it is understood that the particular value forms another aspect. It is further understood that the endpoints of each of the ranges are significant both in relation to the other endpoint, and independently of the other endpoint. It is also understood that there are a number of values disclosed herein, and
15 that each value is also herein disclosed as “about” that particular value in addition to the value itself. It is also understood that throughout the application, data are provided in a number of different formats and that this data represent endpoints and starting points and ranges for any combination of the data points. For example, if a particular data point “10” and a particular data point “15” are disclosed, it is understood that greater than, greater than
20 or equal to, less than, less than or equal to, and equal to 10 and 15 are considered disclosed as well as between 10 and 15. It is also understood that each unit between two particular units are also disclosed. For example, if 10 and 15 are disclosed, then 11, 12, 13, and 14 are also disclosed.

Ranges provided herein are understood to be shorthand for all of the values within the
25 range. For example, a range of 1 to 50 is understood to include any number, combination of numbers, or sub-range from the group consisting 1, 2, 3, 4, 5, 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, or 50 as well as all intervening decimal values between the aforementioned integers such as, for example, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, and 1.9.
30 With respect to sub-ranges, “nested sub-ranges” that extend from either end point of the range are specifically contemplated. For example, a nested sub-range of an exemplary range of 1 to 50 may comprise 1 to 10, 1 to 20, 1 to 30, and 1 to 40 in one direction, or 50 to 40, 50 to 30, 50 to 20, and 50 to 10 in the other direction.

By “reference” is meant a standard or control condition. In one embodiment, the
35 effect of an agent on a cell is compared to the effect of the agent on a control cell.

5 A "reference sequence" is a defined sequence used as a basis for sequence comparison. A reference sequence may be a subset of or the entirety of a specified sequence; for example, a segment of a full-length cDNA or gene sequence, or the complete cDNA or gene sequence. For polypeptides, the length of the reference polypeptide sequence will generally be at least about 16 amino acids, preferably at least about 20 amino acids, more
 10 preferably at least about 25 amino acids, and even more preferably about 35 amino acids, about 50 amino acids, or about 100 amino acids. For nucleic acids, the length of the reference nucleic acid sequence will generally be at least about 50 nucleotides, preferably at least about 60 nucleotides, more preferably at least about 75 nucleotides, and even more preferably about 100 nucleotides or about 300 nucleotides or any integer thereabout or
 15 therebetween.

By "specifically binds" is meant a compound or antibody that recognizes and binds a polypeptide of the invention, but which does not substantially recognize and bind other molecules in a sample.

By "substantially identical" is meant a polypeptide or nucleic acid molecule
 20 exhibiting at least 50% identity to a reference amino acid sequence (for example, any one of the amino acid sequences described herein) or nucleic acid sequence (for example, any one of the nucleic acid sequences described herein). Preferably, such a sequence is at least 60%, more preferably 80% or 85%, and most preferably 90%, 95% or even 99% identical at the amino acid level or nucleic acid to the sequence used for comparison.

25 By "synthetic TSP domain" is meant or fragment thereof having at least about 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% amino acid identity to the amino acid sequence below (SEQ ID NO: 7):

>synthetic TSP domain
 NTENDCPLSHEAYCLHDGVCMYIEALDKYACNCVVG YVGERCQFRDLRWWDAR

30

By "hEGF TSP domain" is meant or fragment thereof having at least about 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% amino acid identity to the amino acid sequence below, as shown in bold above in SEQ ID NO: 6, (SEQ ID NO: 8):

>hEGF TSP domain
 35 NSDSECPLSHDGYCLHDGVCMYIEALDKYACNCVVG YIGERCQYRDLKWWELR

5 By “CTB polypeptide” is meant or fragment thereof having at least about 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% amino acid identity to the amino acid sequence below (SEQ ID NO: 25[[9]]):

>CTB polypeptide
 10 TEQNITDLCAEYHNTQIHTLNDKIESYTESLADKREMAITTFKNGATFQVEVPGSQHIDSQK
 KAIERMKDTRLRIAYLTEAKVEKLCVWNNKTPHATAAISMAN

Where applicable or not specifically disclaimed, any one of the embodiments described herein are contemplated to be able to combine with any other one or more embodiments, even though the embodiments are described under different aspects of the
 15 disclosure.

These and other embodiments are disclosed and/or encompassed by, the following Detailed Description.

BRIEF DESCRIPTION OF THE DRAWINGS

The following detailed description, given by way of example, but not intended to limit
 20 the disclosure solely to the specific embodiments described, may best be understood in conjunction with the accompanying drawings, in which:

FIG. 1 shows the cell line name and origin of several *KRAS* mutation carrying cell lines. DLD1 and LS174T cell lines also carry *PIK3CA* mutations.

FIG. 2 shows the effect on pEGFR, pStat3, pAkt, and pErk $\frac{1}{2}$ signaling, assessed by
 25 western blot, of 2 hour incubation with different concentrations of the anti-IN01 therapeutic antibody, “Ab,” or a control antibody, “C-Ab,” in DLD1 cells.

FIG. 3 shows the effect of 3 day incubation of different concentrations of Trametinib on DLD1 cell viability, with and without EGF.

FIG. 4 shows the effect on pEGFR, pStat3, pAkt, and pErk $\frac{1}{2}$ signaling, assessed by
 30 western blot, of 2 hour incubation with EGF or different concentrations of Trametinib in DLD1 cells.

FIGs. 5A-5B show the effect of anti-IN01 in combination with Trametinib on DLD1 cell viability. **FIG. 5A** shows the effect of 3 day incubation with the anti-IN01 therapeutic

5 antibody, or a control antibody, C-Ab, used in combination with different concentrations of Trametinib on DLD1 cell viability. **FIG. 5B** shows the longer term effects on DLD1 cell viability of the anti-IN01 therapeutic antibody, or a control antibody, C-Ab, used in combination with Trametinib.

FIG. 6 shows the effect on pEGFR, pStat3, pAkt, and pErk $\frac{1}{2}$ signaling, assessed by western blot, of 2 hour incubation with EGF, 10 nM Trametinib, and the anti-IN01 antibody, referred to as "Ab," or a control antibody, referred to as "C," alone or in combination, in DLD1 cells.

FIG. 7 shows the effect of 3 day incubation of different concentrations of Trametinib on LS174T cell viability, with and without EGF.

15 **FIG. 8** shows the effect on pEGFR, pStat3, pAkt, and pErk $\frac{1}{2}$ signaling, assessed by western blot, of a 2 hour incubation with EGF or different concentrations of Trametinib in LS174T cells.

FIG. 9 shows the effect of a 3 day incubation with the anti-IN01 therapeutic antibody, or a control antibody, C-Ab, used in combination with different concentrations of Trametinib on LS174T cell viability.

FIG. 10 shows the effect on pEGFR, pStat3, pAkt, and pErk $\frac{1}{2}$ signaling, assessed by western blot, of a 2 hour incubation with EGF, 5 nM Trametinib, and/or the anti-IN01 antibody, referred to as "Ab," or a control antibody, referred to as "C," in LS174T cells. The anti-IN01 Ab was diluted 1:10 for these experiments.

25 **FIG. 11** shows the development of resistance to Trametinib by LS174T cell with the anti-IN01 therapeutic antibody, or a control antibody, C-Ab, used in combination with 0.5 μ M Trametinib.

FIG. 12 shows the increase in cell growth of HT29, carrying a BRAF V66E mutation, in the presence of EGF.

30 **FIGs. 13A-C** show the effect of the anti-IN01 Ab in combination with Trametinib on HT29 cell signaling and long term viability. **FIG. 13A** shows the effect of a 3 day incubation with the anti-IN01 therapeutic antibody, or a control antibody, C-Ab, used in combination with different concentrations of Trametinib on HT29 cell viability. **FIG. 13B** shows the

5 effects on pEGFR and pErk $\frac{1}{2}$ signaling, assessed by western blot, of a 2 hour incubation with EGF, 0.01 mM Trametinib, and the anti-IN01 antibody, referred to as “Ab8,” alone or in combination, in LS174T cells. The anti-IN01 Ab was diluted 1:10 for these experiments.

10 **FIG. 13 C** shows the longer term effects on HT29 cell viability of the anti-IN01 therapeutic antibody used in combination with EGF and Trametinib. EGF was added at a concentration of 10 ng/mL to the cell culture media.

FIG. 14 shows the effect of a 3 day incubation of different concentrations of Trametinib on H508 cell viability, with and without EGF.

15 **FIG. 15** shows the effect of a 3 day incubation with the anti-IN01 therapeutic antibody, “Ab,” or a control antibody, “C-Ab,” used in combination with different concentrations of Trametinib on H508 cell viability.

FIG. 16 shows the effect of a 3 day incubation of different concentrations of Taselisib on H508 cell viability, with and without EGF.

20 **FIG. 17** shows the effect of a 3 day incubation with the anti-IN01 therapeutic antibody, “Ab,” or a control antibody, “C-Ab,” in combination with different concentrations of Taselisib on H508 cell viability.

FIG. 18 shows the longer term effects on H508 cell viability of the anti-IN01 therapeutic antibody, at a 1/50 dilution, in combination with EGF and 0.1 mM Taselisib.

FIG. 19 shows the longer term effects on H508 cell viability of the anti-IN01 therapeutic antibody, at a 1/50 dilution, in combination with EGF and 0.5 mM Taselisib.

25 **FIG. 20** shows the effects of Alpelisib and Copanlisib with and without EGF in H508 cells. Top, left, shows the effect of 3 day incubation of different concentrations of Alpelisib on H508 cell viability with and without EGF. Top, right, shows the effect on pEGFR, pAkt, and pErk $\frac{1}{2}$ signaling, assessed by western blot, of a 2 hour incubation with EGF and different concentrations of Alpelisib. Bottom, left, shows the effect of 3 day incubation with different concentrations of Copanlisib on H508 cell viability, with and without EGF. Bottom, right, shows the effect on pEGFR, pAkt, and pErk $\frac{1}{2}$ signaling, assessed by western blot, of a 30 2 hour incubation with EGF and different concentrations of Copanlisib.

5 **FIG. 21** shows the effects of Alpelisib and Copanlisib, in combination with the anti-IN01 antibody, on H508 cell viability, in the presence of EGF. FIG. 21, left, shows the effect of a 3 day incubation with the anti-IN01 therapeutic antibody, “Ab,” or a control antibody, “C Ab,” used in combination with different concentrations of Alpelisib on H508 cell viability in the presence of EGF. FIG. 21, right, shows the effect of a 3 day incubation with the anti-IN01
10 therapeutic antibody, “Ab,” or a control antibody, “C Ab,” used in combination with different concentrations of Copanlisib on H508 cell viability in the presence of EGF.

FIG. 22 shows the longer term effects on H508 cell viability of the anti-IN01 therapeutic antibody in combination with EGF and Alpelisib (left) and Copanlisib (right).

FIG. 23 shows the effects of the anti-IN01 therapeutic antibody, “Ab,” or the control
15 antibody, “C-Ab,” in combination with EGF, on HT29 cell viability.

FIG. 24 shows the effects of a 3 day incubation with the anti-IN01 therapeutic antibody, or a control antibody, C-Ab, used in combination with different concentrations of Trametinib on HT29 cell viability.

FIG. 25 shows the effects on pEGFR, pStat3, pAkt, and pErk^{1/2} signaling, assessed by
20 western blot, of a 2 hour incubation with EGF and different concentrations of Trametinib in HT29 cells.

FIG. 26 shows the effects on pEGFR and pErk^{1/2} signaling of a 2 hour incubation with EGF, 0.01 mM Trametinib, and the anti-IN01 antibody, referred to as “Ab8”, alone or in combination, in HT29 cells.

25 **FIG. 27** shows the emergence of resistance over time in PC9 and PC9GR4 cells to kinase inhibitors. Top, left, shows the effects of the anti-IN01 antibody, referred to as “Ab,” and the control antibody, referred to as “C-Ab,” in combination with Osimertinib on PC9 cell viability. Top, right, shows the effects of the anti-IN01 antibody, referred to as “Ab,” and the control antibody, referred to as “C-Ab,” in combination with Osimertinib on PC9GR4 cell
30 viability. Bottom, left, shows the effects of the anti-IN01 antibody, referred to as “Ab,” and the control antibody, referred to as “C-Ab,” in combination with Osimertinib on PC9 cell viability. Bottom, right, shows the effects of the anti-IN01 antibody, referred to as “Ab,” and the control antibody, referred to as “C-Ab,” in combination with Osimertinib on PC9GR4 cell

5 viability. the effects of the anti-IN01 antibody, referred to as “Ab,” and the control antibody, referred to as “C-Ab,”

FIG. 28 shows the emergence of resistance over time of PC9 cells to kinase inhibitors. Top, left, shows the effects on PC9 cell viability of 10% FBS, the anti-IN01 antibody, referred to as “Ab,” and the control antibody, referred to as “C-Ab,” in combination
10 with 0.5 mM Dacomitinib. Top, right, shows the effects on PC9 cell viability of 10% FBS, the anti-IN01 antibody, referred to as “Ab,” and the control antibody, referred to as “C-Ab,” in combination with 1.0 mM Dacomitinib. FIG. 28, bottom, shows the effects on PC9 cell viability of the anti-IN01 antibody, referred to as “Ab,” and the control antibody, referred to as “C-Ab,” in combination with 0.5 mM Dacomitinib.

15 **FIG. 29** shows the emergence of resistance over time of DLD1 cells in response to treatment with the anti-IN01 antibody and a control antibody, in combination with 2.5 mM Trametinib.

FIG. 30 shows a diagram of cell cycle and apoptosis determination via flow cytometry, wherein the sample cells are stained in suspension, and the stained cells are
20 detected via fluorescence excitation.

FIG. 31 shows the relative number of A549 cells in S and G2/M phases of the cell cycle in response to the anti-IN01 antibody, referred to as “Ab,” EGF, and 1nM Trametinib, in combination and alone.

FIG. 32 shows the relative number of A549 cells in apoptosis in response to the anti-
25 IN01 antibody, referred to as “Ab,” EGF, and 1nM Trametinib, in combination and alone.

FIG. 33 shows the relative number of DLD1 cells in S and G2/M phases of the cell cycle in response to the anti-IN01 antibody, referred to as “Ab,” EGF, and 10 nM Trametinib, in combination and alone.

FIG. 34 shows the relative number of DLD1 cells in apoptosis in response to the anti-
30 IN01 antibody, referred to as “Ab,” EGF, and 10 nM Trametinib, in combination and alone.

FIG. 35 shows the relative number of H2228 cells, an ALK translocated cell line, in S and G2/M phases of the cell cycle in response to the anti-IN01 antibody, referred to as “Ab,” EGF, and 1 mM Brigatinib, in combination and alone.

5 **FIG. 36** shows the relative number of H3122 cells, an ALK translocated cell line, in S and G2/M phases of the cell cycle in response to the anti-IN01 antibody, referred to as “Ab,” EGF, and 1 mM Brigatinib, in combination and alone.

FIG. 37 summarizes the main alterations of the H2228, A549, and SW900 model cell lines.

10 **FIG. 38** shows the pEGFR signaling response in SW900 cell lines to patient-derived anti-EGF antibody titers, and to the anti-IN01 antibody, referred to as “Ab8,” in the presence of EGF.

FIG. 39 shows the pEGFR, pAkt, and pErk1/2 signaling response in SW900 cell lines to two different patient-derived anti-EGF antibody titers, and to the anti-IN01 antibody,
15 referred to as “Ab,” in the presence of EGF.

FIG. 40 shows the pEGFR, pAkt, and pErk1/2 signaling response in SW900 cell lines to three different patient-derived anti-EGF antibody titers, and to the anti-IN01 antibody in the presence of EGF.

FIG. 41 shows the western blot quantifications of the pEGFR and pErk1/2 signaling
20 response in SW900 cells to patient-derived anti-EGF antibody titers, and to the anti-IN01 antibody, referred to as “Ab8.”

FIG. 42 shows the western blot quantification of the pEGFR and pErk1/2 signaling response in H288 cells to patient-derived anti-EGF antibody titers, and to the anti-IN01 antibody, referred to as “Ab.”

25 **FIG. 43** shows exemplary alterations present in colorectal cancer cell lines.

FIG. 44 shows exemplary kinase inhibitors used in the instant application.

FIG. 45 shows the effects of Trametinib and Taselisib with and without EGF in DLD1 cells. Top, left, shows the effect of 3 day incubation of different concentrations of Trametinib on DLD1 cell viability, with and without EGF. Top, right, shows the effect on
30 pEGFR, pStat3, pAkt, and pErk $\frac{1}{2}$ signaling, assessed by western blot, of a 2 hour incubation with EGF and different concentrations of Trametinib in DLD1 cells. Bottom, left, shows the effects of a 3 day incubation of different concentrations of Taselisib on DLD1 cell viability,

5 with and without EGF. Bottom, right, shows the effect on pEGFR, pStat3, pAkt, and pErk $\frac{1}{2}$ signaling, assessed by western blot, of a 2 hour incubation with EGF and different concentrations of Taselisib in DLD1 cells.

FIG. 46 shows effects of antibody and kinase inhibitor combination therapy in DLD1 cells. Top, left, shows the effects of a 3 day incubation with the anti-IN01 therapeutic
 10 antibody, or a control antibody, C-Ab, used in combination with different concentrations of Trametinib on DLD1 cell viability. Top, right, shows the effects on pEGFR, pStat3, pAkt, and pErk $\frac{1}{2}$ signaling, assessed by western blot, of a 2 hour incubation with EGF, the anti-IN01 antibody, referred to as “Ab,” and Trametinib, alone or in combination, in DLD1 cells. Bottom, left, shows the effects of 3 day incubation of different concentrations of Trametinib
 15 in combination with the anti-IN01 therapeutic antibody, “Ab,” or a control antibody, “C-Ab,” used in combination with Taselisib. Bottom, right, shows the effects on pEGFR, pStat3, pAkt, and pErk $\frac{1}{2}$ signaling, assessed by western blot, of a 2 hour incubation with EGF, the anti-IN01 antibody, referred to as “Ab,” and Taselisib, alone or in combination, in DLD1 cells.

20 **FIG. 47** shows the effects of 10nM Tramentinib, EGF, and the anti-IN01 antibody, in combination and alone, on the S and G2/M phases of the cell cycle in DLD1 cells.

FIG. 48 shows the effects of 10nM Tramentinib, EGF, and the anti-IN01 antibody, in combination and alone, on apoptosis in DLD1 cells.

FIG. 49 shows the emergence of resistance over time to Trametinib, with Trametinib
 25 alone or in combination with the anti-IN01 antibody, referred to as “Ab,” or a control antibody, referred to as “C,” in DLD1 cells.

FIG. 50 shows the effects of Trametinib with and without EGF in LS174T cells. Left, shows the effect of 3 day incubation with different concentrations of Trametinib on LS174T cell viability, with and without EGF. Right, shows the effect on pEGFR, pStat3, pAkt, and
 30 pErk $\frac{1}{2}$ of 2 hour incubation of EGF and different concentrations of Trametinib in LS174T cells.

FIG. 51 shows the effects of Trametinib in combination with the anti-IN01 antibody in LS174T cells. Left, shows the effects of 3 day incubation with different concentrations of Trametinib, alone and in combination with the anti-IN01 antibody, referred to as “Ab,” or a

5 control antibody, referred to as “C,” on LS174T cell viability. Right, shows the effects on pEGFR, pStat3, pAkt, and pErk ½ signaling, assessed by western blot, of a 2 hour incubation with EGF, 1 nM Trametinib, and/or the anti-IN01 antibody, referred to as “Ab,” or a control antibody, referred to as “C,” in LS174T cells. The anti-IN01 Ab was diluted 1:25 for these experiments.

10 **FIG. 52** shows the longer term effects on LS174T cell viability of the anti-IN01 therapeutic antibody, or a control antibody, C-Ab, used in combination with 0.05 mM Trametinib.

FIG. 53 shows the emergence of resistance over time to Trametinib in LS174T cells. 0.5 mM Trametinib was administered alone or in combination with the anti-IN01 antibody,
15 “Ab” or control antibody, “C-Ab.”

FIG. 54 shows the effects of kinase inhibitors with and without EGF in HT29 cells. Top, left, shows the effects of a 3 day incubation with different concentrations of Trametinib, with and without EGF, on HT29 cell viability. Top, right, shows the effects on pEGFR, pStat3, pAkt, and pErk ½ signaling, assessed by western blot, of a 2 hour incubation with
20 EGF or different concentrations of Trametinib. FIG. 51, bottom, shows the effects of a 3 day incubation with different concentrations of Encorafenib, with and without EGF, on HT29 cell viability.

FIG. 55 shows the effects of antibody and kinase inhibitor combination therapy in HT29 cells, in the presence of EGF. Top, left shows the effect of a 3 day incubation with
25 different concentrations of Trametinib, either alone or in combination with the anti-IN01 antibody “Ab,” or the control antibody “C-Ab,” on HT29 cell viability. Top, right shows the effects of on pEGFR, pAkt, and pErk ½ signaling, assessed by western blot, of a 2 hour incubation with EGF, the anti-IN01 antibody, “Ab,” or Trametinib, alone or in combination. FIG. 52, bottom, shows the effects of a 3 day incubation with different concentrations of
30 Encorafenib, either alone or in combination with the anti-IN01 antibody “Ab,” or the control antibody “C-Ab,” on HT29 cell viability.

FIG. 56 shows the effects of long term incubation of Encorafenib, EGF, and the anti-IN01 antibody “Ab,” either alone or in combination on HT29 cell viability.

5 **FIG. 57** shows the effects of Trametinib and Taselisib with and without EGF in H508 cells. Top right, shows the effects of a 3 day incubation of different concentrations of Trametinib, with and without EGF on H508 cell viability. Top, left, shows the effects on pEGFR, pAkt, and pErk $\frac{1}{2}$ signaling, assessed by western blot, of a 2 hour incubation with EGF and different concentrations of Trametinib. Bottom, left, shows the effects of a 3 day
10 incubation with Taselisib, in the presence and absence of EGF, on H508 cell viability. Bottom, right, shows the effects on pEGFR, pAkt, and pErk $\frac{1}{2}$ signaling, assessed by western blot, of a 2 hour incubation with EGF and different concentrations of Taselisib.

FIG. 58 shows the effects of antibody and kinase inhibitor combination therapy in H508 cells, in the presence of EGF. Top, right, shows the effects of a 3 day
15 incubation with different concentrations of Trametinib, either alone or in combination with the anti-IN01 antibody “Ab,” or the control antibody “C-Ab,” on H508 cell viability. Top, right, shows the effects of on pEGFR, pAkt, and pErk $\frac{1}{2}$ signaling, assessed by western blot, of a 2 hour incubation with EGF, the anti-IN01 antibody, “Ab,” and Trametinib, either alone or in combination. Bottom, right, shows the effects of a 3 day incubation with different
20 concentrations of Taselisib, either alone or in combination with the anti-IN01 antibody “Ab,” or the control antibody “C-Ab,” on H508 cell viability. Bottom, right, shows the effects of on pEGFR, pAkt, and pErk $\frac{1}{2}$ signaling, assessed by western blot, of a 2 hour incubation with EGF, the anti-IN01 antibody, “Ab,” and Taselisib, either alone or in combination.

FIG. 59 shows the effects of antibody and Alpelisib and Copanlisib kinase inhibitors combination therapy in H508 cells, in the presence of EGF. Left, shows the effects of a 3 day
25 incubation with different concentrations of Alpelisib, either alone or in combination with the anti-IN01 antibody “Ab,” or the control antibody “C-Ab,” on H508 cell viability. Right, shows the effects of a 3 day incubation with different concentrations of Copanlisib, either alone or in combination with the anti-IN01 antibody “Ab,” or the control antibody “C-Ab,”
30 on H508 cell viability.

FIG. 60 shows the relative number of H508 cells in S and G2/M phases of the cell cycle in response to the anti-IN01 antibody, referred to as “Ab,” EGF, and kinase inhibitors, in combination and alone. Left, shows the relative number of H508 cells in S and G2/M
35 phases of the cell cycle in response to the anti-IN01 antibody, referred to as “Ab,” EGF, and Alpelisib, in combination and alone. Right, shows the relative number of H508 cells in S and

- 5 G2/M phases of the cell cycle in response to the anti-IN01 antibody, referred to as “Ab,” EGF, and Copanlisib, in combination and alone.

FIG. 61 shows the longer term effects of kinase inhibitors and EGF, alone, and in combination with the anti-IN01 antibody, “Ab” on H508 cell viability. Left, shows the effects of 0.5 mM Taselisib and EGF, alone and in combination with the anti-IN01 antibody, “Ab”
 10 on H508 cell viability. Right, shows the effects of 0.1 mM Copanlisib and EGF, alone and in combination with the anti-IN01 antibody, “Ab” on H508 cell viability.

FIG. 62 shows the emergence of resistance to Taselisib in H508 cells. Taselisib was administered alone or in combination with either the anti-IN01 antibody “Ab,” or the control antibody “C-Ab” and H508 cell growth was monitored over time.

- 15 **FIG. 63** shows exemplary cell lines, relevant alterations, and kinase inhibitors used in the instant application.

FIG. 64 shows the effects of the anti-IN01 antibody without and EGF on HT29 and HCC15 cells. Top, left, shows the effects of 3 day incubation with different dilutions of the anti-IN01 antibody, “Ab,” and the control antibody, “C-Ab,” on HT29 cell viability in the
 20 presence of EGF. Top, right, shows the effects of 3 day incubation with different dilutions of the anti-IN01 antibody, “Ab,” and the control antibody, “C-Ab,” on HCC15 cell viability in the presence of EGF. Bottom, left, shows the effects on pEGFR, pAkt, and pErk ½ signaling, assessed by western blot, of a 2 hour incubation with EGF, different concentrations of the anti-IN01 antibody, “Ab,” or a control antibody, “C-Ab,” in HT29 cells. Bottom, right, shows
 25 the effects of pEGFR, pStat3, pAkt, and pErk ½ signaling, assessed by western blot, of a 2 hour incubation with EGF, different concentrations of the anti-IN01 antibody, “Ab,” or a control antibody, “C-Ab” in HCC15 cells.

FIG. 65 shows the effects of Trametinib and Encorafenib with and without EGF in HT29 cells. Top, left, shows the effects of 3 day incubation with different concentrations of
 30 Trametinib, with and without EGF, on HT29 cell viability. Top, right, shows the effects of 2 hour incubation of EGF and different concentrations of Tramentinib on pEGFR, pStat3, pAkt, and pErk ½ signaling, assessed by western blot in HT29 cells. Bottom, left, shows the effects of different concentrations of Encorafenib, in the presence and absence of EGF on HT29 cell viability. Bottom, right, shows the effects of 2 hour incubation of EGF and

5 different concentrations of Encorafenib on pEGFR, pAkt, and pErk $\frac{1}{2}$ signaling, assessed by western blot in HT29 cells

FIG. 66 shows the effects of Trametinib with and without EGF in HCC15 cells. Left, shows the effects of 3 day incubation with different concentrations of Trametinib, in the presence and absence of EGF, on HCC15 cell viability. Right, shows the effects on pEGFR,
10 pStat3, pAkt, and pErk $\frac{1}{2}$ signaling of 2 hour incubation with EGF and different concentrations of Trametinib.

FIG. 67 shows the effects of kinase inhibitors in combination with the anti-IN01 antibody on HT29 cells, in the presence of EGF. Top, left, shows the effects of a 3 day incubation with different concentrations of Trametinib, alone or in combination with the anti-IN01 antibody, "Ab," or a control antibody, "C-Ab," on HT29 cell viability. Top, right,
15 shows the effects on pEGFR, pAkt, and pErk $\frac{1}{2}$ signaling of 2 hour incubation with EGF, the anti-IN01 antibody, "Ab, and Trametinib, alone or in combination in HT29 cells. Bottom, left, shows the effects of 3 day incubation with different concentrations of Encorafenib, alone or in combination with the anti-IN01 antibody, "Ab," or a control antibody, "C-Ab" in HT29
20 cells. Bottom, right, shows the effects on pEGFR, pAkt, and pErk $\frac{1}{2}$ signaling of 2 hour incubation with EGF, the anti-IN01 antibody, "Ab," and Encorafenib, alone or in combination in HT29 cells.

FIG. 68 shows the effects of Trametinib in combination with the anti-IN01 antibody on HCC15 cells, in the presence of EGF. Left, shows the effects of a 3 day incubation with
25 different concentrations of Trametinib, alone or in combination with the anti-IN01 antibody, "Ab," or a control antibody, "C-Ab," on HCC15 cell viability. Right, shows the effects on pEGFR, pStat3, pAkt, and pErk $\frac{1}{2}$ signaling of 2 hour incubation with EGF, the anti-IN01 antibody, "Ab, and Trametinib, alone or in combination in HCC15 cells.

FIG. 69 shows the long term effects of kinase inhibitors and the anti-IN01 antibody in the presence of 10 ng/mL EGF on HT29 cell viability. Top, left, shows the long term effects
30 of EGF, the anti-IN01 antibody, "Ab," alone or in combination with 0.01 mM Trametinib, on HT29 cell viability. Top, right, shows the long term effects of EGF, the anti-IN01 antibody, "Ab," alone or in combination with 0.1 mM Trametinib, on HT29 cell viability. Bottom, left, shows the long term effects of EGF, the anti-IN01 antibody, "Ab," alone or in combination
35 with 0.01 mM Encorafenib, on HT29 cell viability. Bottom, right, shows the long term effects

5 of EGF, the anti-IN01 antibody, “Ab,” alone or in combination with 0.1 mM Encorafenib, on HT29 cell viability.

FIG. 70 shows the long term effects of different concentrations of EGF in combination with 0.5 mM Encorafenib on HT29 cell viability.

FIG. 71 shows the long term effects of different concentrations of EGF in
10 combination with 0.5 mM Encorafenib, and the anti-IN01 antibody, on HT29 cell viability. Top, left, shows the long term effects of 0.05 ng/mL EGF in combination with 0.5 mM Encorafenib, and the anti-IN01 antibody on HT29 cell viability. Top, right, shows the long term effects of 0.5 ng/mL EGF in combination with 0.5 mM Encorafenib, and the anti-IN01 antibody on HT29 cell viability. Bottom, shows the long term effects of 10 ng/mL EGF in
15 combination with 0.5 mM Encorafenib, and the anti-IN01 antibody on HT29 cell viability.

FIG. 72 shows the long term effects of Trametinib and the anti-IN01 antibody on HCC15 cell viability. Left, shows the long term effects of 0.1 mM Trametinib alone, and in combination with the anti-IN01 antibody on HCC15 cell viability in the presence of EGF. Right, shows the long term effects of 1.0 mM Trametinib alone, and in combination with the
20 anti-IN01 antibody on HCC15 cell viability in the presence of EGF.

FIG. 73 shows the emergence of resistance to Trametinib in HCC15 cells. Left, shows the long term effects of Trametinib in combination with either 1/10 or 1/25 dilutions of the anti-IN01 antibody, “Ab,” or a control antibody, “C-Ab,” on HCC15 cell viability. Right, shows the long term effects of Trametinib in combination with either the anti-IN01 antibody,
25 “Ab,” or a control antibody, “C-Ab,” on HCC15 cell viability.

FIG. 74 shows the H508 cell line alterations and PIK3CA kinase inhibitors used in the instant application.

FIG. 75 shows the effects of kinase inhibitors Alpelisib and Copanlisib with and without EGF in H508 cells. Top, left, shows the effects of 3 day incubation with different
30 concentrations of Alpelisib with and without EGF on H508 cell viability. Top, right, shows the effects on pEGFR, pAkt, and pErk1/2 on 2 hour incubation of EGF and different concentrations of Alpelisib. Bottom, left, shows the effects of 3 day incubation with different concentrations of Copanlisib with and without EGF on H508 cell viability. Bottom, right,

5 shows the effects on pEGFR, pAkt, and pErk1/2 on 2 hour incubation of EGF and different concentrations of Copanlisib.

FIG. 76 shows the effects of kinase inhibitors in combination with the anti-IN01 antibody, “Ab,” or a control antibody, “C-Ab,” on H508 cell viability, in the presence of EGF. Left, shows the effects of a 3 day incubation of different concentrations of Alpelisib in
10 combination with the anti-IN01 antibody, “Ab,” or a control antibody, “C-Ab,” on H508 cell viability. Right, shows the effects of a 3 day incubation of different concentrations of Copanlisib in combination with the anti-IN01 antibody, “Ab,” or a control antibody, “C-Ab,” on H508 cell viability.

FIG. 77 shows the long term effects of kinase inhibitors in combination with the anti-IN01 antibody on H508 cell viability, in the presence of EGF. Left, shows the long term
15 effects of EGF, Alpelisib, and a 1/50 dilution of the anti-IN01 antibody on H508 cell viability. Right, shows the long term effects of EGF, Copanlisib, and a 1/50 dilution of the anti-IN01 antibody on H508 cell viability.

FIG. 78 shows the cell line, histology, kinase inhibitor, and relevant alteration of the
20 RET translocated cell line used in the instant application.

FIG. 79 shows the effects of a 3 day incubation of different concentrations of the BLU667 kinase inhibitor on LC-2/ad cell viability, with and without EGF.

FIG. 80 shows the effects of a 3 day incubation of different concentrations of EGF, and the BLU667 kinase inhibitor, in combination with the anti-IN01 antibody, “Ab,” or a
25 control antibody, “C-Ab,” on LC-2/ad cell viability.

FIG. 81 shows the long term effects of the BLU667 kinase inhibitor, in combination with the anti-IN01 antibody, on LC/2ad cell viability in the presence of EGF. Left, shows the long term effects of EGF and a 1/50 dilution of the anti-IN01 antibody alone or in combination with 0.1 mM BLU667, on LC/2ad cell viability. Left, shows the long term
30 effects of EGF and a 1/50 dilution of the anti-IN01 antibody alone or in combination with 1.0 mM BLU667, on LC/2ad cell viability.

FIG. 82 shows the cell lines used, the tissues from which they were derived, and the relevant MET alterations.

5 **FIG. 83** shows the effects of 3 day incubation with EGF on left, EBC1 and right, Hs746T cell viability.

FIG. 84 shows the effects of 3 day incubation of the anti-IN01 antibody, “Ab,” or control antibody, “C-Ab,” on left, EBC1 and right, Hs746T cell viability with different dilutions of EGF.

10 **FIG. 85** shows the effects of 3 day incubation of top, Capmatinib and bottom left, Tepotinib on EBC1 cell viability, with and without EGF. Bottom right, is shown the effects of 2 hour incubation of different concentrations of Tepotinib on pMET, pEGFR, pAkt, and pErk1/2 activation in EBC1 cells.

15 **FIG. 86** shows the effects of a 3 day incubation with different concentrations of left, Capmatinib and right, Tepotinib, with and without EGF, on Hs746T cell viability,

FIG. 87 shows the effects of 3 day incubation of different concentrations of top, Capmatinib and bottom left, Tepotinib, in combination with the anti-IN01 antibody, “Ab,” or control antibody, “C-Ab,” on EBC1 cell viability, and bottom right, the effects of 2 hour incubation with Tepotinib on pMET signaling in EBC1 cells.

20 **FIG. 88** shows the effects of 3 day incubation of different concentrations of left, Capmatinib and right, Tepotinib, in combination with the anti-IN01 antibody, “Ab,” or control antibody, “C-Ab,” on Hs746T cell viability.

FIG. 89 shows the long-term effects on EBC1 cell viability of incubation with left, 0.25 nM Tepotinib and EGF, alone and in combination with the anti-IN01 antibody, and
25 right, 0.75 nM Tepotinib and EGF, alone and in combination with the anti-IN01 antibody.

FIG. 90 shows the long-term effects on Hs746T cell viability of incubation with left, 0.5 nM Tepotinib and EGF, alone and in combination with the anti-IN01 antibody, and right, 2.0 nM Tepotinib and EGF, alone and in combination with the anti-IN01 antibody

DETAILED DESCRIPTION OF THE DISCLOSURE

30 The disclosure provides compositions and methods for treating neoplasia. In particular, the disclosure provides compositions and methods for enhancing the efficacy of non-tyrosine targeting kinase inhibitors (NTKIs) for treating neoplasia (e.g. colon

5 adenocarcinoma and non-small cell lung carcinoma). In particular, the disclosure provides combination therapies including non-natural synthetic polypeptides (e.g., recombinant proteins) and/or antibodies for inducing immunization to EGF, EGFR, or other growth factors, in combination with NTKIs targeting EGFR effectors, to prevent or minimize resistance to NTKIs and thereby inhibit a neoplasia (e.g. non-small cell lung carcinoma and
10 adenocarcinoma). In particular, the disclosure provides compositions and methods for administering an anti-IN01 antibody in combination with one or more NTKIs, e.g. Trametinib, Taselisib, Alpelisib, Copanlisib, and/or Encorafenib, for treatment of cancer in patients with mutations in KRAS/NRAS, PIK3CA, or BRAF genes in order to delay or prevent resistance to the NTKI.

15 The present disclosure is based, at least in part, on the discovery that anti-IN01 antibodies at physiological concentrations, in combination with NTKIs, significantly delayed the onset of resistance to the NTKIs, decreased cancer cell viability relative to the NTKIs alone, and inhibited the activation of pEGFR and pErk1/2 to a greater extent than NTKIs alone. These data demonstrate that EGF non-natural synthetic polypeptides
20 (NNSPs) and/or antibodies thereof significantly enhanced the effect of EGFR effector NTKIs in inhibiting EGFR effector kinases and EGFR pathway signaling. Treatment of patients exhibiting KRAS/NRAS, PIK3CA, or BRAF mutations with an EGF non-natural synthetic polypeptide an/or antibody in combination with one or more NTKIs provides an important mechanism for delaying or inhibiting the resistance to these compounds, thereby
25 facilitating the treatment of resistant cancers.

Patients with wild type EGFR effector proteins – for example, RAS, BRAF, MEK, and PIK3CA - can be treated with tyrosine kinase inhibitors (TKIs) targeting EGFR such as Cetuximab and Erlotinib. However, there is a large cohort of cancer patients harboring mutations in EGFR effector proteins. Extant treatments are not effective for these patients
30 because the mutations often provide resistance to the TKIs targeting EGFR and the other non-tyrosine targeting kinase inhibitors targeting downstream proteins. Similarly, treatment with TKIs targeting EGFR in patients with wild type EGFR effector proteins often results in the development of resistance due to the selection for oncogenic mutations. Thus, although the extant treatments with TKIs and other non-tyrosine targeting kinase inhibitors exhibit strong
35 anti-tumor properties targeting the EGFR pathway in some patients, they become less potent or entirely ineffective in patients who exhibit resistance. EGFR effector kinases may be

5 referred to as 'bypass pathways', in that they have been identified as mechanisms of both intrinsic and acquired resistance to TKIs and non-tyrosine targeting kinase inhibitors. Without being bound by theory, it is thought that parallel or other kinases in the EGFR signaling cascade compensate for mutated kinases, thereby inducing stronger EGFR signaling effects. Therefore, there is a need for additional therapies to overcome resistance to
10 EGFR pathway non-tyrosine targeting kinase inhibitors (NTKIs) in order to effectively treat EGFR or similar growth factor mediated diseases and cancers.

One such therapy is an initiation of an immune response. Antibodies to EGF, for example, control the division and proliferation of malignant cells by blocking EGFR signaling. The instant disclosure describes combination therapies of non-natural synthetic
15 polypeptides (NNSPs) and/or antibodies thereof, in the immunization to EGF, EGFR, or other growth factors, and in combination with NTKIs targeting EGFR effectors, to prevent or minimize resistance to NTKIs and thereby inhibit tumor cell growth.

In some embodiments, such antibodies or antigen-binding fragments thereof can be used in the methods of treating cancer or other disorders of growth factor regulation. It
20 is contemplated within the scope of the disclosure that anti-EGF antibodies can be actively produced *in vivo* by the administration of a vaccine (i.e. the IN01 peptide) producing an immune response to EGF. It is also contemplated within the scope of the disclosure that passive monoclonal anti-EFG antibodies can be administered (e.g. anti-IN01). It is contemplated that in some embodiments, mutations in other growth factor
25 signaling pathways may play a similar compensatory role in providing resistance to NTKIs.

Overview

Cancer immunology is the study of interactions between an immune system and cancer cells such as, for example, tumors or malignancies. The initiation of an immune response, such as recognition of cancer-specific antigens that are expressed by human tumors
30 and not expressed in normal tissues, is of particular interest. Generally, methods to control the division and proliferation of the malignant cells have focused on isolating these antigens and presenting them so that they are recognized by the immune system as foreign antigens to induce a specific immune response.

There are a significant number of growth factors identified at present, and most, if not
35 all, have been shown to be important mediators of cell proliferation in various cancers in

5 addition to being implicated in other disease conditions. Generally, growth factors are soluble serum proteins that recognize and bind to a group of growth factor receptors located on cell surfaces. Particular growth factors may be specific for a single receptor, or may bind to more than one closely related receptor with varying affinities. Similarly, some receptors bind to only a single growth factor ligand while others can bind to multiple related growth factors,
 10 again usually with differing affinities. Upon binding to its natural receptor, the cytoplasmic domain of the receptor is phosphorylated, and this initiates an intra-cellular signaling cascade that results in modulation of transcription of one or more genes and ultimately to progression through the cell cycle and cell proliferation.

Growth factors and their receptors are essential components of the normal processes
 15 of growth, development and repair, and their tissue distribution profiles and expression levels closely regulate cell growth. Numerous studies have shown that growth factors can stimulate proliferation of a variety of cell types both in vitro and in vivo (Cohen S., Carpenter G., PNAS USA 72, 1317, 1975, Witsch E et al: Physiology: 25(2):85-101, (2010)). Moreover, certain growth factors have been shown to stimulate proliferation in some cancer cell lines.
 20 For example epidermal growth factor (EGF) can stimulate some non-small cell lung carcinoma cells (Osborne C. K. et al. Can Res. 40, 2. 361 (1980)). Other growth factors such as vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), and platelet-derived growth factor (PDGF) are important in several oncology diseases, such as non-small cell lung cancer (NSCLC) (Ballas MS, Chachoua A., Onco Targets and Therapy: 4, 43-58
 25 (2011)), prostate cancer, (Cox ME et al; Prostate 69 (1):33-40 (2009)), and breast cancer (Law J et al, Cancer Res; 68,24: 10238- 10346 (2008)).

Growth factor nucleic acid or peptide sequences anticipated to be used in some embodiments of the instant application include nucleic acid or peptide sequences of full length or portion thereof human or murine EGF, IGF-1, IGF-2, FGF1, FGF2, TGF- α , TGF- β ,
 30 VEGF-A, VEGF-B, VEGF-C, VEGF, D, PDGF, NGF, EGF, HGF, BMP's, KGF, MSF, PDL1, IL's 1-6, Neuregulins 1-4, and Neurotrophins. Similarly, it is anticipated that nucleic acid or peptide sequences of associated full length or portion thereof of growth factor receptors may be employed.

5 *EGFR*

The epidermal growth factor receptor (EGFR) is a member of the ErbB family of receptors, a subfamily of four closely related receptor tyrosine kinases: EGFR (ErbB-1), HER2/neu (ErbB-2), Her 3 (ErbB-3) and Her 4 (ErbB-4). EGFR is a transmembrane protein that is activated by binding of its specific ligands, including epidermal growth factor and
 10 transforming growth factor α (TGF α).

EGFR dimerization stimulates its intrinsic intracellular protein-tyrosine kinase activity. As a result, autophosphorylation of several tyrosine (Y) residues in the C-terminal domain of EGFR occurs. These include Y992, Y1045, Y1068, Y1148 and Y1173. Autophosphorylation of EGFR elicits downstream activation and signaling of several other
 15 effector proteins, initiating several signal transduction cascades, e.g. the MAPK, Akt and JNK pathways, leading to DNA synthesis and cell proliferation. EGFR effectors modulate essential phenotypes such as cell migration, adhesion, and proliferation.

EGFR effectors

A total of 219 reactions and 322 species were identified as downstream of EGFR
 20 signaling, i.e. EGFR effectors: Abi, abl-interactor; ADAM, a disintegrin and metalloproteinase; ADPR, ADP-ribose; Akt, v-akt murine thymoma viral oncogene homolog; AP-1, activator protein-1; Bad, BCL2-antagonist of cell death; cADPR, cyclic ADP-ribose; CAK, cyclin-dependent kinase-activating kinase; CaM, calmodulin; CaMK, calcium/calmodulin-dependent protein kinase; c-Cbl, Casitas B-lineage lymphoma proto-
 25 oncogene; CD, cluster of differentiation; Cdc, cell division cycle; Cdk, cyclin-dependent kinase; c-Fos, v-fos FBJ murine osteosarcoma viral oncogene; Chk, c-src tyrosine kinase (Csk) homologous kinase; c-Jun, v-jun sarcoma virus 17 oncogene homolog; c-Myc, v-myc myelocytomatosis viral oncogene homolog; CREB, cAMP response element-binding protein; c-Src, v-src sarcoma viral oncogene homolog; cyt., cytosol; DAG, diacylglycerol; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; Elk, Ets-like protein; end., endosome; EP, prostaglandin E receptor; Eps, EGF receptor pathway substrate; ER, endoplasmic reticulum; ErbB, erythroblastic leukemia viral (v-erb-b) oncogene homolog; ERK, extracellular signal-regulated kinase; Gab, GRB2-associated binding protein; GPCR, G protein-coupled receptor; Grb, growth factor receptor-bound protein; HB-EGF, heparin-
 30 binding EGF-like growth factor; IP3R, inositol 1,4,5-triphosphate receptor; IP3, inositol

5 1,4,5-triphosphate; JNK, c-Jun N-terminal kinase; KDI, kinase domain I; LARG, leukemia-associated rho guanine nucleotide exchange factor; LIMK, LIM (Lin-11 Isl-1 Mec-3) kinase; LPA, lysophosphatidic acid; LPA1/2, lysophosphatidic acid G protein-coupled receptor 1/2; lyso., lysosome; m., messenger; MAPK, mitogen-activated protein kinase; MEKK, MAPK/ERK kinase kinase; MKK, MAP kinase kinase; MKP, MAP kinase phosphatase;

10 MLK, mixed lineage kinase; NAD, nicotinamide adenine dinucleotide; nuc., nucleus; PAK1, p21/Cdc42/Rac1-activated kinase; PDK, 3-phosphoinositide-dependent protein kinase; PGE2, prostaglandin E2; Pi, phosphoric ion; PI3,4,5-P3, phosphatidylinositol-3,4,5-triphosphate; PI3,4-P2, phosphatidylinositol-3,4-bisphosphate; PI3K, phosphatidylinositol-3-kinase; PI4,5-P2, phosphatidylinositol-4,5-bisphosphate; PI4-P, phosphatidylinositol-4-

15 phosphate; PI5K, phosphatidylinositol-5-kinase; PIP, phosphatidylinositol polyphosphate; PKB, protein kinase B; PKC, protein kinase C; pl.m., plasma membrane; PLC, phospholipase C; PLD, phospholipase D; PP, protein phosphatase; PTB, phosphotyrosine-binding domain; PTEN, phosphatase and tensin homolog; Pyk, proline-rich tyrosine kinase; Rab5a, RAS-associated protein RAB5a; Raf, v-raf-1 murine leukemia viral oncogene homolog; Ras, rat

20 sarcoma viral oncogene homolog; RasGAP, Ras GTPase-activating protein; Rb, retinoblastoma; RGS, regulator of G-protein signaling; Rin, Ras interaction; RN-tre, related to the N-terminus of tre; RSK, ribosomal protein S6 kinase; RYR, ryanodine receptor; S, serine; S1P, sphingosine-1-phosphate; S1P1/2/3, sphingolipid G protein-coupled receptor 1/2/3; SERCA, sarcoplasmic/endoplasmic reticulum calcium ATPase; Shc, Src homology 2

25 domain containing transforming protein; SHP, Shp-2 tyrosine phosphatase; SOS, son of sevenless homolog; SPRY, Sprouty; STAT, signal transducer and activator of transcription; T, threonine; TGF α , transforming growth factor alpha; Ubc, ubiquitin-conjugating enzyme; Y, tyrosine (Oda, K et al. *Molecular Systems Biology* 2005). Thus, it is anticipated that antibody and NTKI combination therapies targeting EGFR and its downstream effectors may

30 serve as therapies to a wide range of diseases and disorders.

Ras proteins

Ras proteins are proto-oncogenes that are frequently mutated in human cancers. They are encoded by 3 ubiquitously expressed genes: HRAS, KRAS, and NRAS. These proteins are GTPases that function as molecular switches in regulating pathways that are responsible

35 for proliferation and cell survival. KRAS (Kirsten rat sarcoma viral oncogene homolog) mutations are present in more than 25% of all human tumors, and KRAS is one of the most

5 frequently activated oncogenes. Studies during the last quarter century have characterized the Ras proteins as essential components of signaling networks controlling cellular proliferation, differentiation, or survival. The oncogenic mutations of the H-ras, N-ras, or K-ras genes frequently found in human tumors are known to throw off balance the normal outcome of those signaling pathways, thus leading to tumor development. Oncogenic mutations in a
 10 number of other upstream or downstream components of Ras signaling pathways (including membrane RTKs or cytosolic kinases) have been detected more recently in association with a variety of cancers. Interestingly, the oncogenic Ras mutations and the mutations in other components of Ras/MAPK signaling pathways appear to be mutually exclusive events in most tumors, indicating that deregulation of Ras-dependent signaling is the essential
 15 requirement for tumorigenesis. Finally, even without being a causative force, defective Ras signaling has been cited as a contributing factor to many other human illnesses, including diabetes and immunological and inflammatory disorders. KRAS G12S, KRAS G12C, KRAS G13D, KRAS G12D, and NRAS Q61K are exemplary mutations, among others, known to cause uninhibited growth and cell division demonstrative of carcinomas.

20 *PIK3CA*

Phosphoinositide kinases (PIKs) are lipid kinases that phosphorylate the inositol ring of phosphoinositides, thus acting as signal transducers, and which are mutated in a variety of human cancers. Depending on the phosphorylation site on the carbohydrate, PIKs are categorized into three families: phosphoinositide 3-kinases (PI3Ks), phosphoinositide 4-
 25 kinases (PIP4Ks) and phosphoinositide 5-kinases (PIP5Ks). It is estimated that PIK3CA is somatically mutated in 32% of colorectal cancers, 40% of human breast cancers, 12% of ovarian cancers, and 36% of uterine endometrioid cancers. In the brain, PIK3CA mutations are found in 14% of anaplastic oligodendrogliomas, 5% of GBMs, 5% of medulloblastomas, and 3% of anaplastic astrocytomas. PIK3CA E545K is an exemplary mutation known to
 30 contribute to carcinoma growth.

BRAF

B-Raf proto-oncogene, serine/threonine kinase is involved in many cellular processes, including cell proliferation, differentiation, and transcriptional regulation. BRAF is estimated to be altered in 5% of all colon adenocarcinoma, cutaneous melanoma, lung adenocarcinoma,

5 melanoma, and thyroid gland papillary carcinoma. BRAF V600E and BRAF G596R, among others, are further exemplary mutations known to drive carcinomas.

KRAS, NRAS, PIK3CA, and BRAF proteins downstream of EGFR are exemplary proteins wherein mutations provide resistance to TKIs targeting EGFR and NTKIs targeting EGFR effectors. It is contemplated that in some embodiments, mutations in other growth
10 factor signaling pathways may play a similar compensatory role in providing resistance to NTKIs.

Synthetic Proteins/Molecules

The present disclosure provides a homogeneous synthetic protein/molecule for improving the presentation of the maximum number of growth factor epitopes, tumor antigen
15 epitopes, and/or receptor binding sites as elements of an immunogenic synthetic protein/molecule. In one illustrative embodiment, a synthetic protein/molecule expressing all or portions of an immunogenic carrier domain (e.g., cholera toxin B (CT-B)), and a synthetic epidermal growth factor (sEGF), and/or a receptor is described. In alternative illustrative embodiments, the protein may express other immunogenic synthetic or recombinant
20 proteins/molecules that are modeled based upon known immunogenic proteins. It is contemplated within the scope of the disclosure that such synthetic proteins/molecules may express polypeptides that are highly immunogenic to the human immune system. Preferably, the synthetic proteins/molecules confer additional properties to the chimeric protein such as, for example, high expression yield and ease of manufacture, oral stability and the ability to
25 cross from gut to blood stream, and/or previous safe use in humans.

In an illustrative embodiment, the synthetic proteins/molecules disclosed herein may include or express growth factor epitopes as multiple copies of a whole or part of a single growth factor, or copies of whole or part of more than one different growth factor. These growth factor epitopes may be naturally occurring or synthetic (e.g., artificial). For example,
30 BVN22E (hereinafter IN01), an illustrative non-natural synthetic protein (NNSP) described herein, may have a molecular weight of about 120 kD. IN01 (SEQ ID NO: 2) consists of two repeated synthetic EGF (Targeted Signaling Pathway (SEQ ID NO: 7) domains, Glycine-Serine repeat linkers, and a CTB sequence (SEQ ID NO: 9). In illustrative embodiments, the growth factor epitopes described herein may correspond to one or more domains within the
35 growth factor (e.g., EGF targeted signaling pathway (TSP) domains).

5 According to the disclosure, the expressed growth factor epitopes should be folded in a manner that allows their natural conformation to be substantially retained and presented to components of the host immune system in such a way as to elicit a robust host immune response to the epitopes. Such epitope folding may be facilitated by suitable naturally occurring proteins that support the ability of a domain or domains within a synthetic
10 proteins/molecules to properly fold and induce an immune response and may include, but are not limited to, cholera toxin B sub-unit, E. coli heat-labile LT and LT-II enterotoxin B subunits, veratoxin, pertussis toxin, C. jejuni enterotoxin, Shiga toxin, listeria toxin, tetanus toxoid, diphtheria toxoid, N. meningitidis outer membrane protein, bacteriophage coat protein, adenovirus and other viral coat proteins. As described herein, non-natural synthetic
15 polypeptides (e.g., BVN22E, IN01) may be used that fulfill the requirements of conferring immunogenicity to the whole protein and allowing appropriate folding and presentation of growth factors, receptors, tumor antigens or epitopes thereof to the host immune system.

 According to the disclosure, the synthetic proteins/molecules provided herein—whether growth factors or parts thereof, cellular receptors or parts thereof, or tumor antigens
20 or parts thereof—are related to a broad range of cellular pathways involved in chronic disease, growth factor based or receptor based cancers, and/or solid tumors for use as tumor antigens within the said synthetic proteins. The proteins are in the form of a synthetic proteins/molecules and may be useful in treating chronic diseases, for example, breast, lung, bladder, ovarian, vulva, colonic, pulmonary, brain, colorectal, intestinal, head and neck, and
25 esophagus cancers.

EGFR-EFFECTOR NTKI Compounds

 As described herein, such EGFR-EFFECTOR NTKI compounds inhibit kinases downstream of EGFR, and are useful for the treatment of neoplasias, such as non-small cell lung carcinoma and colon adenocarcinoma, particularly in combination with an alkylating
30 agent, such as cisplatin. Without wishing to be bound by theory, these combination therapies may be particularly effective against neoplastic cells because they reduce or eliminate the ability of the neoplastic cells to become resistant to the NTKIs.

 In certain embodiments, a compound of the invention can prevent, inhibit, or disrupt, or reduce by at least 10%, 25%, 50%, 75%, or 100% the activity of an EGFR effector kinase.

5 In certain embodiments, a compound of the invention is a small molecule having a molecular weight less than about 1,300 daltons, less than 1000 daltons, less than 800 daltons, less than 600 daltons, less than 500 daltons, less than 400 daltons, or less than about 300 daltons. Examples of compounds of the invention include EGFR-EFFECTOR NTKI compounds of the disclosure may include, but are not limited to, Trametinib, Taselisib, 10 Alpelisib, Copanlisib, Idealisib, Duvelisib, Buparlisib, Umbralisib, PX-866 Dactolisib, CUDC-907, Voxtalib (SAR245409, XL765) ME-401, IPI-549, SF1126, INK1117 Pictilisib, XL147 (SAR245408), GSK1059615, ZSTK474, PWT33597, IC87114, TG100-115, CAL 263, RP6503, GNE-477 AEZS-136, Vemurafenib, Dabrafenib, GDC-0879, PLX-4720 Cobimetinib, Binimetinib, Selumetinib, PD-325901, PD035901, CI-1040, TAK-733, 15 Perifosine, Palomid 529 are other exemplary non-tyrosine targeting kinase inhibitors (NTKIs) targeting EGFR pathway kinases.

Trametinib (MW 615.4 g/mol) is an orally bioavailable inhibitor of mitogen-activated protein kinase kinase (MEK MAPK/ERK kinase). Trametinib specifically binds to and inhibits MEK 1 and 2, resulting in an inhibition of growth factor-mediated cell 20 signaling and cellular proliferation in various cancers. MEK 1 and 2, dual specificity threonine/tyrosine kinases often upregulated in various cancer cell types, play a key role in the activation of the RAS/RAF/MEK/ERK signaling pathway that regulates cell growth. Trametinib is indicated for the treatment of unresectable or metastatic melanoma with BRAF V600E or V600K mutations, as detected by an FDA-approved test.

25 Trametinib is a pyridopyrimidine that in extant methods is used, in DMSO, to treat patients with unresectable or metastatic melanoma with BRAF V600E or V600K mutations, and who have not received prior BRAF inhibitor treatment. Trametinib is readily absorbed. When an oral administration of trametinib was given to patients with BRAF V600 mutation-positive melanoma, peak plasma concentration occurred 1.5 hours 30 post-dose (T_{max}). A single 2 mg oral dose has a bioavailability of 72%. When a dose of 2mg/day is given, the peak plasma concentration (C_{max}) is 22.2 ng/mL. Trametinib is metabolized predominantly via deacetylation alone or with mono-oxygenation or in combination with glucuronidation biotransformation pathways in vitro. Deacetylation is likely mediated by hydrolytic enzymes, such as carboxyl-esterases or amidases. The 35 predominant circulating component in the plasma is the parent drug. Elimination half-life = 3.9-4.8 days. IUPAC name N-[3-[3-cyclopropyl-5-(2-fluoro-4-iodoanilino)-6,8-dimethyl-2,4,7-trioxopyrido[4,3-d]pyrimidin-1-yl]phenyl]acetamide.

5 Taselisib (MW 460.5 g/mol) is an orally bioavailable inhibitor of the class I phosphatidylinositol 3-kinase (PI3K) alpha isoform (PIK3CA). Taselisib selectively inhibits PIK3CA and its mutant forms in the PI3K/Akt/mTOR pathway, which may result in tumor cell apoptosis and growth inhibition in PIK3CA-expressing tumor cells. By specifically targeting class I PI3K alpha, this agent may be more efficacious and less
10 toxic than pan PI3K inhibitors. Dysregulation of the PI3K/Akt/mTOR pathway is frequently found in solid tumors and causes increased tumor cell growth, survival, and resistance to both chemotherapy and radiotherapy. PIK3CA, which encodes the p110-alpha catalytic subunit of the class I PI3K, is mutated in a variety of cancer cell types and plays a key role in cancer cell growth and invasion. IUPAC name 2-methyl-2-[4-[2-(5-
15 methyl-2-propan-2-yl-1,2,4-triazol-3-yl)-5,6-dihydroimidazo[1,2-d][1,4]benzoxazepin-9-yl]pyrazol-1-yl]propenamide.

 Alpelisib (MW 441.5 g/mol) is a phosphatidylinositol 3-kinase (PI3K) inhibitor with potent antitumor activity. It works by selectively inhibiting class I PI3K p110 α , which is the catalytic subunit of PI3K, a lipid kinase that plays a role in various biological
20 processes, including proliferation, survival, differentiation, and metabolism. Alpelisib was designed to target this enzyme that appears to be mutated at a rate of nearly 30% in human cancers, leading to hyperactivation. Approved by the FDA in May 2019, Alpelisib is the first approved PI3K inhibitor indicated for the treatment of hormone receptor (HR)-positive, human epidermal growth factor receptor 2 (HER2)-negative, PIK3CA-mutated,
25 advanced or metastatic breast cancer for postmenopausal women and male patients. In extant methods, to initiate Alpelisib therapy, the presence of a PIK3CA mutation in the tissue and/or liquid biopsy sample collection is required via FDA-approved diagnostic tests. Alpelisib is marketed under the trade name Piqray and is available as oral tablets. Patients taking Alpelisib experience a dose dependent benefit from treatment with a 51%
30 advantage of a 200mg daily dose over a 100mg dose and a 22% advantage of 300mg once daily over 150mg twice daily. Patients requiring a lower dose may benefit from twice daily dosing. The mean half-life of Alpelisib is 8 to 9 hours. IUPAC name (2S)-1-N-[4-methyl-5-[2-(1,1,1-trifluoro-2-methylpropan-2-yl)pyridin-4-yl]-1,3-thiazol-2-yl]pyrrolidine-1,2-dicarboxamide.

35 Copanlisib (MW 480.5 g/mol) is a selective pan-Class I phosphoinositide 3-kinase (PI3K/Phosphatidylinositol-4,5-bisphosphate 3-kinase/phosphatidylinositide 3-kinase)

5 inhibitor that was first developed by Bayer Healthcare Pharmaceuticals, Inc. The drug targets the enzyme that plays a role in regulating cell growth and survival. Copanlisib was granted accelerated approval on September 14, 2017 under the market name Aliqopa for the treatment of adult patients with relapsed follicular lymphoma and a treatment history of at least two prior systemic therapies. Follicular lymphoma is a slow-growing type of
10 non-Hodgkin lymphoma that is caused by unregulated proliferation and growth of lymphocytes. The active ingredient in Aliqopa intravenous therapy is Copanlisib dihydrochloride. Copanlisib has demonstrated potent anti-tumor and pro-apoptotic activity in various tumor cell lines and xenograft models. In clinical trials, 59% of patients receiving Copanlisib achieved complete or partial shrinkage of their tumors after
15 a median of 12.2 months. Higher systemic levels of Copanlisib are associated with elevated plasma glucose levels. Following a steady state exposure at 0.8 mg/kg, the mean peak plasma concentration (C_{max}) of copanlisib is 463 ng/mL with the range of 105 to 1670 ng/mL. The geometric mean terminal elimination half-life of Copanlisib is 39.1 hours (range: 14.6 to 82.4; SD: 15.0). The *in vitro* human plasma protein binding of
20 Copanlisib is 84.2%, with albumin being the main binding protein. IUPAC name 2-amino-N-[7-methoxy-8-(3-morpholin-4-ylpropoxy)-2,3-dihydro-1H-imidazo[1,2-c]quinazolin-5-ylidene]pyrimidine-5-carboxamide.

Encorafenib (MW 540 g/mol), also known as BRAFTOVI, is a kinase inhibitor. Encorafenib inhibits BRAF which plays a role in regulating the MAP kinase/ERK
25 signaling pathway, impacting cell division, differentiation, and secretion. Mutations in this gene, most frequently the V600E mutation, are the most commonly identified cancer-causing mutations in melanoma, and have been isolated in various other cancers as well, including non-Hodgkin lymphoma, colorectal cancer, thyroid carcinoma, non-small cell lung carcinoma, hairy cell leukemia and adenocarcinoma of the lung. On June 27, 2018,
30 the Food and Drug Administration approved Encorafenib and Binimetinib (BRAFTOVI and MEKTOVI, Array BioPharma Inc.) in combination for patients with unresectable or metastatic melanoma with a BRAF V600E or V600K mutation, as detected by an FDA-approved test. After oral administration, the median T_{max} of encorafenib is 2 hours. At least 86% of the dose is absorbed. The apparent clearance is 14 L/h (54%) at day 1,
35 increasing to 32 L/h (59%) at steady-state. IUPAC name is methyl N-[(2S)-1-[[4-[3-[5-chloro-2-fluoro-3-(methanesulfonamido)phenyl]-1-propan-2-yl]pyrazol-4-yl]pyrimidin-2-yl]amino]propan-2-yl]carbamate.

5 The term “pharmaceutically acceptable salt” also refers to a salt prepared from a compound of the invention having an acidic functional group, such as a carboxylic acid functional group, and a pharmaceutically acceptable inorganic or organic base. Suitable bases include, but are not limited to, hydroxides of alkali metals such as sodium, potassium, and lithium; hydroxides of alkaline earth metal such as calcium and magnesium; hydroxides
10 of other metals, such as aluminum and zinc; ammonia, and organic amines, such as unsubstituted or hydroxy substituted mono , di , or trialkylamines; dicyclohexylamine; tributyl amine; pyridine; N methyl,N ethylamine; diethylamine; triethylamine; mono , bis , or tris (2 hydroxy lower alkyl amines), such as mono , bis , or tris (2 hydroxyethyl)- amine, 2 hydroxy tert butylamine, or tris (hydroxymethyl)methylamine, N, N, di lower alkyl N
15 (hydroxy lower alkyl) amines, such as N,N dimethyl N (2 hydroxyethyl)- amine, or tri (2 hydroxyethyl)amine; N methyl D glucamine; and amino acids such as arginine, lysine, and the like. The term “pharmaceutically acceptable salt” also refers to a salt prepared from a compound disclosed herein, e.g., NSC-30049, having a basic functional group, such as an amino functional group, and a pharmaceutically acceptable inorganic or organic acid.
20 Suitable acids include, but are not limited to, hydrogen sulfate, citric acid, acetic acid, oxalic acid, hydrochloric acid, hydrogen bromide, hydrogen iodide, nitric acid, phosphoric acid, isonicotinic acid, lactic acid, salicylic acid, tartaric acid, ascorbic acid, succinic acid, maleic acid, besylic acid, fumaric acid, gluconic acid, glucaronic acid, saccharic acid, formic acid, benzoic acid, glutamic acid, methanesulfonic acid, ethanesulfonic acid, benzenesulfonic acid,
25 and p-toluenesulfonic acid.

Adjuvant

Certain illustrative embodiments as provided herein include composition and method of use of synthetic proteins/molecules according to the disclosure within vaccine compositions and immunological adjuvant compositions, including pharmaceutical
30 compositions, that contain, in addition to synthetic proteins/molecules at least one adjuvant, which refers to a component of such compositions that has adjuvant activity. An adjuvant having such adjuvant activity includes a composition that, when administered to a subject such as a human (e.g., a human patient), a non-human primate, a mammal or another higher eukaryotic organism having a recognized immune system, is capable of altering (i.e.,
35 increasing or decreasing in a statistically significant manner, and in certain preferred embodiments, enhancing or increasing) the potency and/or longevity of an immune response.

5 In certain illustrative embodiments disclosed herein a desired antigen and or antigens contain within a protein carrier, and optionally one or more adjuvants, may so alter, e.g., elicit or enhance, an immune response that is directed against the desired antigen and or antigens which may be administered at the same time or may be separated in time and/or space (e.g., at a different anatomic site) in its administration, but certain illustrative embodiments are not
10 intended to be so limited and thus also contemplate administration of synthetic proteins/molecules in a composition that does not include a specified antigen but which may also include but is not limited to one or more co-adjuvant, an imidazoquinoline immune response modifier.

Pharmaceutical Compositions

15 In certain illustrative embodiments, the pharmaceutical composition is a vaccine composition that comprises both the synthetic proteins/molecules according to the disclosure and may further comprise one or more components, as provided herein, that are selected from TLR agonist, co-adjuvant (including, e.g., a cytokine, an imidazoquinoline immune response modifier and/or a dSLIM) and the like and/or a recombinant expression construct, in
20 combination with a pharmaceutically acceptable carrier, excipient or diluent.

Illustrative carriers will be nontoxic to recipients at the dosages and concentrations employed. For vaccines comprising synthetic proteins/molecules, about 0.01 µg/kg to about 100 mg/kg body weight will be administered, typically by the intradermal, subcutaneous, intramuscular or intravenous route, or by other routes. In embodiments, vaccines comprising
25 synthetic proteins/molecules may be administered from about 0.01-0.1 µg/kg to about 95 mg/kg body weight; or from about 0.1-1.0 µg/kg to about 90 mg/kg body weight; or from about 0.5-5.0 µg/kg to about 80 mg/kg body weight; or from about 1.0-10.0 µg/kg to about 60 mg/kg body weight; or from about 5.0-20.0 µg/kg to about 50 mg/kg body weight; or from about 20-50 µg/kg to about 25 mg/kg body weight; or from about 50-100 µg/kg to about 20
30 mg/kg body weight. In other embodiments this dose may be about 1, 5, 10, 25, 50, 75, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1050, 1100, 1150, 1200, 1250, 1300, 1350, 1400, 1450, 1500, 1600, 1700, 1800, 1900, 2000, 2500, 3000, 3500, 4000, 4500, or 5000 µg/kg body weight.

It will be evident to those skilled in the art that the number and frequency of
35 administration will be dependent upon the response of the host. "Pharmaceutically acceptable

5 carriers” for therapeutic use are well known in the pharmaceutical art, and are described, for example, in Remington's Pharmaceutical Sciences, Mack Publishing Co. (A. R. Gennaro edit. 1985). For example, sterile saline and phosphate-buffered saline at physiological pH may be used. Preservatives, stabilizers, dyes and even flavoring agents may be provided in the pharmaceutical composition. For example, sodium benzoate, ascorbic acid and esters of p-
10 hydroxybenzoic acid may be added as preservatives. In addition, antioxidants and suspending agents may be used.

The pharmaceutical compositions may be in any form which allows for the composition to be administered to a patient. For example, the composition may be in the form of a solid, liquid or gas (aerosol). Typical routes of administration include, without limitation,
15 oral, topical, parenteral (e.g., sublingually or buccally), sublingual, rectal, vaginal, and intranasal (e.g., as a spray). The term parenteral as used herein includes iontophoretic sonophoretic, passive transdermal, microneedle administration and also subcutaneous injections, intravenous, intramuscular, intrasternal, intracavernous, intrathecal, intrameatal, intraurethral injection or infusion techniques. In a particular embodiment, a composition as
20 described herein (including vaccine and pharmaceutical compositions) is administered intradermally by a technique selected from iontophoresis, microcavitation, sonophoresis or microneedles.

The pharmaceutical composition is formulated so as to allow the active ingredients contained therein to be bioavailable upon administration of the composition to a patient.
25 Compositions that will be administered to a patient take the form of one or more dosage units, where for example, a tablet may be a single dosage unit, and a container of one or more compounds of the invention in aerosol form may hold a plurality of dosage units.

For oral administration, an excipient and/or binder may be present. Examples are sucrose, kaolin, glycerin, starch dextrins, sodium alginate, carboxymethylcellulose and ethyl
30 cellulose. Coloring and/or flavoring agents may be present. A coating shell may be employed.

The composition may be in the form of a liquid, e.g., an elixir, syrup, solution, emulsion or suspension. The liquid may be for oral administration or for delivery by injection, as two examples. When intended for oral administration, preferred compositions contain one or more of a sweetening agent, preservatives, dye/colorant and flavor enhancer.
35 In a composition intended to be administered by injection, one or more of a surfactant,

5 preservative, wetting agent, dispersing agent, suspending agent, buffer, stabilizer and isotonic agent may be included.

A liquid pharmaceutical composition as used herein, whether in the form of a solution, suspension or other like form, may include one or more of the following carriers or excipients: sterile diluents such as water for injection, saline solution, preferably
10 physiological saline, Ringer's solution, isotonic sodium chloride, fixed oils such as squalene, squalane, mineral oil, a mannide monooleate, cholesterol, and/or synthetic mono or diglycerides which may serve as the solvent or suspending medium, polyethylene glycols, glycerin, propylene glycol or other solvents; antibacterial agents such as benzyl alcohol or methyl paraben; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such
15 as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic. An injectable pharmaceutical composition is preferably sterile.

In a particular embodiment, a pharmaceutical or vaccine composition of the invention
20 comprises a stable aqueous suspension of less than 0.2 μm and further comprises at least one component selected from the group consisting of phospholipids, fatty acids, surfactants, detergents, saponins, fluorodated lipids, and the like.

It may also be desirable to include other components in a vaccine or pharmaceutical composition, such as delivery vehicles including but not limited to aluminum salts, water-in-
25 oil emulsions, biodegradable oil vehicles, oil-in-water emulsions, biodegradable microcapsules, and liposomes. Examples of additional immunostimulatory substances (co-adjuvants) for use in such vehicles are also described above and may include N-acetylmuramyl-L-alanine-D-isoglutamine (MDP), glucan, IL-12, GM-CSF, gamma interferon and IL-12.

30 While any suitable carrier known to those of ordinary skill in the art may be employed in the pharmaceutical compositions of this invention, the type of carrier will vary depending on the mode of administration and whether a sustained release is desired. For parenteral administration, such as subcutaneous injection, the carrier preferably comprises water, saline, alcohol, a fat, a wax or a buffer. For oral administration, any of the above carriers or a solid
35 carrier, such as mannitol, lactose, starch, magnesium stearate, sodium saccharine, talcum,

5 cellulose, glucose, sucrose, and magnesium carbonate, may be employed. Biodegradable microspheres (e.g., polylactic galactide) may also be employed as carriers for the pharmaceutical compositions of this invention.

Pharmaceutical compositions may also contain diluents such as buffers, antioxidants such as ascorbic acid, low molecular weight (less than about 10 residues) polypeptides,
10 proteins, amino acids, carbohydrates including glucose, sucrose or dextrans, chelating agents such as EDTA, glutathione and other stabilizers and excipients. Neutral buffered saline or saline mixed with nonspecific serum albumin are exemplary appropriate diluents. Preferably, product may be formulated as a lyophilizate using appropriate excipient solutions (e.g., sucrose) as diluents.

15 In an illustrative embodiment, the epitope or receptor supporting domain of the synthetic protein/molecule, whether derived from a natural or synthetic polypeptide sequence, should have the capacity to self-assemble into oligomeric multimers under appropriate chemical/environmental conditions, or to be reduced to monomers under alternative conditions. Ideally, multimerisation domains will assemble into stable multimers with a
20 discreet number of sub-units, for example dimers, trimers, tetramers, pentamers, etc., such that a product of homogeneous size is generated. Examples of natural polypeptides include, but are not limited to, leucine zippers, lac repressor protein, streptavidin/avidin, cholera toxin B sub- unit, Pseudomonas trimerization domain, and viral capsid proteins.

In one illustrative embodiment the anti-EGF antibodies used in the pre-clinical
25 studies are actively produced by immunizations with a rEGF-rP64k conjugate, CIMAvax-EGF vaccine as described in *Manufacturing Process Development for an Epidermal Growth Factor-Based Cancer Vaccine*, Rodriguez et al., (Supplement to Biopharm International October 2008, the contents of which are incorporated in their entirety by reference) formulated with Montanide adjuvant. It is contemplated within the scope of the
30 disclosure that other vaccine formulations that produce an immune response to EGF or EGFR may be used. It is also within the Scope of the disclosure that vaccines producing an immune response to other growth factors or their receptors may also be used. In particular, immunogenic proteins as set forth in W02013/076580 and W02014/140894, the content of each incorporated in their entirety by reference, may be used to produce anti-EGF antibodies according
35 to the disclosure.

5 Formulation of Therapeutic NTKI Compositions

The administration of a compound (e.g., a NTKI) in combination with a non-natural synthetic polypeptide (e.g., IN01) for the treatment of a neoplasia may be by any suitable means that results in a concentration of the therapeutic that, combined with other components, is effective in ameliorating, reducing, or stabilizing a neoplasia. The compound may be contained
10 in any appropriate amount in any suitable carrier substance, and is generally present in an amount of 1-95% by weight of the total weight of the composition. The composition may be provided in a dosage form that is suitable for parenteral (e.g., subcutaneously, intravenously, intramuscularly, or intraperitoneally) administration route. The pharmaceutical compositions may be formulated according to conventional pharmaceutical practice (see, e.g., Remington:
15 The Science and Practice of Pharmacy (20th ed.), ed. A. R. Gennaro, Lippincott Williams & Wilkins, 2000 and Encyclopedia of Pharmaceutical Technology, eds. J. Swarbrick and J. C. Boylan, 1988-1999, Marcel Dekker, New York).

Human dosage amounts can initially be determined by extrapolating from the amount of compound used in mice, as a skilled artisan recognizes it is routine in the art to modify the
20 dosage for humans compared to animal models. In certain embodiments it is envisioned that the dosage may vary from between about 1 µg compound/Kg body weight to about 5000 mg compound/Kg body weight; or from about 5 mg/Kg body weight to about 4000 mg/Kg body weight or from about 10 mg/Kg body weight to about 3000 mg/Kg body weight; or from about 50 mg/Kg body weight to about 2000 mg/Kg body weight; or from about 100 mg/Kg body
25 weight to about 1000 mg/Kg body weight; or from about 150 mg/Kg body weight to about 500 mg/Kg body weight. In other embodiments this dose may be about 1, 5, 10, 25, 50, 75, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1050, 1100, 1150, 1200, 1250, 1300, 1350, 1400, 1450, 1500, 1600, 1700, 1800, 1900, 2000, 2500, 3000, 3500, 4000, 4500, or 5000 mg/Kg body weight. In other embodiments, it is
30 envisaged that doses may be in the range of about 5 mg compound/Kg body to about 20 mg compound/Kg body. In other embodiments the doses may be about 8, 10, 12, 14, 16 or 18 mg/Kg body weight. Of course, this dosage amount may be adjusted upward or downward, as is routinely done in such treatment protocols, depending on the results of the initial clinical trials and the needs of a particular patient.

35 Pharmaceutical compositions according to the invention may be formulated to release the active compound substantially immediately upon administration or at any predetermined

5 time or time period after administration. The latter types of compositions are generally known as controlled release formulations, which include (i) formulations that create a substantially constant concentration of the drug within the body over an extended period of time; (ii) formulations that after a predetermined lag time create a substantially constant concentration of the drug within the body over an extended period of time; (iii) formulations that sustain
10 action during a predetermined time period by maintaining a relatively, constant, effective level in the body with concomitant minimization of undesirable side effects associated with fluctuations in the plasma level of the active substance (sawtooth kinetic pattern); (iv) formulations that localize action by, e.g., spatial placement of a controlled release composition adjacent to or in contact with the thymus; (v) formulations that allow for convenient dosing,
15 such that doses are administered, for example, once every one or two weeks; and (vi) formulations that target a neoplasia by using carriers or chemical derivatives to deliver the therapeutic agent to a particular cell type (e.g., neoplastic cell). For some applications, controlled release formulations obviate the need for frequent dosing during the day to sustain the plasma level at a therapeutic level.

20 Any of a number of strategies can be pursued in order to obtain controlled release in which the rate of release outweighs the rate of metabolism of the compound in question. In one example, controlled release is obtained by appropriate selection of various formulation parameters and ingredients, including, e.g., various types of controlled release compositions and coatings. Thus, the therapeutic is formulated with appropriate excipients into a
25 pharmaceutical composition that, upon administration, releases the therapeutic in a controlled manner. Examples include single or multiple unit tablet or capsule compositions, oil solutions, suspensions, emulsions, microcapsules, microspheres, molecular complexes, nanoparticles, patches, and liposomes.

Although the instant disclosure suggests ranges of dosing, one skilled in the art would
30 understand that any therapeutically effective dose as set forth in the product labeling is within the scope of this disclosure. Likewise, any dosing that is based upon the understanding and knowledge of a medical practitioner in the field of oncology is contemplated within the scope of the disclosure.

Selection of a Treatment Method

35 After a subject is diagnosed as having a neoplasia (e.g., NSLC or CRC) a method of

5 treatment is selected. In colorectal cancer (CRC), for example, a number of standard treatment regimens are available. In general, CRCs are one of the most aggressive forms of cancer, and advanced CRCs are rarely susceptible to conventional treatment methods. For aggressive CRC, few therapeutic options are available, and such tumors often correlate with poor clinical outcomes, such as metastasis or death. A subject having aggressive CRC is
10 identified as likely to benefit from treatment with a composition of the invention comprising a non-natural synthetic polypeptide (NNSP) of IN01 or antibody thereof, in combination with Trametinib, Taselisib, Alpelisib, Copanlisib, and/or Encorafenib. Thus, the invention provides methods for selecting a therapy for a subject, the method involving identifying a subject as having aggressive neoplasia, such as CRC, or aggressive forms of NSLC or
15 glioblastoma, and administering to the subject a therapeutic combination of the invention.

Even when a subject with neoplasia (e.g., colon cancer, lung cancer, and brain cancer, such as glioblastoma) is identified as having a good clinical outcome, the subject is also likely to benefit from treatment with the methods of the invention (e.g., lower side effects). When methods of the invention indicate that a neoplasia is very aggressive, an aggressive
20 method of treatment should be selected. Aggressive therapeutic regimens typically include one or more of the following therapies: radical mastectomy, radiation therapy, hormone therapy, and chemotherapy. Such methods may be used in combination with the therapeutic methods described herein, particularly for the treatment of pancreatic cancer, which is prone to relapse.

25 While the homogeneous synthetic proteins/molecules expressing or incorporating one or more tumor antigens, synthetic growth factors, and/or receptors have been described and illustrated in connection with certain embodiments, many variations and modifications will be evident to those skilled in the art and may be made without departing from the spirit and scope of the disclosure. The disclosure is thus not to be limited to the precise details of
30 methodology or construction set forth above as such variations and modification are intended to be included within the scope of the disclosure.

It is contemplated that any order of administration of the non-natural synthetic polypeptide (NNSP) of IN01 or antibody thereof, relative to the NTKI Trametinib, Taselisib, Alpelisib, Copanlisib, and/or Encorafenib may be preferred in treating a patient. For example,
35 it may be beneficial to administer the non-natural synthetic polypeptide of IN01 or antibody thereof prior to administering the NTKI, e.g. Trametinib, Taselisib, Alpelisib, Copanlisib,

5 and/or Encorafenib. It may also be beneficial to administer the Trametinib, Taselisib, Alpelisib, Copanlisib, and/or Encorafenib prior to administering the non-natural synthetic polypeptide of IN01 or antibody thereof.

The terms "decrease," "reduce," "reduced", "reduction", "decrease," and "inhibit" are all used herein generally to mean a decrease by a statistically significant amount relative to a
10 reference. However, for avoidance of doubt, "reduce," "reduction" or "decrease" or "inhibit" typically means a decrease by at least 10% as compared to a reference level and can include, for example, a decrease by at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about
15 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, up to and including, for example, the complete absence of the given entity or parameter as compared to the reference level, or any decrease between 10-99% as compared to the absence of a given treatment.

The terms "increased", "increase" or "enhance" or "activate" are all used herein to
20 generally mean an increase by a statically significant amount; for the avoidance of any doubt, the terms "increased", "increase" or "enhance" or "activate" means an increase of at least 10% as compared to a reference level, for example an increase of at least about 20%, or at least about 30%, or at least about 40%, or at least about 50%, or at least about 60%, or at least about 70%, or at least about 80%, or at least about 90% or up to and including a 100%
25 increase or any increase between 10-100% as compared to a reference level, or at least about a 2-fold, or at least about a 3-fold, or at least about a 4-fold, or at least about a 5-fold or at least about a 10-fold increase, or any increase between 2-fold and 10-fold or greater as compared to a reference level.

The term "isolated" or "partially purified" as used herein refers, in the case of a
30 nucleic acid or polypeptide, to a nucleic acid or polypeptide separated from at least one other component nucleic acid or polypeptide) that is present with the nucleic acid or polypeptide as found in its natural source and/or that would be present with the nucleic acid or polypeptide when expressed by a cell, or secreted in the case of secreted polypeptides. A chemically synthesized nucleic acid or polypeptide or one synthesized using in vitro
35 transcription/translation is considered "isolated." The terms "purified" or "substantially purified" refer to an isolated nucleic acid or polypeptide that is at least 95% by weight the

5 subject nucleic acid or polypeptide, including, for example, at least 96%, at least 97%, at least 98%, at least 99% or more.

As used herein, the terms "proteins" and "polypeptides" are used interchangeably herein to designate a series of amino acid residues connected to the other by peptide bonds between the alpha-amino and carboxy groups of adjacent residues. The terms "protein", and
10 "polypeptide", which are used interchangeably herein, refer to a polymer of protein amino acids, including modified amino acids (e.g., phosphorylated, glycosylated, etc.) and amino acid analogs, regardless of its size or function. "Protein" and "polypeptide" are often used in reference to relatively large polypeptides, whereas the term "peptide" is often used in reference to small polypeptides, but usage of these terms in the art overlaps. The terms
15 "protein" and "polypeptide" are used interchangeably herein when referring to an encoded gene product and fragments thereof. Thus, exemplary polypeptides or proteins include gene products, naturally occurring proteins, homologs, orthologs, paralogs, fragments and other equivalents, variants, fragments, and analogs of the foregoing.

As used herein the term, "Antibody" includes any immunoglobulin molecule that
20 recognizes and specifically binds to a target, such as a protein, polypeptide, peptide, carbohydrate, polynucleotide, lipid, etc., through at least one antigen recognition site within the variable region of the immunoglobulin molecule. As used herein, the term is used in the broadest sense and encompasses intact polyclonal antibodies, intact monoclonal antibodies, antibody fragments (such as Fab, Fab', F(ab').sub.2, and Fv fragments), single chain Fv
25 (scFv) mutants, multi-specific antibodies such as bispecific antibodies generated from at least two intact antibodies, fusion proteins comprising an antibody portion, and any other modified immunoglobulin molecule comprising an antigen recognition site so long as the antibodies exhibit the desired biological activity. An antibody can be of any the five major classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM, or subclasses (isotypes) thereof (e.g. IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2), based on the identity of their heavy-chain constant
30 domains referred to as alpha, delta, epsilon, gamma, and mu, respectively. The different classes of immunoglobulins have different and well known subunit structures and three-dimensional configurations. Antibodies can be naked or conjugated to other molecules such as cytotoxics, toxins, radioisotopes, etc. Antibodies can be administered by actively
35 producing them *in vivo* or passive administering monoclonal antibodies.

"Polynucleotide," or "nucleic acid," as used interchangeably herein, refer to polymers

5 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.

10 "Antibodies" (Abs) and "immunoglobulins" (Igs) are glycoproteins having the same structural characteristics. While antibodies exhibit binding specificity to a specific antigen, immunoglobulins include both antibodies and other antibody-like molecules which generally lack antigen specificity. Polypeptides of the latter kind are, for example, produced at low levels by the lymph system and at increased levels by myelomas.

15 The terms "antibody" and "immunoglobulin" are used interchangeably in the broadest sense and include monoclonal antibodies (e.g., full length or intact monoclonal antibodies), polyclonal antibodies, monovalent, multivalent antibodies, multispecific antibodies (e.g., bispecific antibodies so long as they exhibit the desired biological activity) and may also include certain antibody fragments (as described in greater detail herein). An antibody can be
20 chimeric, human, humanized and/or affinity matured.

Depending on the amino acid sequences of the constant domains of their heavy chains, antibodies (immunoglobulins) can be assigned to different classes. There are five major classes of immunoglobulins: IgA, IgD, IgE, IgG and IgM, and several of these may be further divided into subclasses (isotypes), e.g., IgG-1, IgG-2, IgA-1, IgA-2, and etc. The
25 heavy chain constant domains that correspond to the different classes of immunoglobulins are called α , δ , ϵ , γ , and μ , and respectively. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known and described generally in, for example, Abbas et al. Cellular and Mol. Immunology, 4th ed. (2000). An antibody may be part of a larger fusion molecule, formed by covalent or non-covalent
30 association of the antibody with one or more other proteins or peptides.

The terms "full length antibody," "intact antibody" and "whole antibody" are used herein interchangeably, to refer to an antibody in its substantially intact form, not antibody fragments as defined below. The terms particularly refer to an antibody with heavy chains that contain the Fc region.

35 "Antibody fragments" comprise only a portion of an intact antibody, wherein the

5 portion preferably retains at least one, preferably most or all, of the functions normally associated with that portion when present in an intact antibody. In one embodiment, an antibody fragment comprises an antigen binding site of the intact antibody and thus retains the ability to bind antigen. In another embodiment, an antibody fragment, for example one that comprises the Fc region, retains at least one of the biological functions normally
10 associated with the Fc region when present in an intact antibody, such as FcRn binding, antibody half-life modulation, ADCC function and complement binding. In one embodiment, an antibody fragment is a monovalent antibody that has an in vivo half-life substantially similar to an intact antibody. For example, such an antibody fragment may comprise an antigen binding arm linked to an Fc sequence capable of conferring in vivo stability to the
15 fragment.

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 comprise essentially identical amino acid sequence except for possible naturally occurring mutations that may be present in minor amounts. Monoclonal
20 antibodies are highly specific, being directed against a single antigen. Furthermore, in contrast to polyclonal antibody preparations that typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen.

The monoclonal antibodies herein specifically include "chimeric" antibodies in which
25 a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so
30 long as they exhibit the desired biological activity (U.S. Pat. No. 4,816,567; and Morrison et al., *Proc. Natl. Acad. Sci. USA* 81:6851-6855 (1984)).

A "human antibody" is one which possesses an amino acid sequence which corresponds to that of an antibody produced by a human and/or has been made using any of the techniques for making human antibodies as disclosed herein. This definition of a human
35 antibody specifically excludes a humanized antibody comprising non-human antigen-binding residues.

5 "Tumor", as used herein, refers to all neoplastic cell growth and proliferation, whether malignant or benign, and all pre-cancerous and cancerous cells and tissues. The terms "cancer", "cancerous", "cell proliferative disorder", "proliferative disorder" and "tumor" are not mutually exclusive as referred to herein.

10 The terms "cancer" and "cancerous" refer to or describe the physiological condition in mammals that is typically characterized by unregulated cell growth/proliferation. Examples of cancer include but are not limited to, carcinoma, lymphoma, blastoma, sarcoma, and leukemia. More particular examples of such cancers include squamous cell cancer, small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung, squamous carcinoma of the lung, cancer of the peritoneum, hepatocellular cancer, gastrointestinal cancer, pancreatic
15 cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, breast cancer, colon cancer, colorectal cancer, endometrial or uterine carcinoma, salivary gland carcinoma, kidney cancer, liver cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma and various types of head and neck cancer.

20 As used herein, "treatment" refers to clinical intervention in an attempt to alter the natural course of the individual or cell being treated, and can be performed either for prophylaxis or during the course of clinical pathology. Desirable effects of treatment include preventing occurrence or recurrence of disease, alleviation of symptoms, diminishment of any direct or indirect pathological consequences of the disease, preventing or decreasing inflammation and/or tissue/organ damage, decreasing the rate of disease progression,
25 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 disorder.

30 A "pharmaceutical excipient" shall mean those commonly utilized within the pharmaceutical art and in particular those found "Handbook of excipients", (Raymond C. Rowe, Paul J. Sheskey, Paul J. Weller-4th Edition, 2003), the contents of which are incorporated in their entirety.

35 A "therapeutically effective amount" of a substance/molecule of the invention 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

5 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 would be less than the therapeutically effective amount.

10 A "chemotherapeutic agent" is a chemical compound useful in the treatment of cancer. Examples of chemotherapeutic agents include alkylating agents such as thiotepa and CYTOXAN® cyclophosphamide; alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, triethylenephosphoramide, triethylenethiophosphoramide and trimethylolomelamine; acetogenins (especially bullatacin and bullatacinone); delta-9-tetrahydrocannabinol (dronabinol, MARINOL®); beta-lapachone; lapachol; colchicines; betulinic acid; a camptothecin (including the synthetic analogue topotecan (HYCAMTIN®), CPT-11 (irinotecan, CAMPTOSAR®), acetylcamptothecin, scoplectin, and 9-aminocamptothecin); bryostatin; callystatin; CC-1065 (including its adozelesin, carzelesin and bizelesin synthetic analogues); podophyllotoxin; podophyllinic acid; teniposide; cryptophycins (particularly cryptophycin 1 and cryptophycin 8); dolastatin; duocarmycin (including the synthetic analogues, KW-2189 and CB1-TM1); eleutherobin; pancratistatin; a sarcodictyin; spongistatin; nitrogen mustards such as chlorambucil, 25 chlornaphazine, cholophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, and ranimustine; antibiotics such as the enediyne antibiotics (e.g., calicheamicin, especially calicheamicin gammall and calicheamicin omegall (see, e.g., Agnew, *Chem Intl. Ed. Engl.*, 33: 183-186 (1994)); dynemicin, including 30 dynemicin A; an espmeramicin; as well as neocarzinostatin chromophore and related chromoprotein enediyne antibiotic chromophores), aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, carabycin, carminomycin, carzinophilin, chromomycinis, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, doxorubicin (including ADRIAMYCIN®, morpholino-doxorubicin, cyanomorpholino-doxorubicin, 2-pyrrolino-doxorubicin, doxorubicin HCl liposome injection (DOXIL®) and deoxydoxorubicin), epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins such as

5 mitomycin C, mycophenolic acid, nogalamycin, olivomycins, peplomycin, potfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites such as methotrexate, gemcitabine (GEMZAR®), tegafur (UFTORAL®), capecitabine (XELODA®), an epothilone, and 5-fluorouracil (5-FU); folic acid analogues such as denopterin, methotrexate, pteropterin, trimetrexate; purine

10 analogs such as fludarabine, 6-mercaptopurine, thiamiprine, thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine; androgens such as calusterone, drom ostanol one propionate, epitiostanol, mepitiothane, testolactone; anti-adrenals such as aminoglutethimide, mitotane, trilostane; folic acid replenisher such as frolinic acid; aceglatone; aldophosphamide

15 glycoside; aminolevulinic acid; eniluracil; amsacrine; bestrabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elfornithine; elliptinium acetate; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidainine; maytansinoids such as maytansine and ansamitocins; mitoguazone; mitoxantrone; mopidanmol; nitraerine; pentostatin; phenamet; pirarubicin; losoxantrone; 2-ethylhydrazide; procarbazine; PSK® polysaccharide complex

20 (JHS Natural Products, Eugene, Oreg.); razoxane; rhizoxin; sizofiran; spirogermanium; tenuazonic acid; triaziquone; 2,2',2''-trichlorotriethylamine; trichothecenes (especially T-2 toxin, verracurin A, roridin A and anguidine); urethan; vindesine (ELDISINE®, FILDESIN®); dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); thiotepa; taxoids, e.g., paclitaxel (TAXOL®), albumin-

25 engineered nanoparticle formulation of paclitaxel (ABRAXANETM), and doxetaxel (TAXOTERE®); chloranbucil; 6-thioguanine; mercaptopurine; methotrexate; platinum analogs such as cisplatin and carboplatin; vinblastine (VELBAN®); platinum; etoposide (VP-16); ifo sfamide ; mitoxantrone; vincristine (ONCOVIN®); oxaliplatin; leucovovin; vinorelbine (NAVELBINE®); novantrone; edatrexate; daunomycin; aminopterin;

30 ibandronate; topoisomerase inhibitor RFS 2000; difluoromethylomithine (DMF0); retinoids such as retinoic acid; pharmaceutically acceptable salts, acids or derivatives of any of the above; as well as combinations of two or more of the above such as CHOP, an abbreviation for a combined therapy of cyclophosphamide, doxorubicin, vincristine, and prednisolone, and FOLFOX, an abbreviation for a treatment regimen with oxaliplatin (ELOXATINTM)

35 combined with 5-FU and leucovovin.

"Patient response" can be assessed using any endpoint indicating a benefit to the patient, including, without limitation, (1) inhibition, to some extent, of disease progression,

5 including slowing down and complete arrest; (2) reduction in the number of disease episodes and/or symptoms; (3) reduction in lesional size; (4) inhibition (i.e., reduction, slowing down or complete stopping) of disease cell infiltration into adjacent peripheral organs and/or tissues; (5) inhibition (i.e. reduction, slowing down or complete stopping) of disease spread; (6) decrease of cell proliferation, invasion or metastasis, which may, but does not have to,
10 result in the regression or ablation of a disease lesion; (7) relief, to some extent, of one or more symptoms associated with the disorder; (8) increase in the length of disease-free presentation following treatment; and/or (9) decreased mortality at a given point of time following treatment.

By "tissue or cell sample" is meant a collection of similar cells obtained from a tissue
15 of a subject or patient. The source of the tissue or cell sample may be solid tissue as from a fresh, frozen and/or preserved organ or tissue sample or biopsy or aspirate; blood or any blood constituents; bodily fluids such as cerebral spinal fluid, amniotic fluid, peritoneal fluid, or interstitial fluid; cells from any time in gestation or development of the subject. The tissue sample may also be primary or cultured cells or cell lines. Optionally, the tissue or cell
20 sample is obtained from a disease tissue/organ. The tissue sample may contain compounds which are not naturally intermixed with the tissue in nature such as preservatives, anticoagulants, buffers, fixatives, nutrients, antibiotics, or the like.

The efficacy of a given treatment for cancer can be determined by the skilled clinician. However, a treatment is considered "effective treatment," as the term is used
25 herein, if any one or all of the signs or symptoms of e.g., a tumor are altered in a beneficial manner or other clinically accepted symptoms are improved, or even ameliorated, e.g., by at least 10% following treatment with an agent as described herein. Efficacy can also be measured by a failure of an individual to worsen as assessed by hospitalization or need for medical interventions (i.e., progression of the disease is
30 halted). Methods of measuring these indicators are known to those of skill in the art and/or described herein. An effective amount for the treatment of a disease means that amount which, when administered to a mammal in need thereof, is sufficient to result in effective treatment as that term is defined herein, for that disease. Efficacy of an agent can be determined by assessing physical indicators of, for example cancer, e.g., tumor
35 size, tumor mass, tumor density, angiogenesis, tumor growth rate, etc. In addition, efficacy of an agent can be measured by a decrease in circulating MIC peptides or

- 5 fragments thereof in a subject being treated with an agent comprising an antibody or antigen-binding portion thereof as described herein or a nucleic acid encoding an antibody or antigen-binding portion thereof as described herein.

Kits or Pharmaceutical Systems

The present compositions may be assembled into kits or pharmaceutical systems for use in ameliorating a neoplasia (e.g., CRC or NSLC). Kits or pharmaceutical systems according to this aspect of the invention comprise a carrier means, such as a box, carton, tube or the like, having in close confinement therein one or more container means, such as vials, tubes, ampoules, bottles and the like. The kits or pharmaceutical systems of the invention may also comprise associated instructions for using the agents of the invention.

15 Kits of the invention include at least one or more alkylating agents (e.g., cisplatin, and at least one or more agents that bind to an EGFR effector kinase, e.g. Trametinib, in combination with an IN01 non-natural synthetic polypeptide and/or an anti-IN01 antibody, thereby reducing the activity of both EGFR and MEK proteins. If desired, the kit also includes an agent that binds to ribonucleotide reductase (RR) and reduces

20 nucleotide synthesis (e.g., gemcitabine). The kit may include instructions for administering the alkylating agent in combination with one or more agents that bind to an EGFR effector kinase, e.g. Trametinib, in combination with an IN01 non-natural synthetic polypeptide (NNSP) and/or an anti-IN01 antibody, reducing the activity of both EGFR and MEK proteins, thereby increasing the efficacy of the alkylating agent relative to the

25 efficacy of the alkylating agent administered alone. Methods for measuring the efficacy of alkylating agents are known in the art and are described herein (e.g., measuring the IC50).

The practice of the present invention employs, unless otherwise indicated, conventional techniques of molecular biology (including recombinant techniques), microbiology, cell biology, biochemistry and immunology, which are well within the purview of the skilled artisan. Such techniques are explained fully in the literature, such as, "Molecular Cloning: A Laboratory Manual", second edition (Sambrook, 1989); "Oligonucleotide Synthesis" (Gait, 1984); "Animal Cell Culture" (Freshney, 1987); "Methods in Enzymology" "Handbook of Experimental Immunology" (Weir, 1996);

35 "Gene Transfer Vectors for Mammalian Cells" (Miller and Calos, 1987); "Current Protocols in Molecular Biology" (Ausubel, 1987); "PCR: The Polymerase Chain

5 **Reaction**”, (Mullis, 1994); “**Current Protocols in Immunology**” (Coligan, 1991). These techniques are applicable to the production of the polynucleotides and polypeptides of the invention, and, as such, may be considered in making and practicing the invention. Particularly useful techniques for particular embodiments will be discussed in the sections that follow.

10 The description of embodiments of the disclosure is not intended to be exhaustive
or to limit the disclosure to the precise form disclosed. While specific embodiments of,
and examples for, the disclosure are described herein for illustrative purposes, various
equivalent modifications are possible within the scope of the disclosure, as those skilled
in the relevant art will recognize. The teachings of the disclosure provided herein can be
15 applied to other procedures or methods as appropriate. The various embodiments described
herein can be combined to provide further embodiments. Aspects of the disclosure can be
modified, if necessary, to employ the compositions, functions and concepts of the above
references and application to provide yet further embodiments of the disclosure. These and
other changes can be made to the disclosure in light of the detailed description.

Specific elements of any of the foregoing embodiments can be combined or substituted for elements in other embodiments. Furthermore, while advantages associated with certain embodiments of the disclosure have been described in the context of these embodiments, other embodiments may also exhibit such advantages, and not all embodiments need necessarily exhibit such advantages to fall within the scope of the disclosure.

25 **EXAMPLES**

The present disclosure is further illustrated by the following examples, which should not be construed as limiting. The contents of all references, GenBank Accession and Gene numbers, and published patents and patent applications cited throughout the application are hereby incorporated by reference. Those skilled in the art will recognize that the disclosure may be practiced with variations on the disclosed structures, materials, compositions and methods, and such variations are regarded as within the scope of the disclosure.

5 Example 1: DLD1 (G13D KRAS) and LS174T (G13D KRAS) KRAS mutant cell lines response to anti-IN01 antibody NTKI combination therapies

The anti-IN01 (BVN22E) antibody was developed from SEQ ID NO: 2, which consists of two repeated synthetic EGF Targeted Signaling Pathway (SEQ ID NO: 7) domains, Glycine-Serine repeat linkers, and a CTB sequence (SEQ ID NO: 25[[9]]). The
 10 anti-IN01 antibody was developed to be used in combination with non-tyrosine targeting kinase inhibitors targeting EGFR downstream effectors in order to inhibit cancer cell growth. In this example, KRAS mutation carrying cell lines DLD1, which carries a G13D mutation in KRAS and an E545K mutation in PIK3CA, and LS174T which carries a G12D mutation in KRAS and a mutation in PIK3CA were used (FIGs. 1 and 43).

15 FIGs. 2-6 show the effects of the anti-IN01 antibody in combination with Trametinib in DLD1 cells. Phosphorylation of EGFR is strongly inhibited by the anti-IN01 antibody. The combination of anti-IN01 antibody with Trametinib enhances the effect of Trametinib alone, in that there is significant effect on pEGFR and pERK1/2 activation (FIG. 6). The combined therapy also has a pronounced effect on pSTAT3 and pAKT (FIG. 6). FIGs. 29 and 49 show
 20 that over time resistance to Trametinib develops, but is lessened by the anti-IN01 antibody. FIG. 45 shows that DLD1 cells demonstrate similar cell signaling responses to Taselisib and Trametinib. FIG. 46 shows that the anti-IN01 antibody greatly decreases cell viability of DLD1 cells in combination with either Trametinib or Taselisib.

FIGs. 33, 34, 47, and 48 show that the number of DLD1 cells in S and G2/M phases
 25 was lower with anti-IN01 and Trametinib combination therapy, and the number of DLD1 cells in apoptosis was higher, as assessed by flow cytometry (FIG. 30).

FIGs. 7-11 show the effects of the anti-IN01 antibody in combination with Trametinib
 I n LS174T cells. Compared to the effects on cell viability in DLD1 cells, the
 30 combinatorial therapy is less pronounced in the LS174T cells. However, the activation of pAkt, pEGFR, and pErk1/2 are almost completely inhibited in LS174T cells (FIG. 10). Notably, as shown in FIG. 53, Trametinib resistance is significantly delayed when the anti-IN01 antibody is co-administered in LS174T cell lines.

Notably, the anti-IN01 antibody consistently reverses the stimulatory effect of EGF on these KRAS mutant cell lines. As shown EGF stimulates KRAS cell line growth, while

5 the anti-IN01 antibody, in combination with Trametinib or Taselisib, inhibits EGF induced cell growth and delays resistance to the non-tyrosine targeting kinase inhibitors.

Example 2: A549 (G12S KRAS) and HCC15 (Q61K NRAS) Ras mutant cell lines response to anti-IN01 antibody NTKI combination therapies

The anti-IN01 (BVN22E) antibody was developed from SEQ ID NO: 2, which
 10 consists of two repeated synthetic EGF Targeted Signaling Pathway (SEQ ID NO: 7) domains, Glycine-Serine repeat linkers, and a CTB sequence (SEQ ID NO: 25[[9]]). The anti-IN01 antibody was developed to be used in combination with non-tyrosine targeting kinase inhibitors targeting EGFR downstream effectors in order to inhibit cancer cell growth. In this example, the A549 cell line, which carries a KRAS G12S mutation, and the
 15 HCC15 cell line, which carries a Q61K mutation in NRAS were used.

FIGs. 31 and 32 show that the number of A549 cells in S and G2/M phases was lower with anti-IN01 and Trametinib combination therapy, and the number of A549 cells in apoptosis was higher, as assessed by flow cytometry. FIGs. 66, 68, 72, and 73 show the effects of Trametinib alone, and in combination with anti-IN01 antibodies in HCC15 cells,
 20 wherein anti-IN01 antibodies enhance the effect of Trametinib in lowering cell viability.

Example 3: HT29 (V600E BRAF) and H508 (G596R BRAF and E545K PIK3CA) mutant cell lines response to anti-IN01 antibody NTKI combination therapies

The anti-IN01 (BVN22E) antibody was developed from SEQ ID NO: 2, which consists of two repeated synthetic EGF Targeted Signaling Pathway (SEQ ID NO: 7)
 25 domains, Glycine-Serine repeat linkers, and a CTB sequence (SEQ ID NO: 25[[9]]). The anti-IN01 antibody was developed to be used in combination with non-tyrosine targeting kinase inhibitors targeting EGFR downstream effectors in order to inhibit cancer cell growth. In this example, HT29 and H508 cells which carry BRAF and PIK3CA mutations were used (FIGs. 43, 63, and 74).

30 FIGs. 12, 13, and 54-56 show the decreased cell viability of HT29 cells with Trametinib or Encorafenib when used in combination with the anti-IN01 antibody. FIGs. 14-22, 57-62, 72, 73, and 75-77 show the decreased cell viability of H508 and HT29 cells with Trametinib, Taselisib, Alpelisib, and Copanlisib with the anti-IN01 antibody. FIGs. 18 and 19 notably demonstrate that effective inhibition of cell viability by Taselsib in combination with

5 the anti-IN01 antibody was achieved at concentrations below that of current patient therapies. FIG. 14 also shows that the anti-IN01 antibody in combination with Trametinib strongly enhanced the inhibition of pEGFR and pErk1/2 activation over Trametinib alone in HT29 cells.

FIG. 20 shows that Alpelisib and Copanlisib, which are PIK3CA inhibitors, inhibit
10 pAkt, but do not affect pEGFR or pErk1/2 signaling. However, FIG. 21 shows that these inhibitors exhibit effects on cell viability in combination with the anti-IN01 antibody similar to that of Taselisib. Similarly, the anti-IN01 antibody had a greater effect on H508 cell viability than Alpelisib or Copanlisib alone at low concentrations (FIG. 22). These results indicate that Taselisib provides more targeted and likely more effective tumor growth
15 inhibition.

Taselisib targets PIK3CA, Encorafenib targets BRAF, while Trametinib is a MEK inhibitor. The similar effects of PIK3CA, BRAF, and KRAS inhibitors in achieving decreased cell proliferation and EGFR effector signaling, particularly in combination with the anti-IN01 antibody, indicate that targeting multiple pathways downstream of EGFR
20 demonstrates more therapeutic options and greater efficacy in treating cancer.

Example 4: PC9/PC9GR4 (exon 19 deletion in EGFR) mutant cell lines response to anti-IN01 antibody TKI combination therapies

The anti-IN01 (BVN22E) antibody was developed from SEQ ID NO: 2, which consists of two repeated synthetic EGF Targeted Signaling Pathway (SEQ ID NO: 7)
25 domains, Glycine-Serine repeat linkers, and a CTB sequence (SEQ ID NO: 25[[9]]). The anti-IN01 antibody was developed to be used in combination with tyrosine targeting kinase inhibitors targeting EGFR in order to inhibit cancer cell growth. In this example, PC9/PC9GR cells were used.

FIGs. 27 and 28 show that emergence of resistance to Osimertinib and Dacomitinib
30 was lowered when the anti-IN01 antibody was co-administered to the cells.

5 **Example 5: H228 and H3122 EML4-ALK translocation mutant cell lines response to anti-IN01 antibody TKI combination therapies**

The anti-IN01 (BVN22E) antibody was developed from SEQ ID NO: 2, which consists of two repeated synthetic EGF Targeted Signaling Pathway (SEQ ID NO: 7) domains, Glycine-Serine repeat linkers, and a CTB sequence (SEQ ID NO: 25[[9]]). The
10 anti-IN01 antibody was developed to be used in combination with tyrosine targeting kinase inhibitor Brigatinib targeting EGFR in order to inhibit cancer cell growth. In this example, the H228 and H3122 cells were used.

FIGs. 35 and 36 show the decrease in G2/M phase H2228 and H3122 cells, respectively, in response to Brigatinib in combination with the anti-IN01 antibody, as
15 assessed by flow cytometry.

FIG. 42 shows the signaling response to patient titers of anti-IN01 antibody in H228 cells.

Example 6: LC-2/ad (RET translocation) mutant cell line response to anti-IN01 antibody/ TKI combination therapies

20 The RET proto-oncogene gain of function is associated the development of various types of human cancer, including medullary thyroid carcinoma, multiple endocrine neoplasias type 2A and 2B, pheochromocytoma and parathyroid hyperplasia. RET is phosphorylated downstream of EGFR with EGF induction. Although EGF stimulates RET cell line growth, the anti-IN01 antibody significantly enhanced BLU667 inhibition of this
25 effect in LC-2/ad (RET translocated) cells (FIGs. 78-81).

Example 7: SW900 wt KRAS/wt ALK cell line response to anti-IN01 antibody patient titers

FIGs. 38-41 show the signaling response of SW900 cells to different patient titers of the anti-IN01 antibody, demonstrating that active immunization using the anti-IN01 peptide
30 (SEQ ID NO: 2) has significant effects on EGFR and downstream signaling, known to lead to decreased cell division and viability.

5 **Example 8: Anti-epidermal growth factor vaccine antibodies increase the antitumor activity of kinase inhibitors (TKIs) in non- small cell lung cancer cell lines**

Advanced NSCLC patients harboring *MET* amplification and *MET* exon 14 deletion (*MET* Δ ex14) derived benefit from treatment with MET tyrosine kinase inhibitors (TKIs). In patients with alterations in *RAS* family genes, no targeted therapy was yet approved and trials
10 of MEK inhibitors, such as Trametinib, in NSCLC failed to show clinical benefit. Herein, antibodies generated by vaccination (anti-EGF antibodies) potentiated the effects of EGFR TKIs in epidermal growth factor receptor mutant (*EGFR*-mut), *ALK* and *RET* translocated cells growing *in vitro* and prevent the emergence of resistance to EGFR TKIs. The goal was to determine the effects of anti-EGF VacAbs, MET TKIs, MEK inhibitors and combinations in
15 cell lines harboring *MET* and *NRAS* alterations.

Anti-EGF VacAbs were generated in rabbits, purified and titrated. Cell lines were treated with anti-EGF VacAbs alone and in combination with MET and MEK inhibitors in *MET* amplified, *MET* Δ ex14 and *NRAS* mutant NSCLC cell lines (EBC-1, Hs746T and HCC15). Cell viability and long-term growth curves were determined by MTT, changes of total and
20 phosphorylated proteins by Western blot, cell cycle and apoptotic effects by flow cytometry and emergence of resistance by direct microscopic examination in low density cultures.

Anti-EGF antibodies suppressed EGF and TGF α -induced cell proliferation and inhibited EGFR phosphorylation and downstream ERK signaling in all cell lines tested. In combination, the anti-EGF antibodies significantly enhanced the antitumor activity of
25 Capmatinib and Tepotinib in EBC and Hs746T and Trametinib in HCC15 cells. In these cell lines, western blotting demonstrated that the combination of anti-EGF antibodies with the inhibitors tested effectively suppressed EGFR downstream pathways. Cell cycle experiments were performed to further investigate these effects. The addition of EGF significantly increased the number of cells in S+G2/M, as expected, while anti-EGF antibodies and the inhibitors
30 reversed this stimulation.

Example 9: Anti-IN01 antibody in combination with TKI inhibitors Tepotinib and Capmatinib in MET amplified and METex14 cell lines

The anti-IN01 (BVN22E) antibody was developed from SEQ ID NO: 2, which consists
35 of two repeated synthetic EGF Targeted Signaling Pathway (SEQ ID NO: 7) domains, Glycine-

5 Serine repeat linkers, and a CTB sequence (SEQ ID NO: 25[[9]]). In this example, EBC1 and Hs746T cell lines, which carry *MET* amplifications and *MET* exon 14 deletions were used (FIG. 82).

EBC1 and Hs746T short term cell viability was tested in the presence of (FIGs. 83- 88) EGF, the anti-IN01 antibody, "Ab," and different concentrations of Capmatinib and Tepotinib.
10 The effects of different concentrations of Tepotinib on pMET, pEGFR, pAkt, and pErk1/2 activation in EBC1 cells and Tepotinib on pMET signaling in EBC1 cells were also observed (FIGs. 85 and 87).

The long-term effects on EBC1 cell viability of incubation with 0.75 nM and 0.25 nM Tepotinib, EGF, and the anti-IN01 antibody were also observed in EBC1 cells.

15 The long-term effects on EBC1 cell viability of incubation with 2.0 nM and 0.5 nM Tepotinib, EGF, and the anti-IN01 antibody were observed in Hs746T cells.

In summary, the anti-IN01 antibody which inhibits activation of the epidermal growth factor receptor (EGFR) was shown to significantly enhance the effects of NTKIs targeting EGFR effector proteins, including but not limited to Trametinib, Taselisib, Alpelisib,
20 Copanlisib, and Erlotinib by reducing cell signaling downstream of EGFR that regulates tumor cell growth and viability, and notably, delaying the onset of resistance to the NTKI compared to the NTKI alone. Patient therapies derived from the IN01 non-natural synthetic polypeptide (NNSP), and/or antibody thereof, in combination with NTKIs, thus have the potential to diminish or eradicate previously untreatable cancers. It is further contemplated that antibodies
25 to other growth factors and their receptors, used in combination with non-tyrosine targeting kinase inhibitors, would similarly enhance the effect of the NTKI and delay the onset of resistance.

INCORPORATION BY REFERENCE

All documents cited or referenced herein and all documents cited or referenced in the
30 herein cited documents, together with any manufacturer's instructions, descriptions, product specifications, and product sheets for any products mentioned herein or in any document incorporated by reference herein, are hereby incorporated by reference, and may be employed in the practice of the disclosure.

5

EQUIVALENTS

It is understood that the detailed examples and embodiments described herein are given by way of example for illustrative purposes only, and are in no way considered to be limiting to the disclosure. Various modifications or changes in light thereof will be suggested to persons skilled in the art and are included within the spirit and purview of this application and are considered within the scope of the appended claims. Additional advantageous features and functionalities associated with the systems, methods, and processes of the present disclosure will be apparent from the appended claims. Moreover, those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the disclosure described herein. Such equivalents are intended to be encompassed by the following claims.

5

CLAIMS

We claim:

1. A method of treating cancer in a subject, comprising administering to the subject an active regimen of a non-tyrosine targeting kinase inhibitor (NTKI) and a non-natural synthetic polypeptide (NNSP), comprising a full length, or fragment thereof, of a growth factor or growth factor receptor, an immunogenic polypeptide, and a linker, wherein the polypeptide is separated from the immunogenic polypeptide by the linker.
2. A method of treating cancer in a subject, comprising administering to the subject an active regimen of a non-tyrosine targeting kinase inhibitor (NTKI) and an antibody directed to a growth factor or growth factor receptor.
3. A method of treating non-small cell lung carcinoma (NSCLC) or colorectal cancer (CRC) in a subject, comprising administering to the subject a non-tyrosine targeting kinase inhibitor (NTKI) and a non-natural synthetic polypeptide (NNSP), comprising a full length, or fragment thereof, of a growth factor or growth factor receptor, an immunogenic polypeptide, and a linker, wherein the polypeptide is separated from the immunogenic polypeptide by the linker.
4. A method of treating non-small cell lung carcinoma (NSCLC) or colorectal cancer (CRC) in a subject, comprising administering to the subject a non-tyrosine targeting kinase inhibitor (NTKI) and an antibody directed to a growth factor or growth factor receptor.
5. The method of any one of claims 1-4, wherein the growth factor is EGF.
6. The method of any one of claims 1-4, wherein the growth factor receptor is EGFR.
7. The method of claim 1 or 3, wherein the immunogenic polypeptide comprises a full length, or portion thereof, of a cholera toxin B (CT-B) protein.
8. The method of claim 1 or 3, wherein the NNSP comprises SEQ ID NO: 7 or SEQ ID NO: 8, or wherein the NNSP is derived from a cell that expresses SEQ ID NO: 7 or SEQ ID NO: 8.
9. The method of claim 1 or 3, wherein the NNSP comprises SEQ ID NO: 2 or SEQ ID NO: 6, or wherein the NNSP is derived from a cell expressing SEQ ID NO: 2 or SEQ ID NO: 6.

- 5 10. The method of claim 2 or 4, wherein the antibody is derived from a hybridoma cell.
11. The method of any one of claims 1-4, wherein the NTKI is Trametinib, Taselisib, Alpelisib, Copanlisib, Idealisib, Duvelisib, Buparlisib, Umbralisib, PX-866, Dactolisib, CUDC-907, Voxtalib (SAR245409, XL765) ME-401, IPI-549, SF1126, INK1117 Pictilisib, XL147 (SAR245408), GSK1059615, ZSTK474, PWT33597, IC87114, TG100-115, CAL
10 263, RP6503, GNE-477 AEZS-136, Encorafenib, Vemurafenib, Dabrafenib, GDC-0879, PLX-4720, Cobimetinib, Binimetinib, Selumetinib, PD-325901, PD035901, CI-1040, TAK-733, Perifosine, Palomid 529 or pharmaceutically acceptable salts thereof.
12. The method of claim 5, wherein the EGF is human EGF or murine EGF.
13. The method of claim 1 or 3, wherein the linker is selected from the group consisting of
15 SSG, GSSG (SEQ ID NO: 9), SSGGG (SEQ ID NO: 10), SGG, GGSGG (SEQ ID NO: 11), GGGGS (SEQ ID NO: 12), SSGGGSGG (SEQ ID NO: 13), SSGGGGSGGG (SEQ ID NO: 14), TSGGGSG (SEQ ID NO: 15), TSGGGGSGG (SEQ ID NO: 16), SSGGSGGGSG (SEQ ID NO: 17), SSGGGSGGSSG (SEQ ID NO: 18), GGSGGTSGGGSG (SEQ ID NO: 19), SGGTSGGGGSGG (SEQ ID NO: 20), GGSGGTSGGGGSGG (SEQ ID NO: 21),
20 SSGGGGSGGGSSG (SEQ ID NO: 22), SSGGGSGGSSGGG (SEQ ID NO: 23), and SSGGGGSGGGSSGGG (SEQ ID NO: 24).
14. The method of claim 1 or 3, wherein the linker is GSSG (SEQ ID NO: 9) or GGSGGTSGGGGSGG (SEQ ID NO: 21).
15. The method of claim 1 or 3, wherein the NNSP includes a full length, or fragment
25 thereof, of at least two different growth factors or two different growth factor receptors.
16. The method of claim 1 or 2, wherein the active regimen is administered to the subject according to a therapeutically effective amount repeated thrice, twice or once a week, once in two weeks, once in three weeks or at least once monthly, in combination with the administration of a NTKI in a continuous regimen based on an average daily dose in the
30 range of 10 to 400 mg, wherein the method prevents acquiring resistance to the NTKI treatment.
17. The method of claim 16, wherein the average daily dose is selected from the group consisting of 10 mg, 20 mg, 30 mg, 40 mg, 50 mg, 60 mg, 70 mg, 80 mg, 90 mg, 100 mg, 110 mg, 120 mg, 130 mg, 140 mg, 150 mg, 160 mg, 170 mg, 180 mg, 190 mg, 200 mg, 210

5 mg, 220 mg, 230 mg, 240 mg, 250 mg, 260 mg, 270 mg, 280 mg, 290 mg, 300 mg, 310 mg, 320 mg, 330 mg, 340 mg, 350 mg, 360 mg, 370 mg, 380 mg, 390 mg, and 400 mg.

18. The method of claim 1 or 2, wherein the active regimen is administered to the subject according to a therapeutically effective amount repeated thrice, twice or once a week, once in two weeks, once in three weeks or at least once monthly, prior to administration of a NTKI in
10 a continuous regimen based on an average daily dose in the range of 10 to 400 mg, wherein the method prevents acquiring resistance to the NTKI treatment.

19. The method of claim 1 or 2, wherein the active regimen is administered to the subject according to a therapeutically effective amount repeated thrice, twice or once a week, once in two weeks, once in three weeks or at least once monthly, after administration of a NTKI in a
15 continuous regimen based on an average daily dose in the range of 10 to 400 mg, wherein the method prevents acquiring resistance to the NTKI treatment.

20. The method of claim 1 or 3, wherein the NNSP produces pathway immunization that results in inhibition of an EGF/EGFR pathway.

21. The method of claim 2 or 4, wherein the antibody produces pathway immunization that
20 results in inhibition of an EGF/EGFR pathway.

22. The method of any one of claims 1-4, wherein the subject expresses mutated forms of HRAS, NRAS, KRAS, PIK3CA, BRAF, and/or MEK proteins.

23. The method of any one of claims 1-4, wherein the NTKI targets HRAS, NRAS, KRAS, PIK3CA, BRAF, and/or MEK proteins.

25 24. The method of any one of claims 1-4, wherein the subject has acquired resistance to NTKI treatment and the method overcomes resistance to NTKI treatment.

25. The method of any one of claims 1-4, wherein the cancer is associated with a mutant form of HRAS, NRAS, KRAS, PIK3CA, BRAF, and/or MEK proteins.

26. The method of any one of claims 1-4, further comprising the steps of:

30 obtaining a tissue sample from the subject;

identifying one or more mutations in the tissue sample from the subject; and

5 conducting the administering steps if the one or more mutations are identified as being in one or more growth factor receptor effector proteins.

27. The method of claim 26, wherein the one or more effector proteins is HRAS, NRAS, KRAS, PIK3CA, BRAF, and/or MEK proteins.

28. The method of claim 27, wherein the one or more mutations is selected from the
10 following: KRAS G12S, KRAS G12C, KRAS G13D, KRAS G12D, EML4-ALK (V3; E6:A20), BRAF V600E, BRAF G596R, PIK3CA E545K, and NRAS Q61K.

29. The method of treating a subject of claim 5, wherein the EGF is human EGF or murine EGF.

15 30. The method of claim 2 or 4, wherein the antibody is administered at about 0.01-0.1 µg/kg to about 95 mg/kg body weight; or from about 0.1-1.0 µg/kg to about 90 mg/kg body weight; or from about 0.5-5.0 µg/kg to about 80 mg/kg body weight; or from about 1.0-10.0 µg/kg to about 60 mg/kg body weight; or from about 5.0-20.0 µg/kg to about 50 mg/kg body weight; or from about 20-50 µg/kg to about 25 mg/kg body weight; or from about 50-100 µg/kg to
20 about 20 mg/kg body weight. In other embodiments this dose may be about 1, 5, 10, 25, 50, 75, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1050, 1100, 1150, 1200, 1250, 1300, 1350, 1400, 1450, 1500, 1600, 1700, 1800, 1900, 2000, 2500, 3000, 3500, 4000, 4500, or 5000 µg/kg body weight.

25

30

Cell lines	Origin	KRAS mutation
A549	Lung Adenocarcinoma NSCLC	G12S
H29	Lung Adenocarcinoma NSCLC	G12C
DLD1	Colon Adenocarcinoma	G13D, also PIK3CA mut and P53
LS174T	Colon Adenocarcinoma	G12D, also PIK3CA mut

FIG. 1

DLD1 x C-Ab x Ab - 2 h

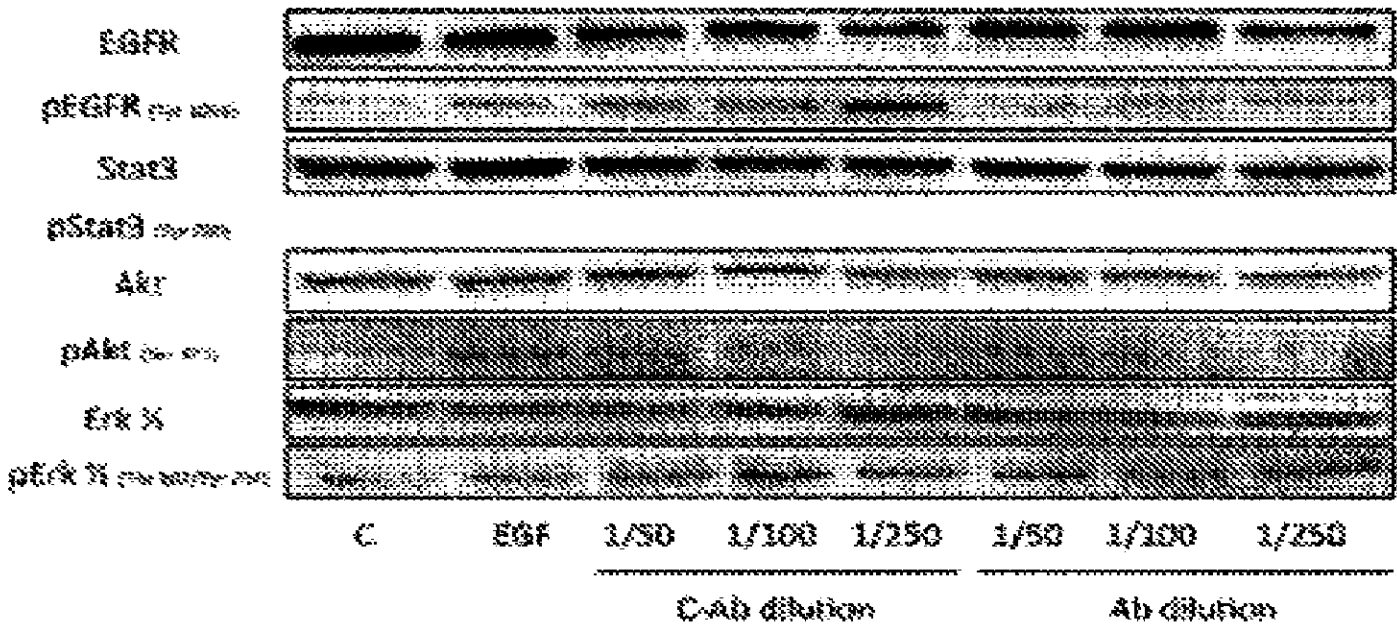


FIG. 2

EGF effects in trametinib activity in DLD1 cell line

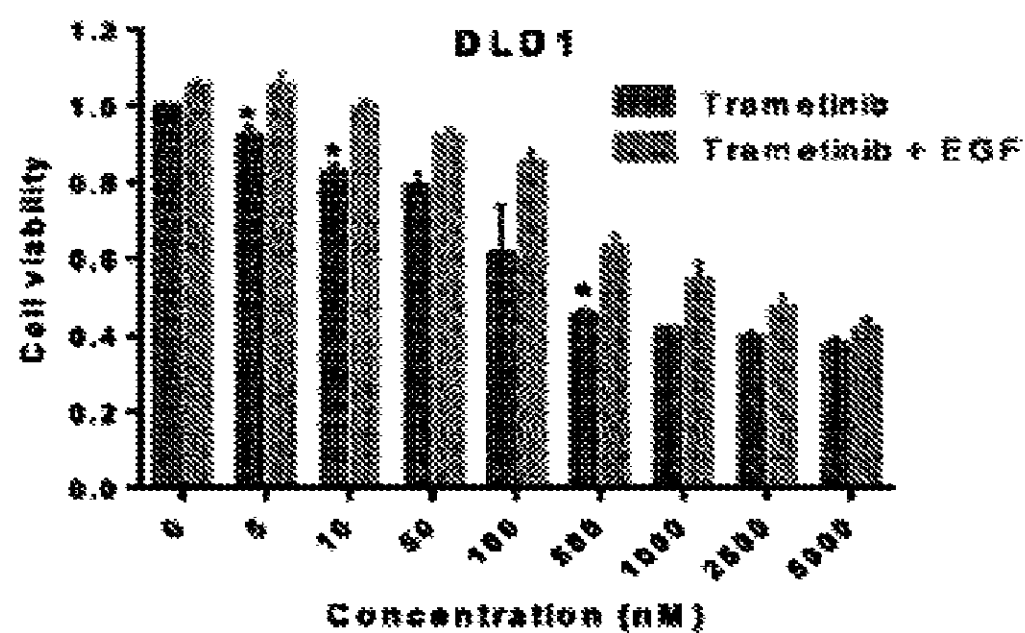


FIG. 3

DLD1 x Trametinib - 2 h

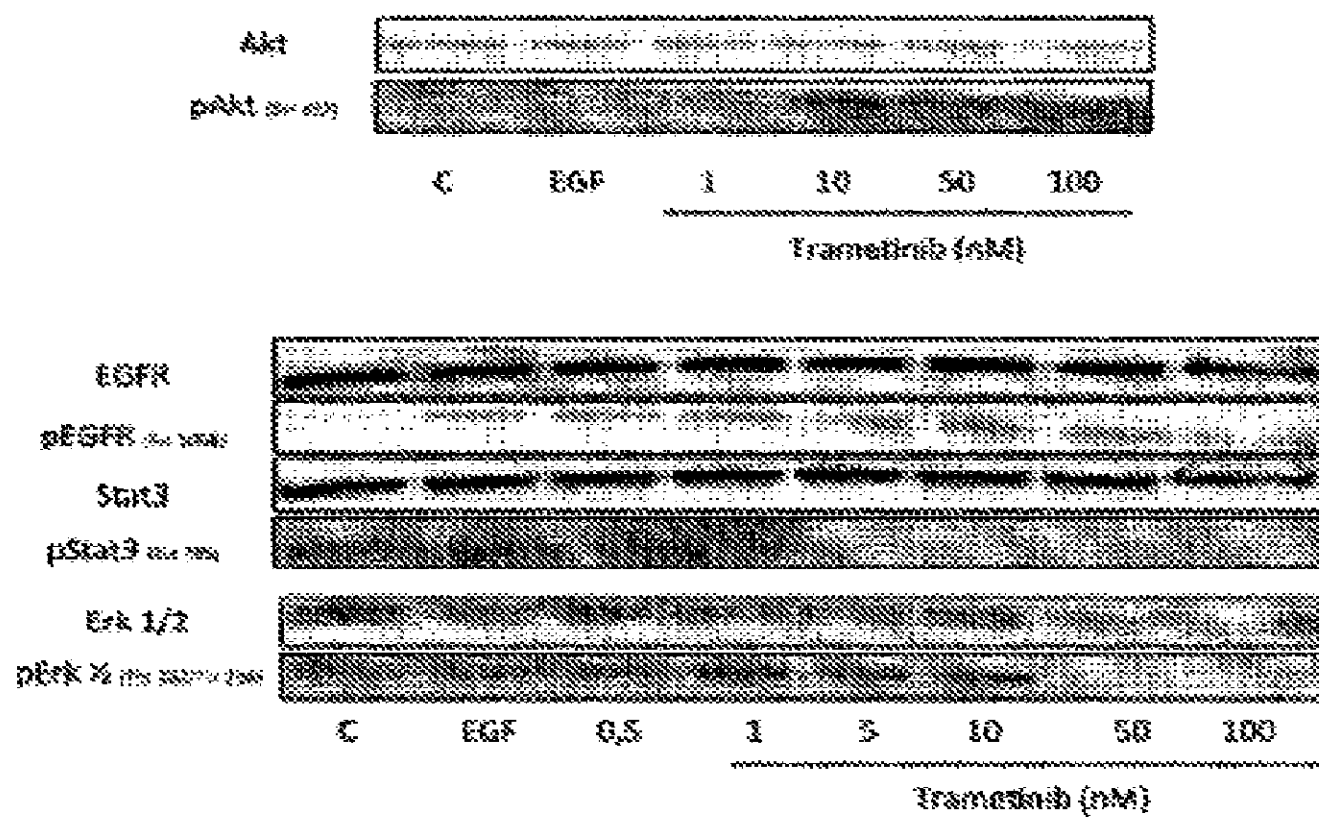


FIG. 4

Ab and trametinib combination in DLD1 cell line

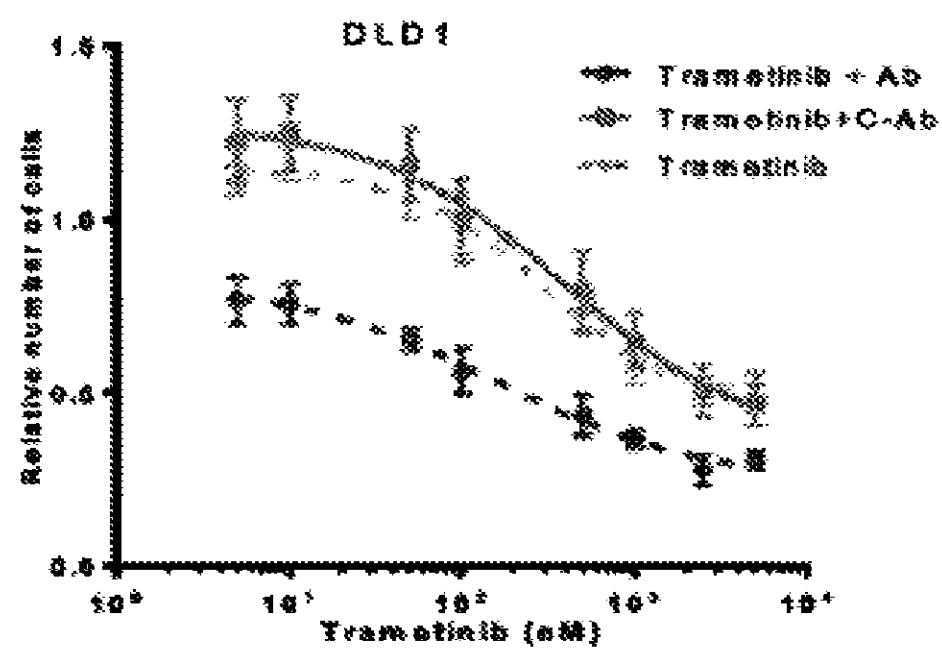


FIG. 5A

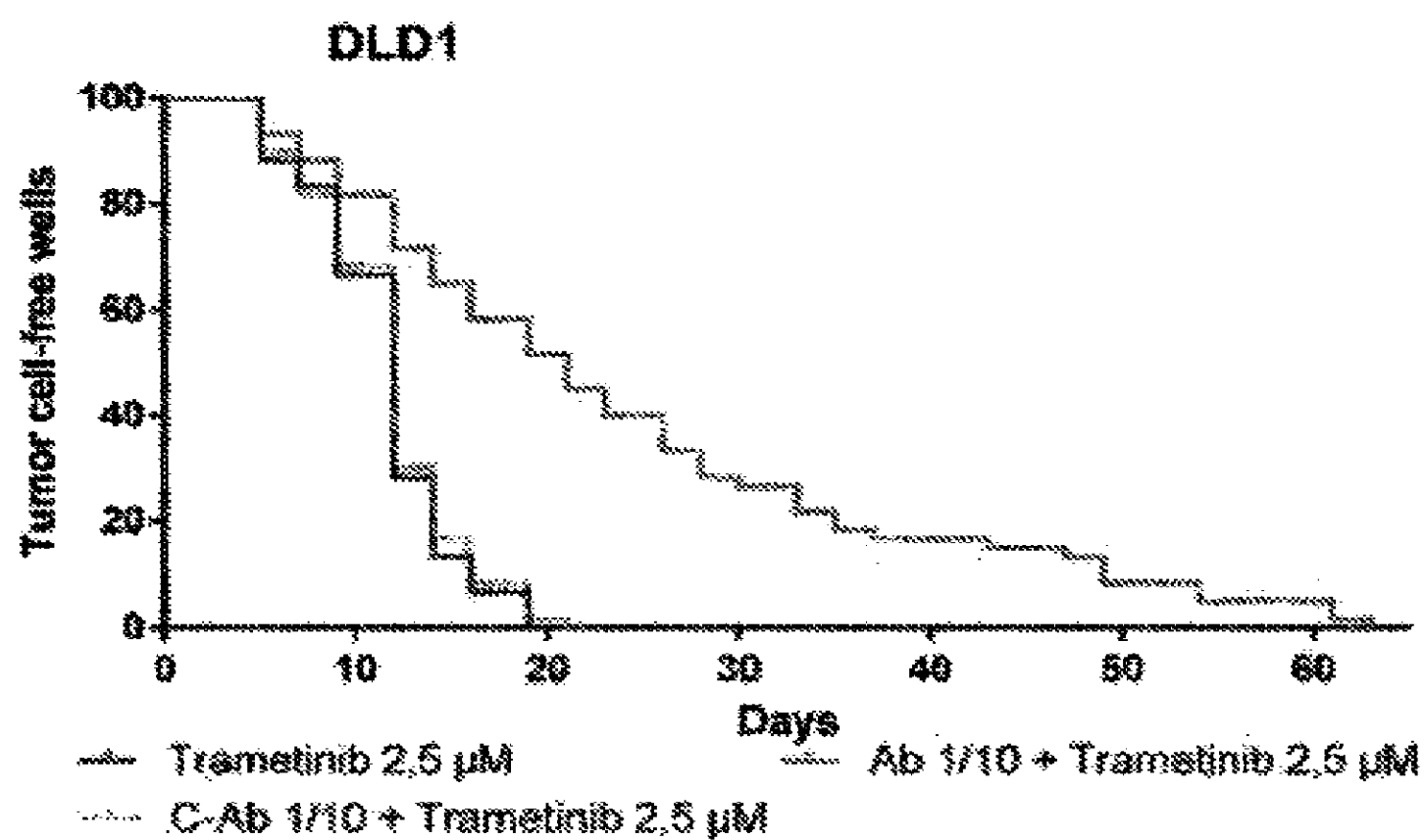


FIG. 5B

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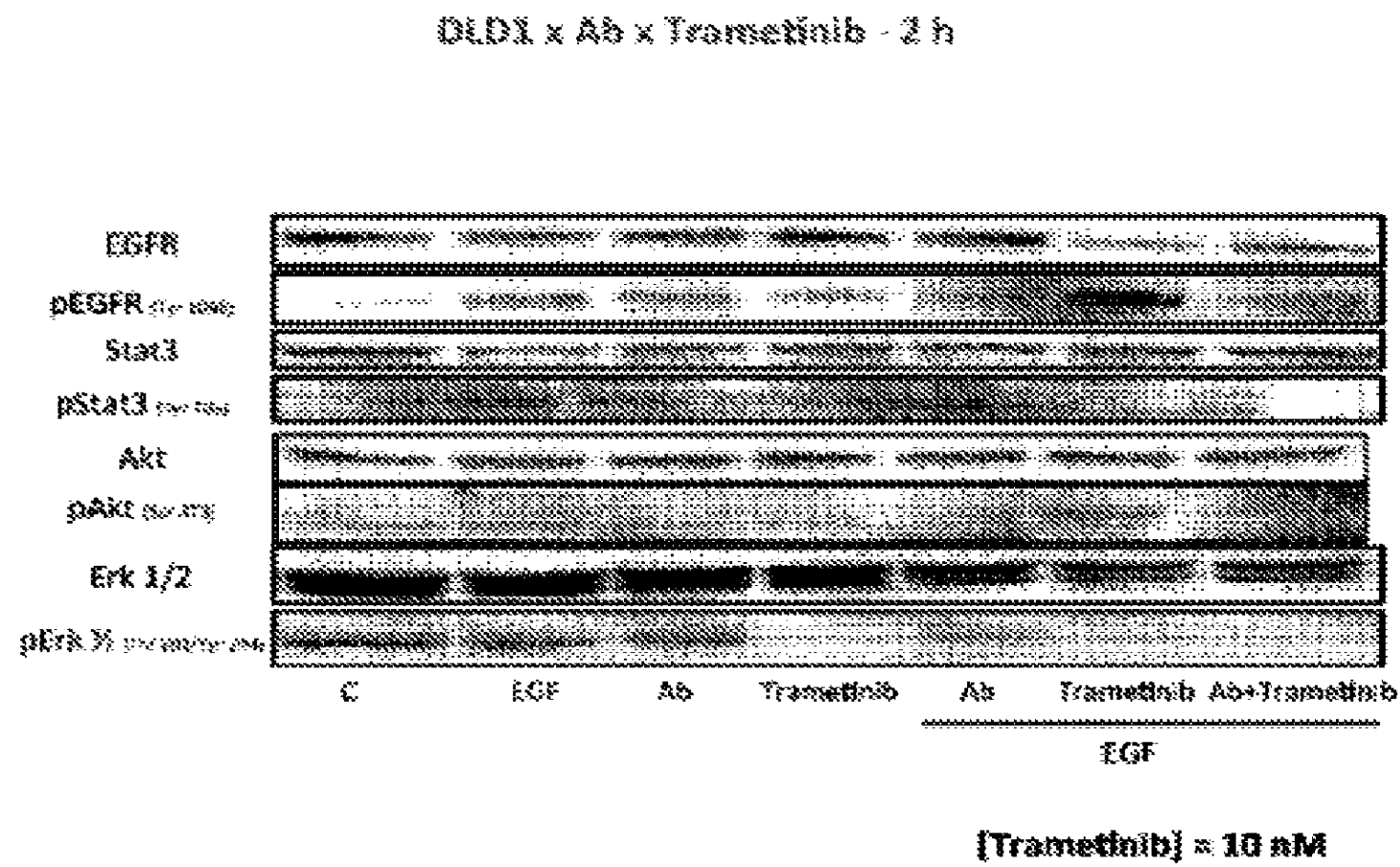


FIG. 6

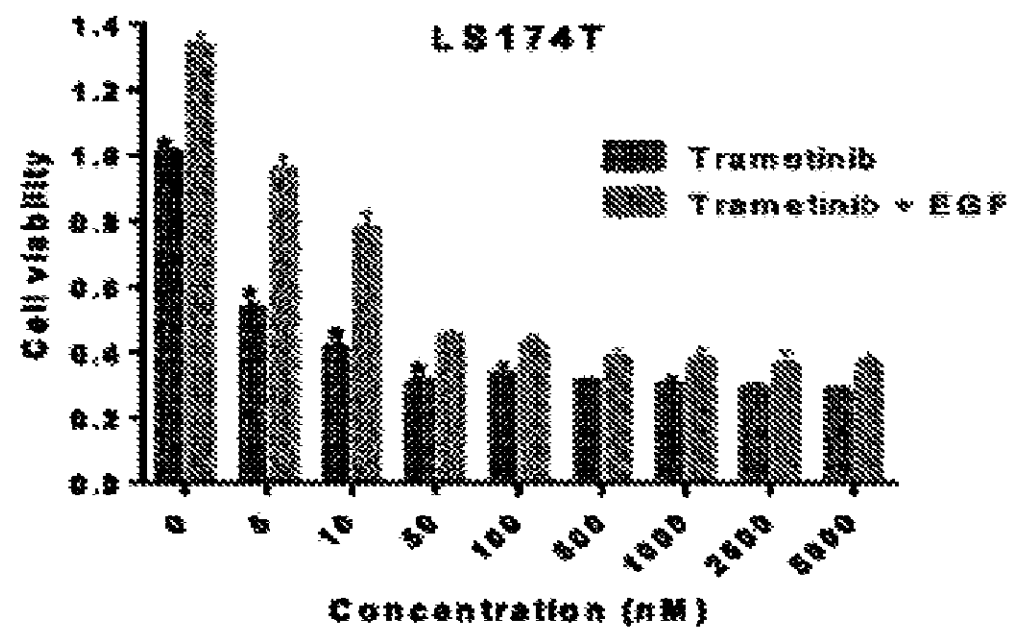


FIG. 7

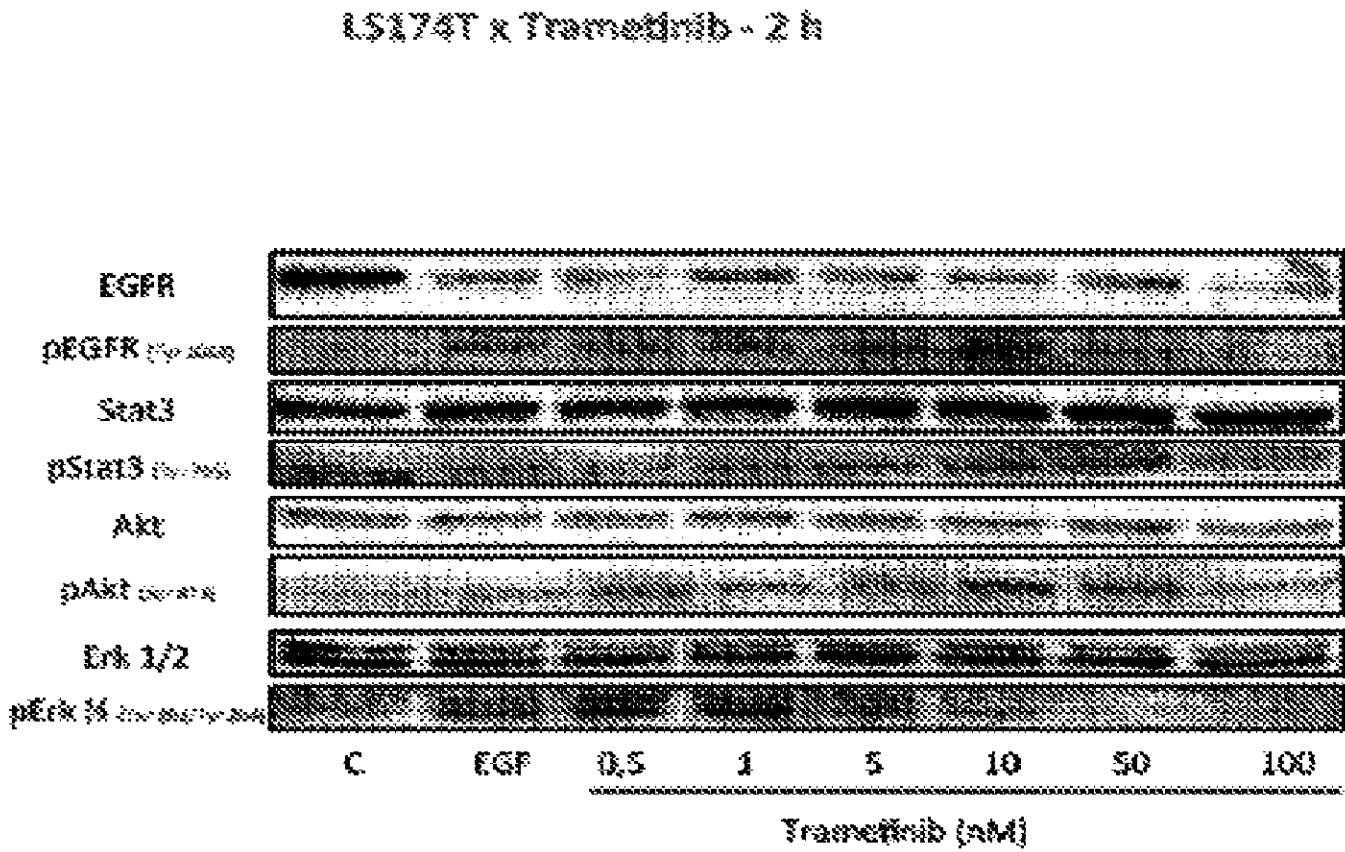


FIG. 8

Ab and trametinib combination in LS174T cell line

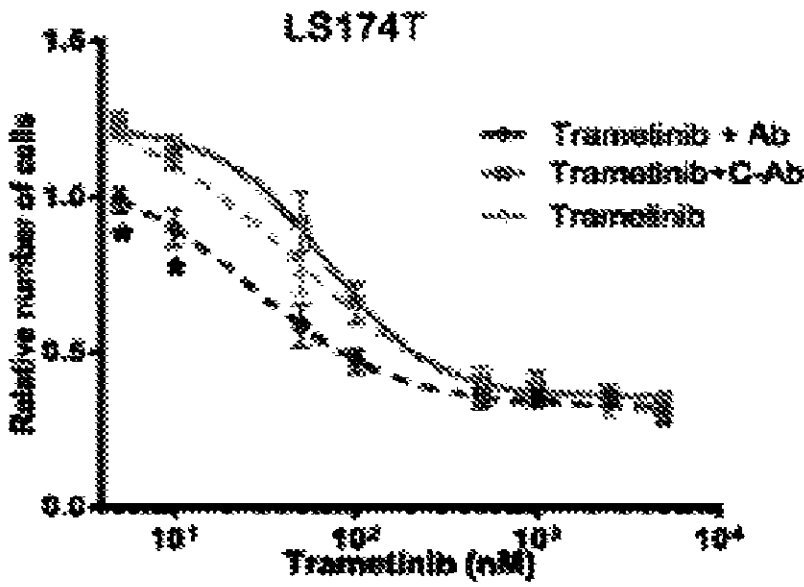


FIG. 9

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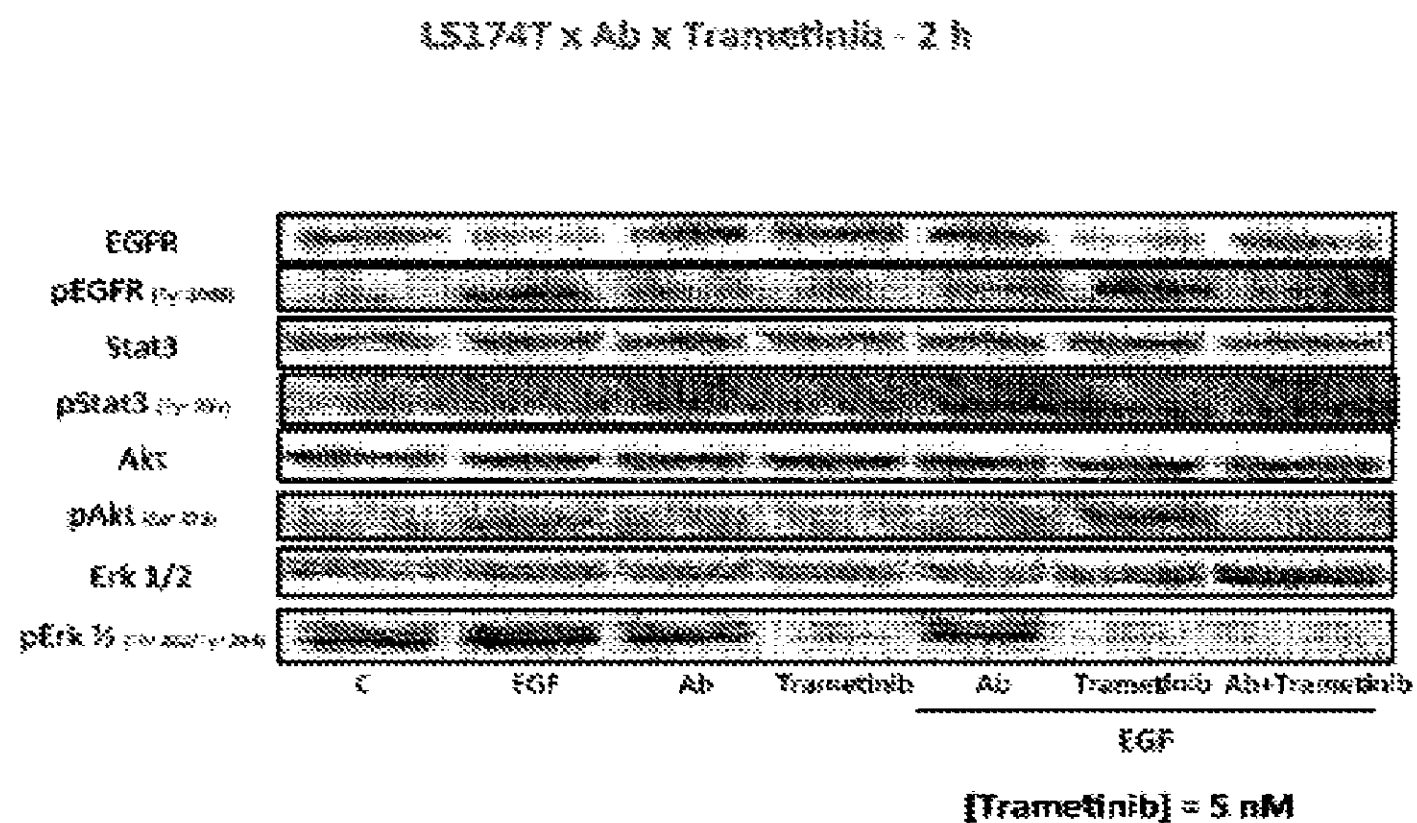


FIG. 10

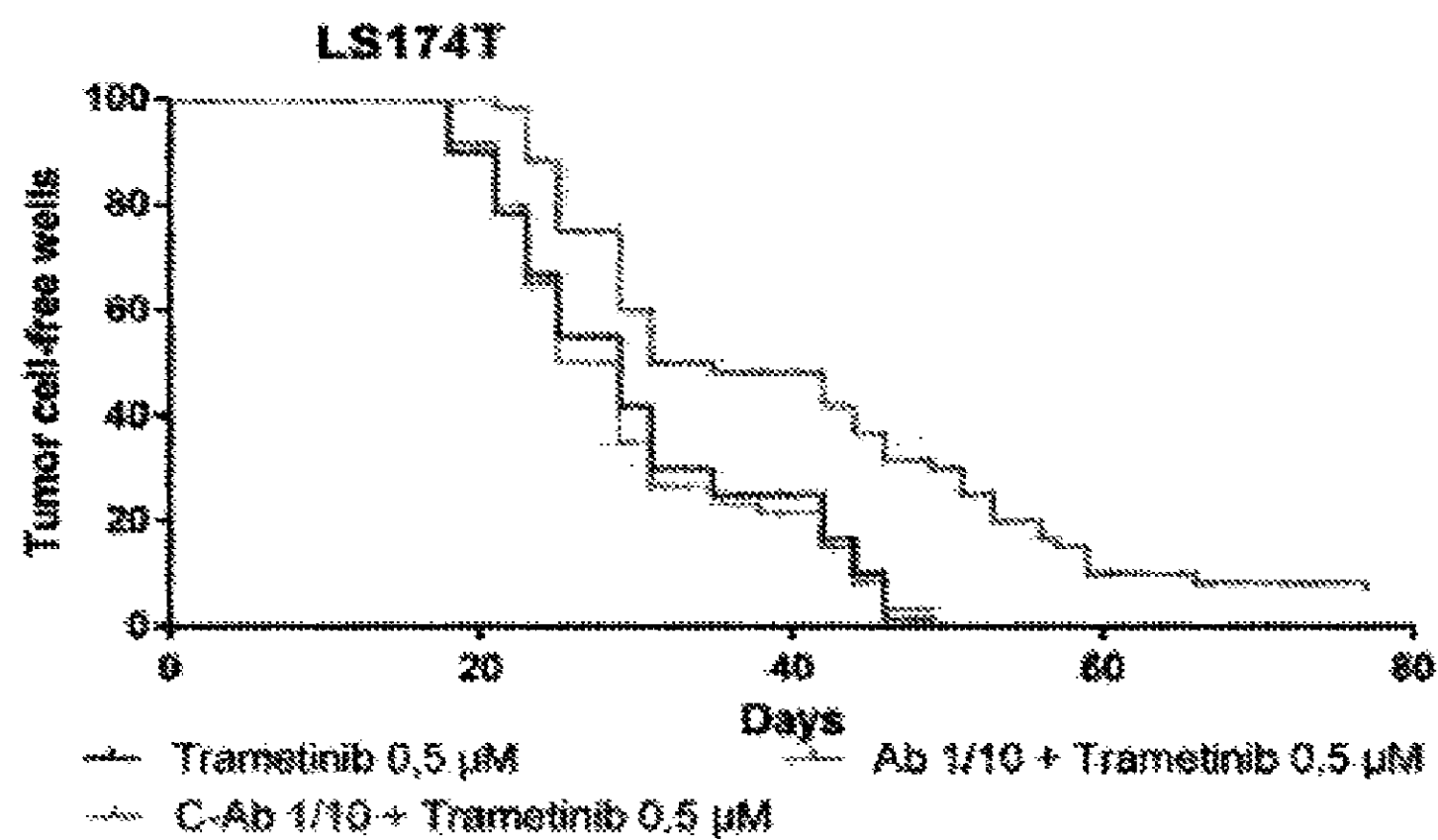


FIG. 11

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Effect of EGF in HT29 cell line

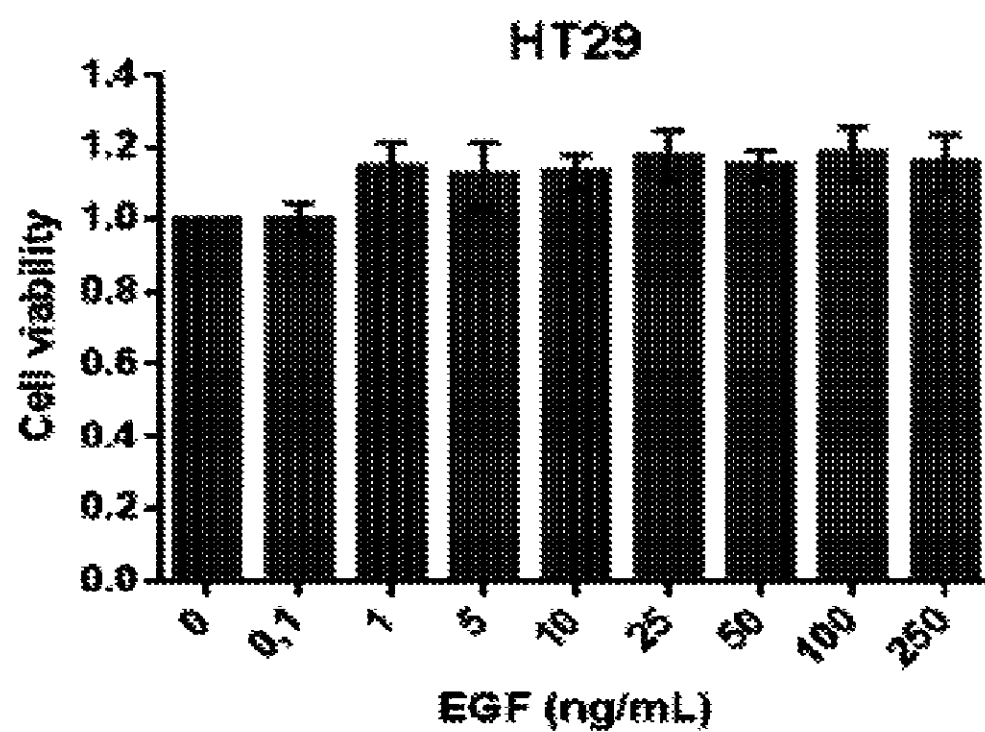


FIG. 12

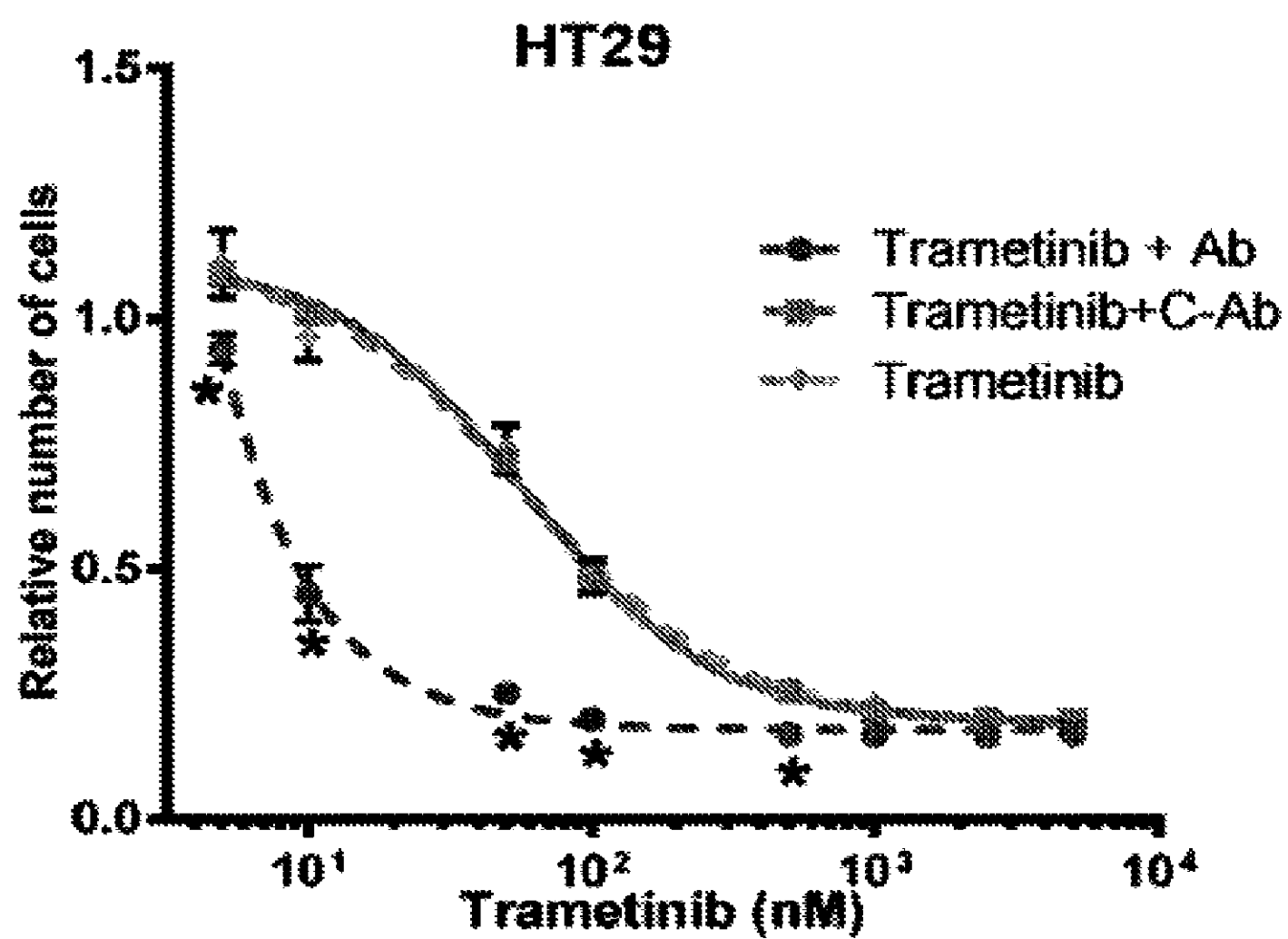


FIG. 13A

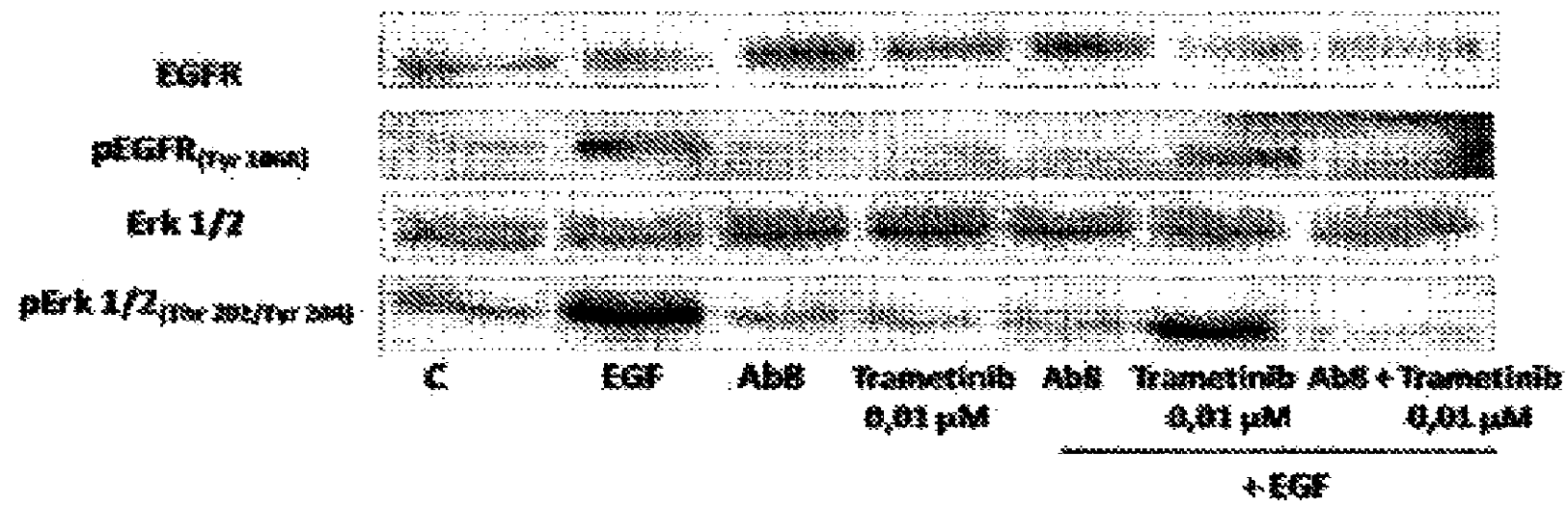


FIG. 13B

Growth curve of trametinib in combination with anti-EGF VacAbs
in HT29 at 14 days

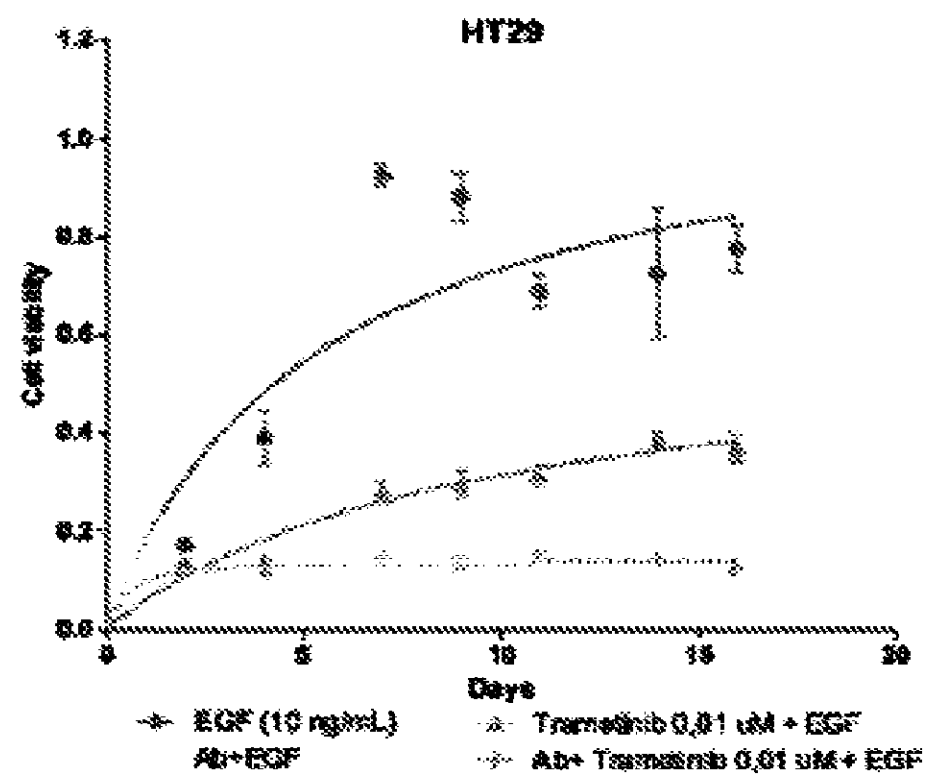


FIG. 13C

Effects of EGF on the activity of trametinib in the H508 cell line

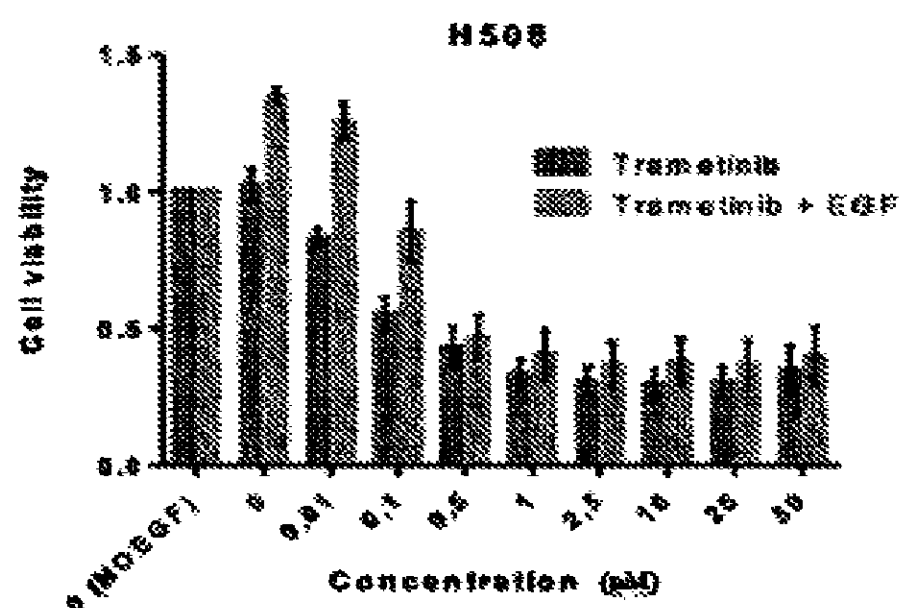


FIG. 14

Anti-EGF VecAbs and trametinib combination in H508 cell line

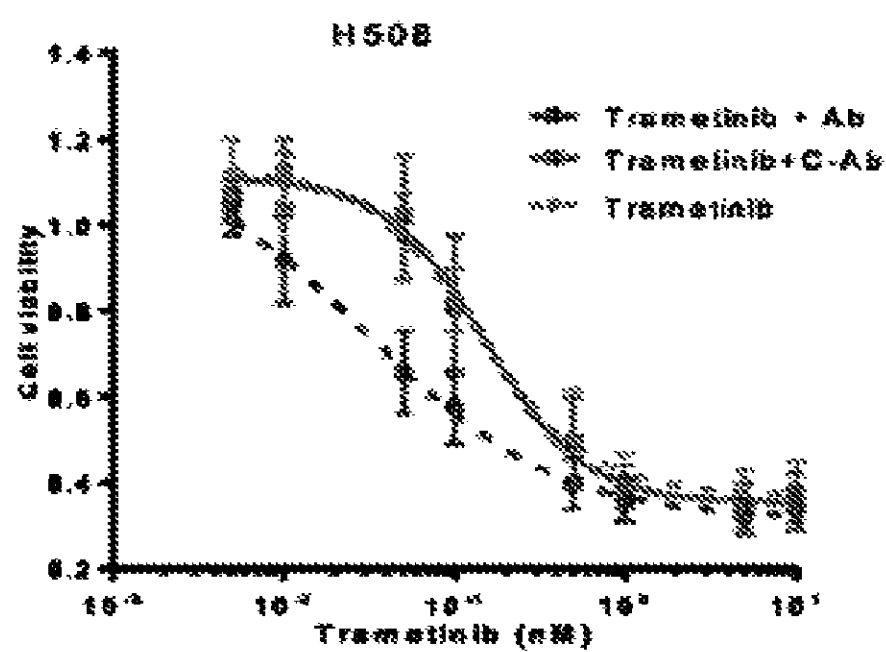


FIG. 15

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Effects of EGF on the activity of taselisib in the H508 cell line

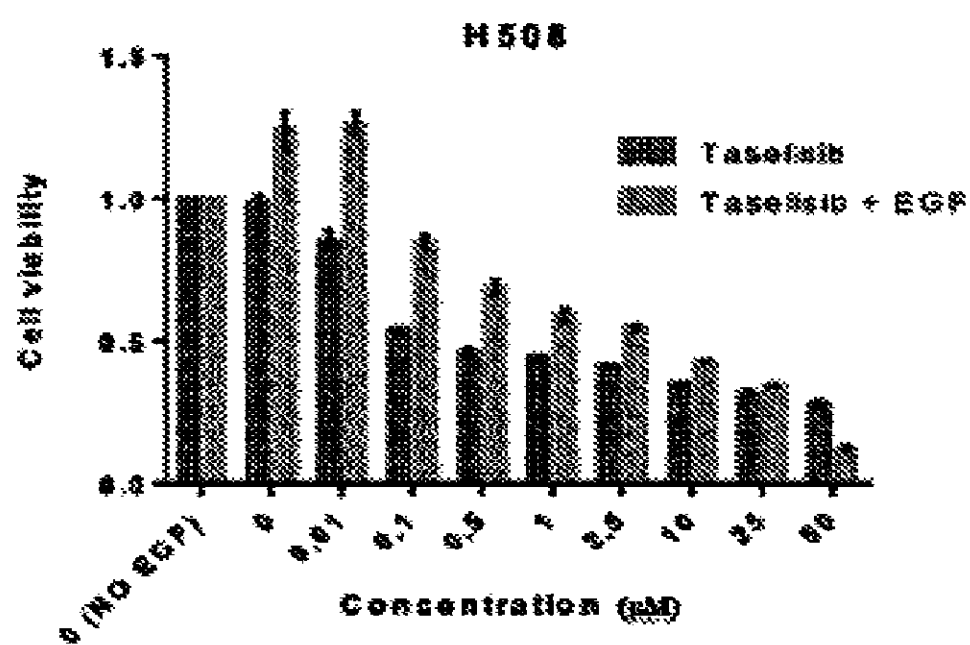


FIG. 16

Anti-EGF VacAbs and taselisib combination in H508 cell line

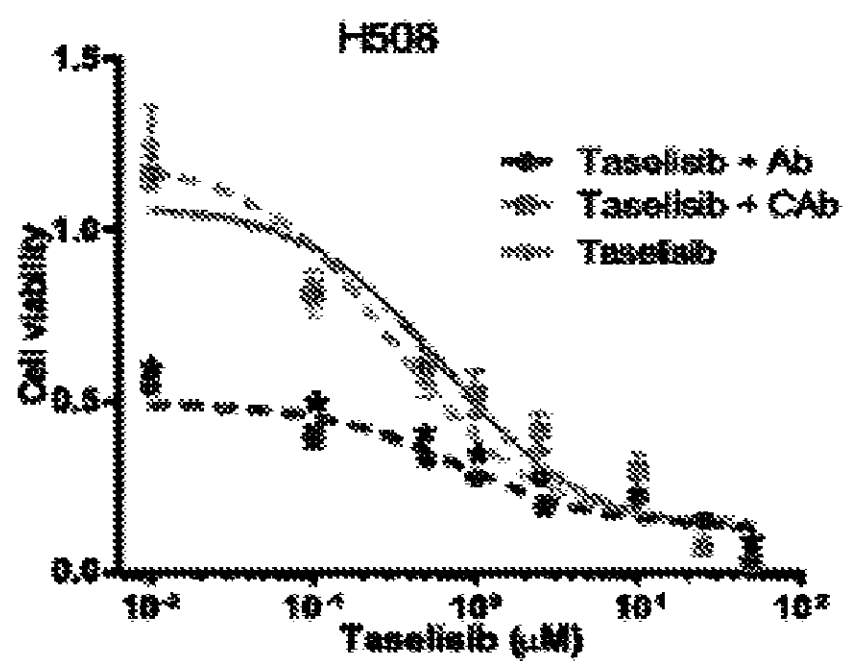


FIG. 17

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Growth curve of taselisib 0.01 μ M in combination with anti-EGF VacAbs
in H508 at 14 days

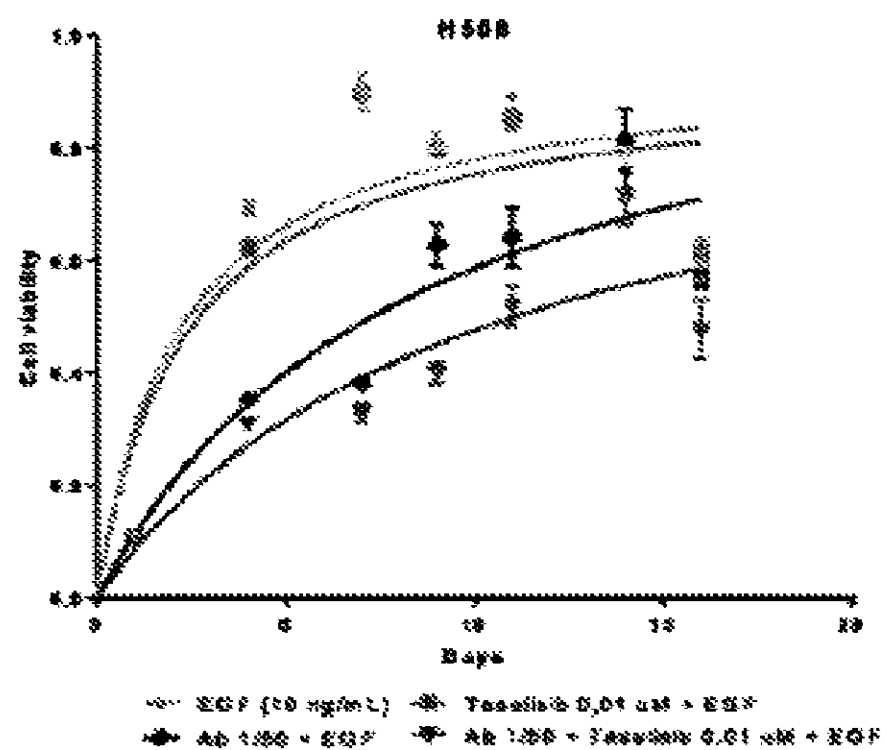


FIG. 18

Growth curve of taselisib 0.5 μ M in combination with anti-EGF VacAbs
in H508 at 14 days

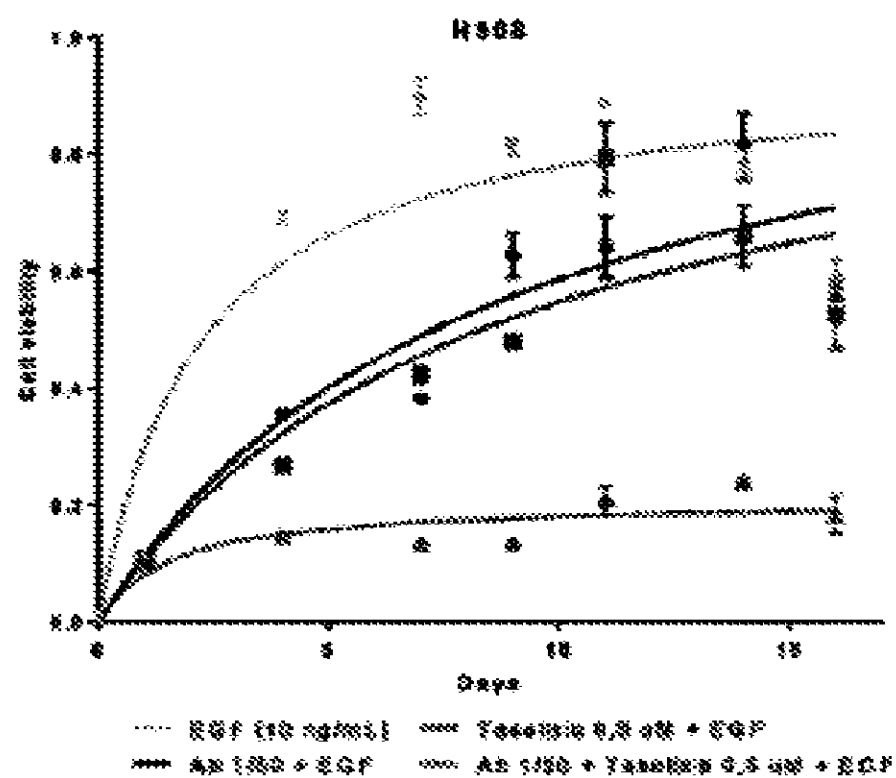


FIG. 19

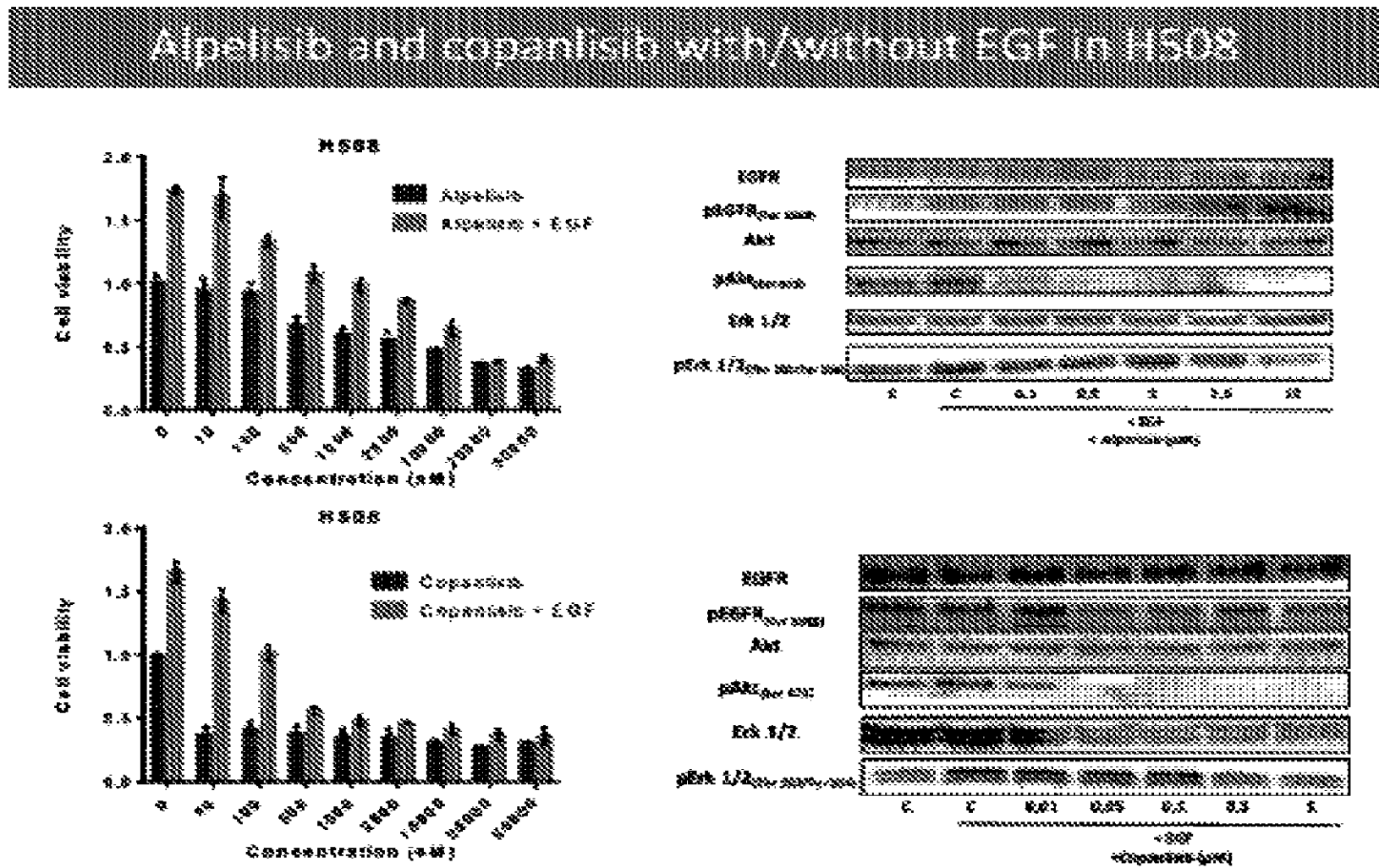


FIG. 20

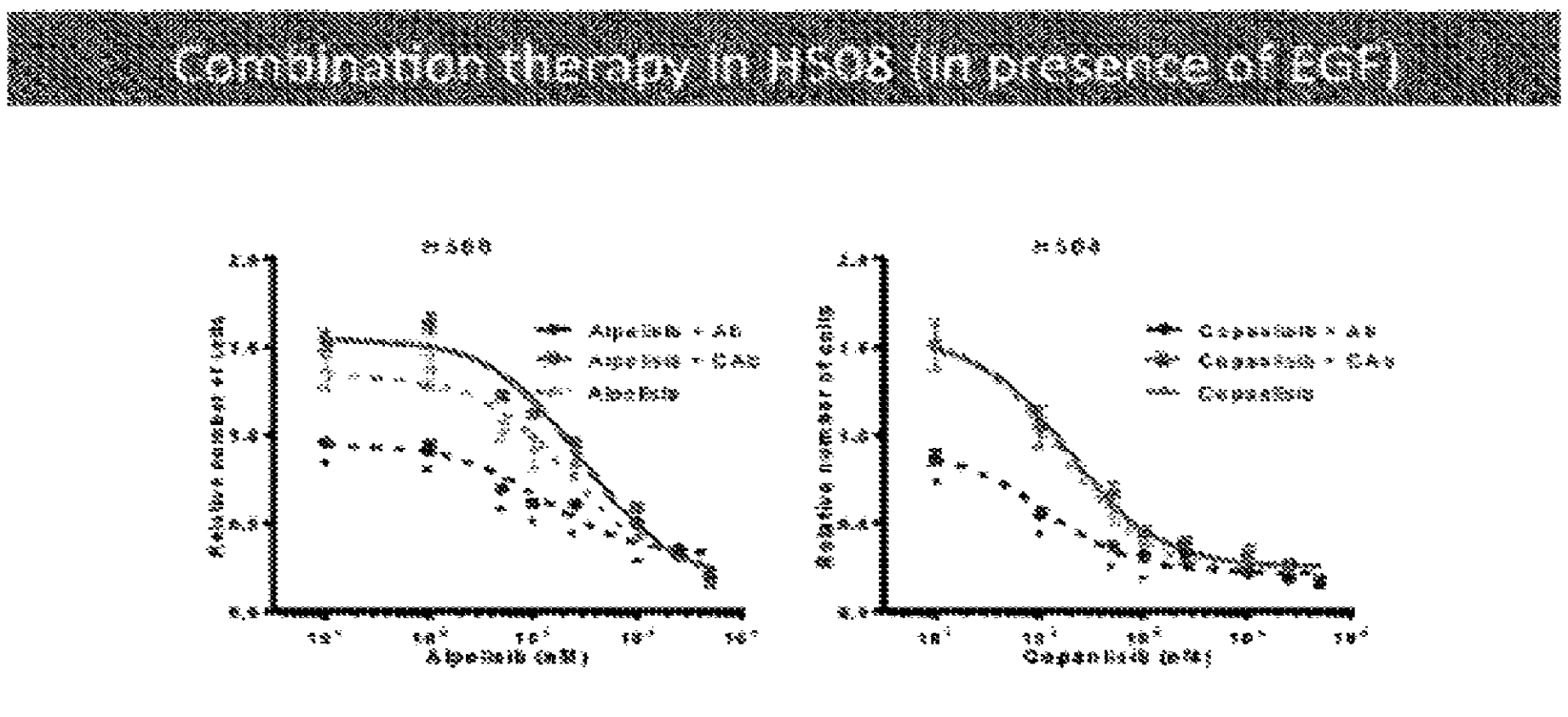


FIG. 21

inhibitory effects of the combination (H503)

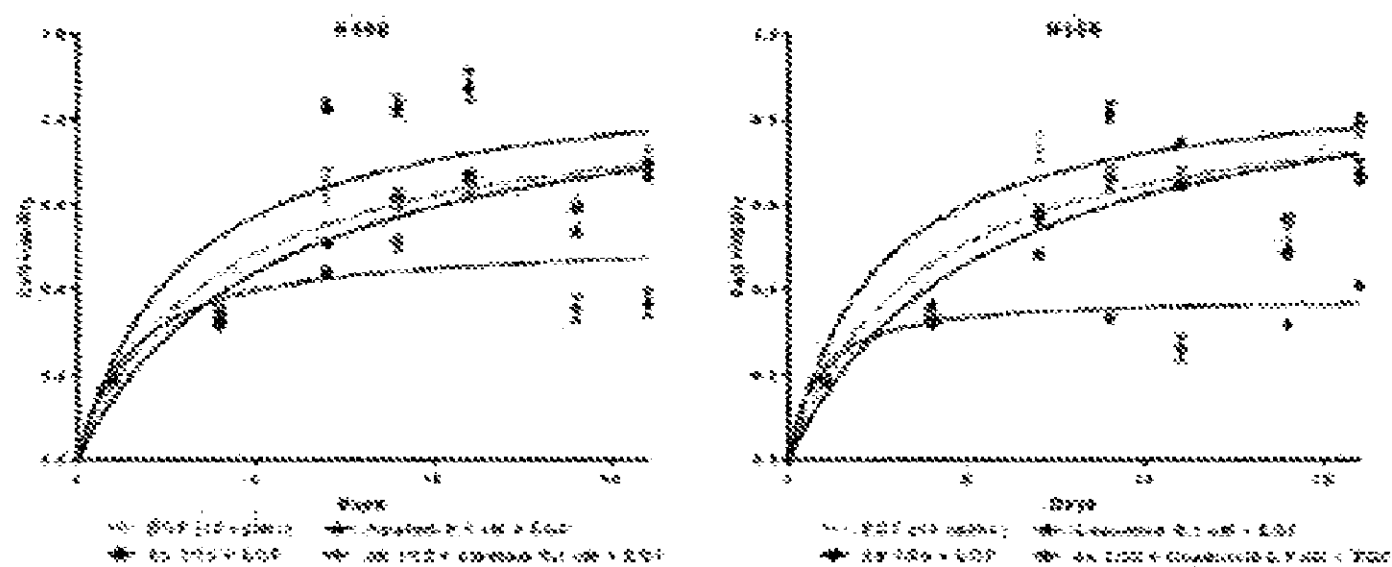


FIG. 22

Anti-EGF VacAbs activity in HT29 cell line

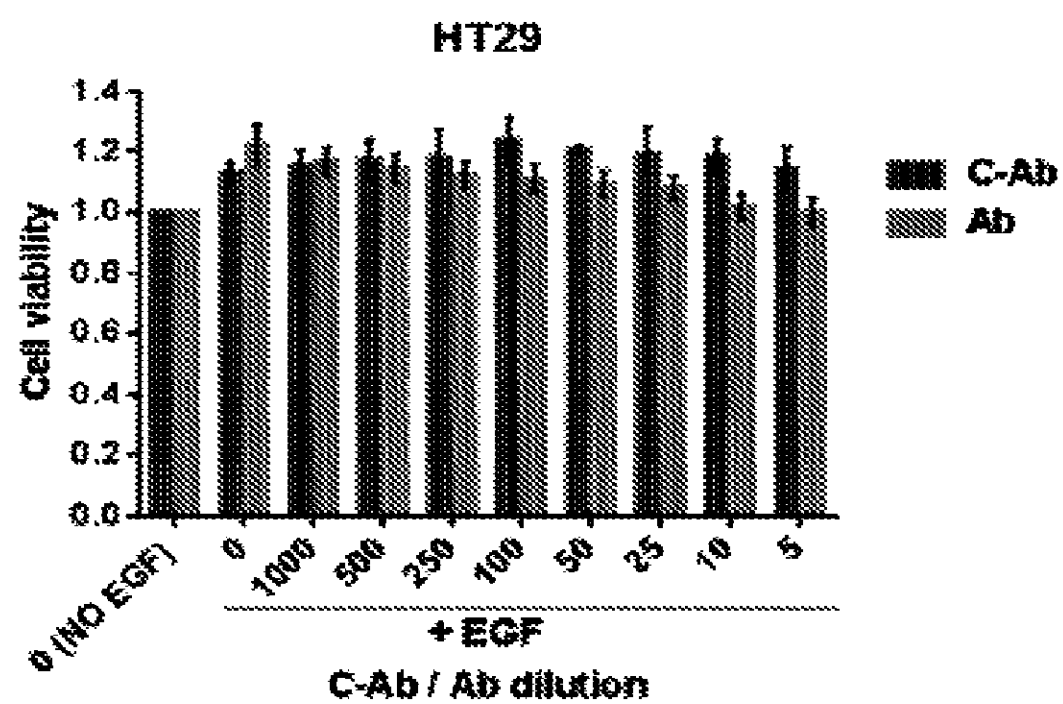


FIG. 23

IF

Anti-EGF VacAbs and trametinib combination in HT29 cell line

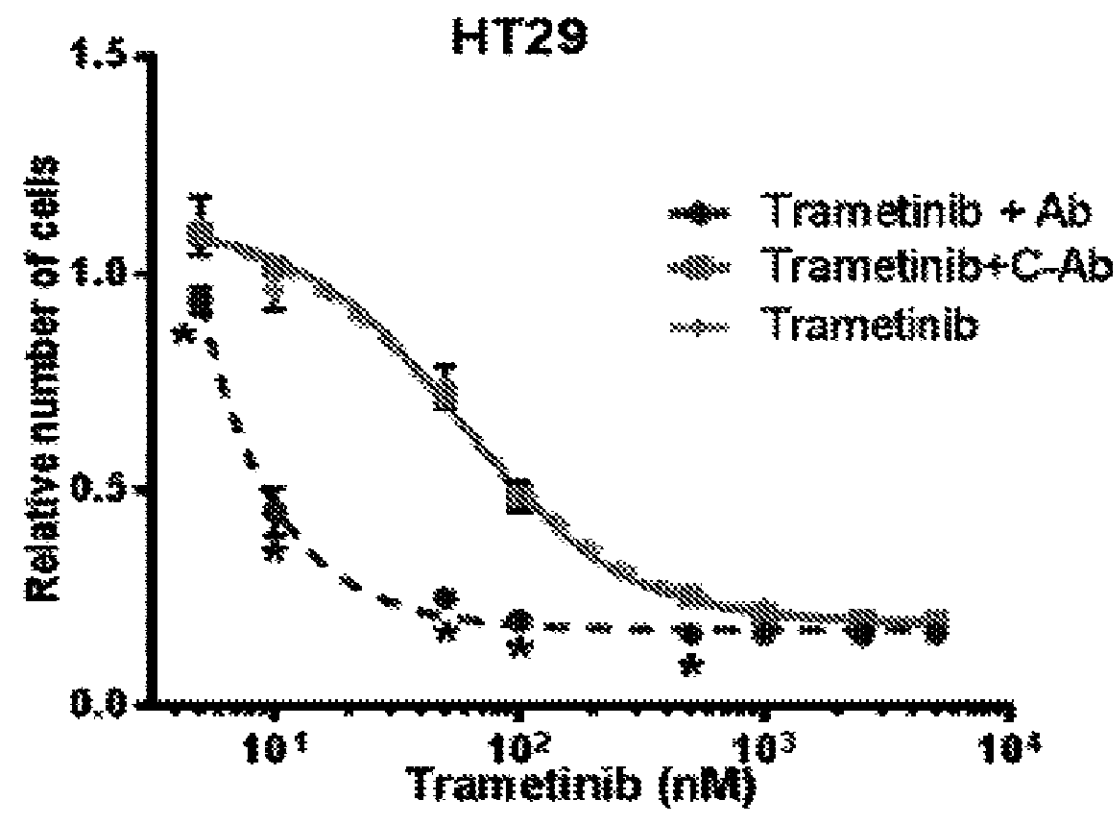


FIG. 24

Dose-Response of trametinib at 2 hours of incubation in HT29 cell line

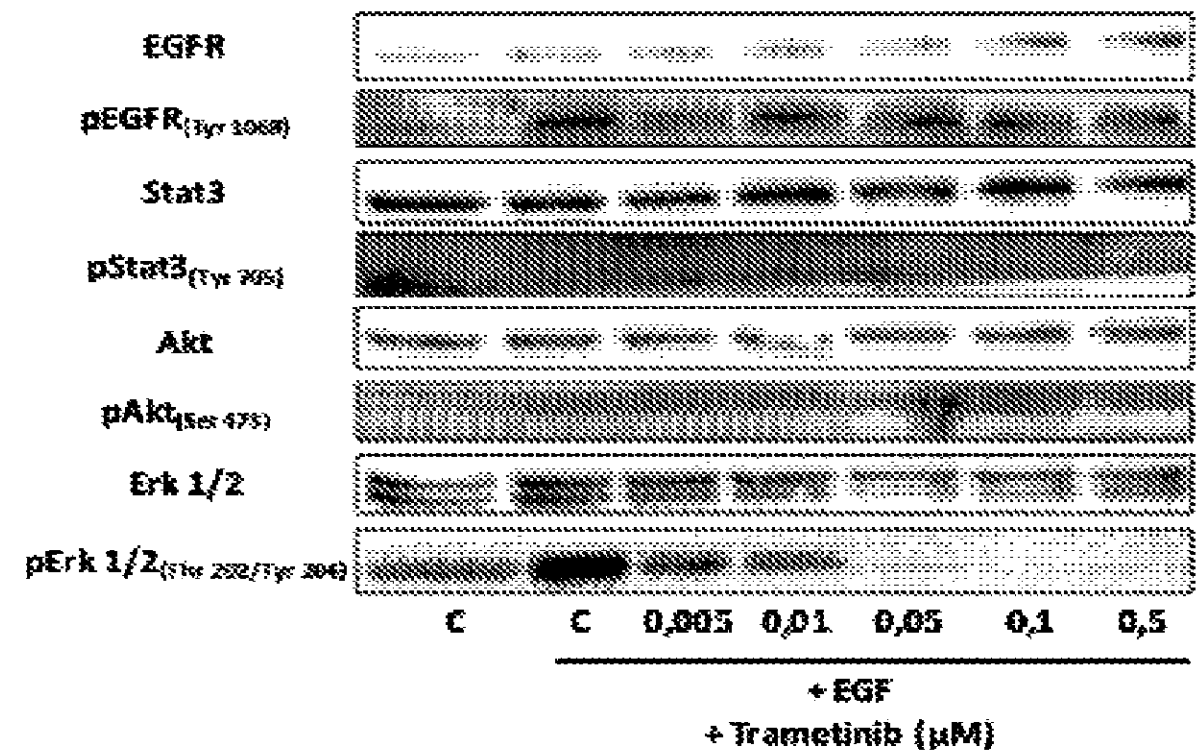


FIG. 25

Anti-EGF VacAbs with trametinib at 2 hours of incubation in HT29 cell line

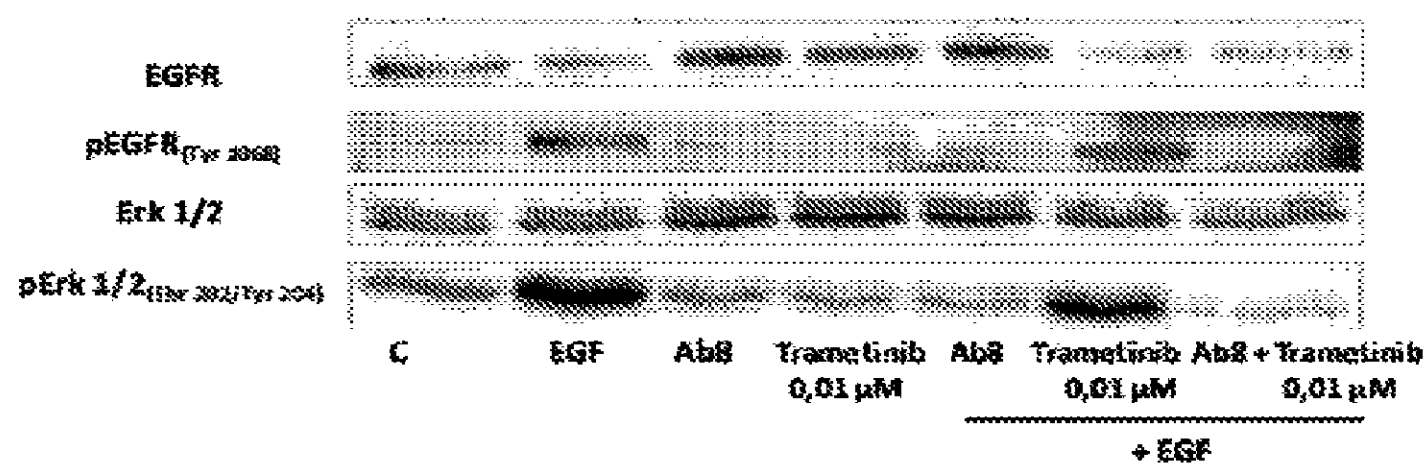


FIG. 26

PC9 and PC9GR4: Osimertinib

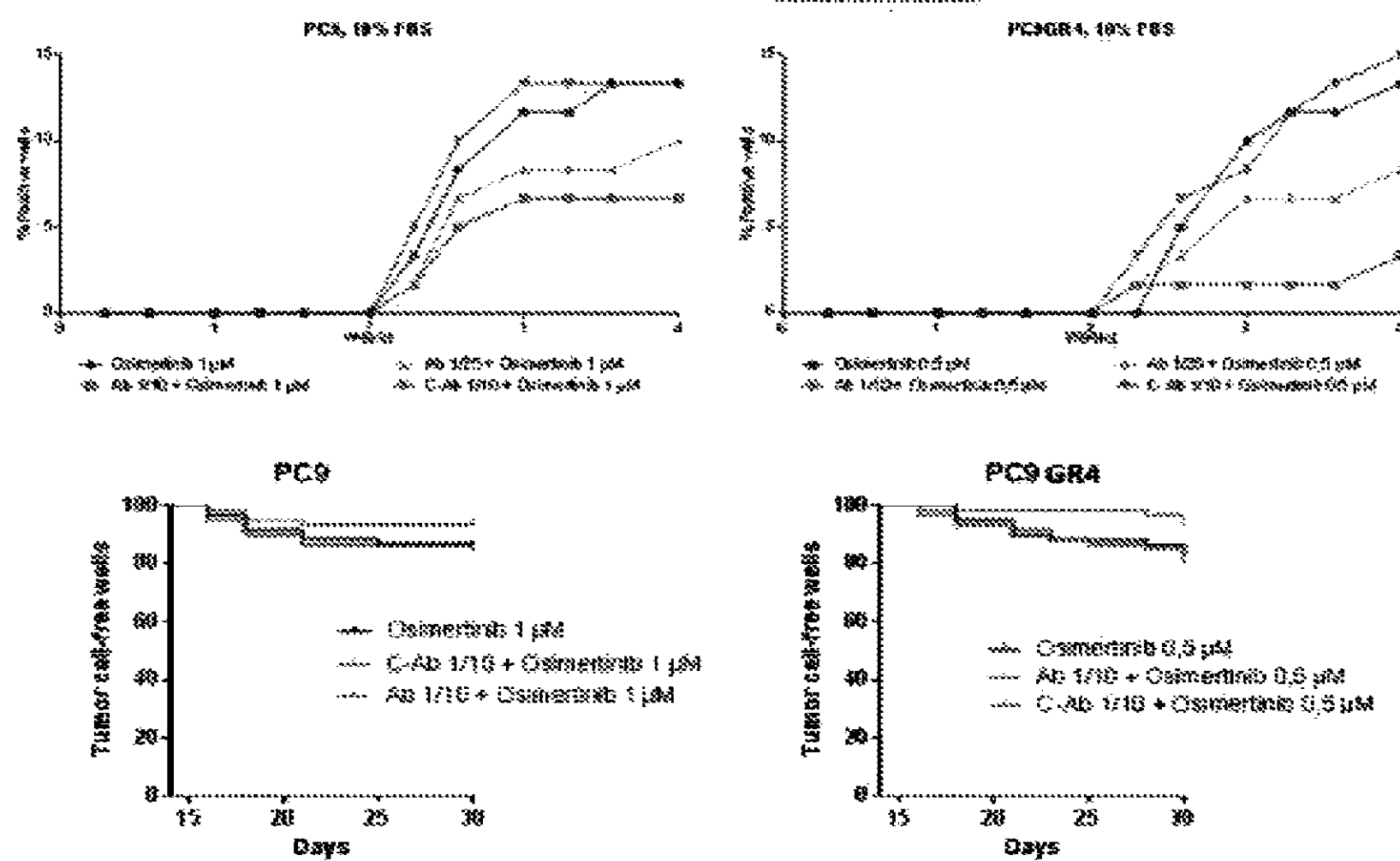


FIG. 27

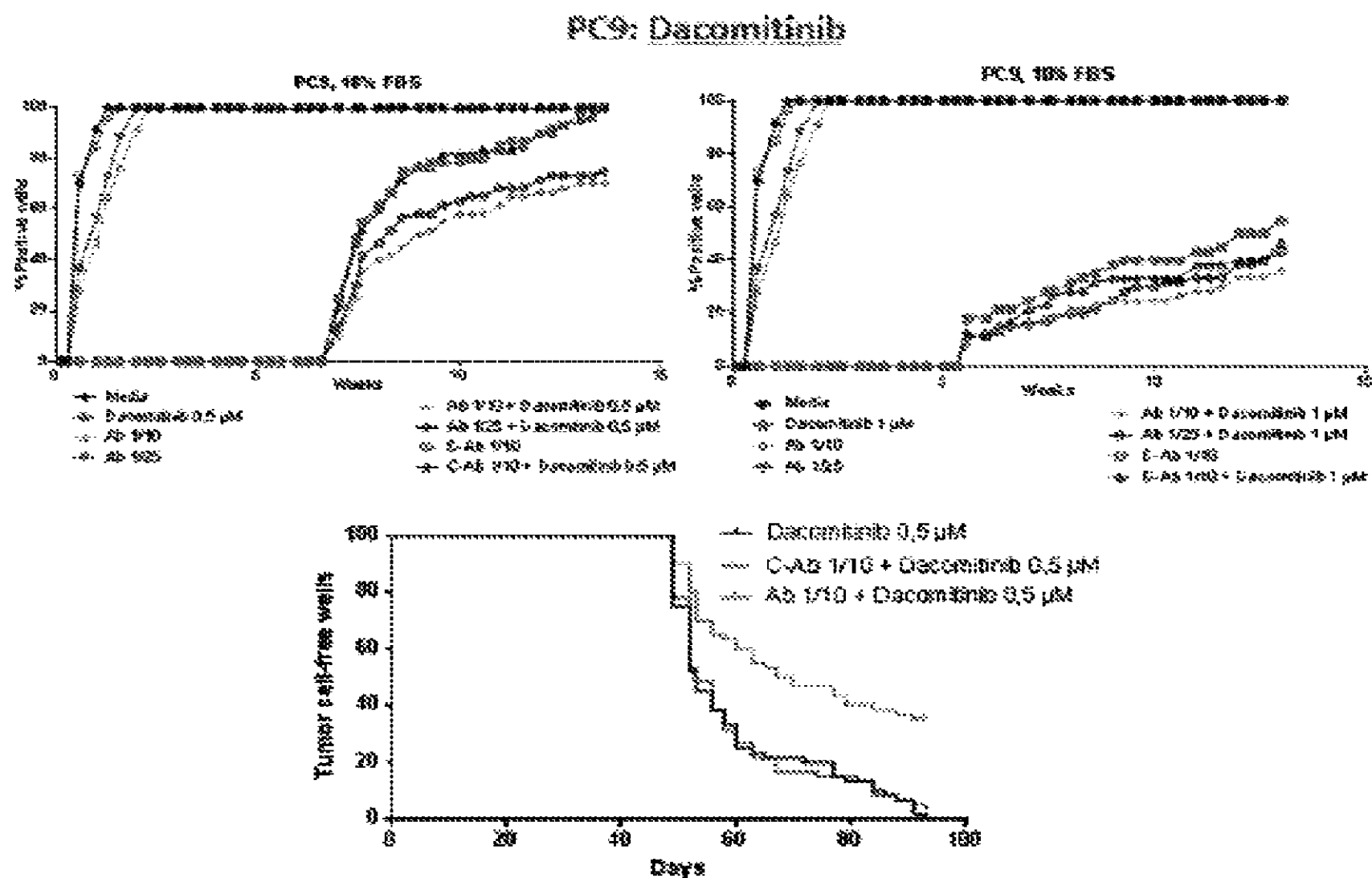


FIG. 28

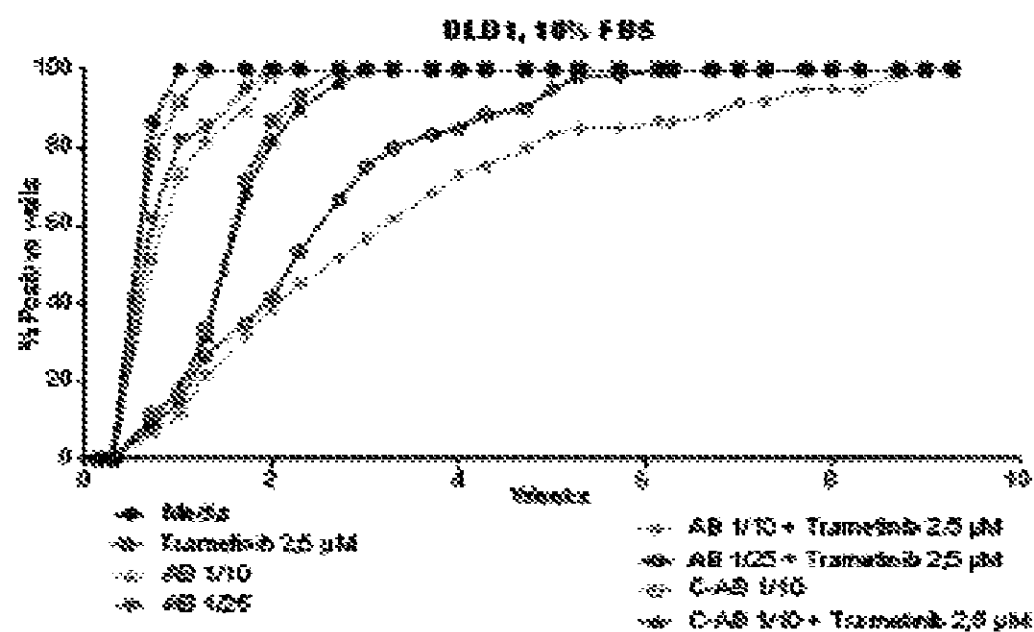
DLD1: Trametinib

FIG. 29

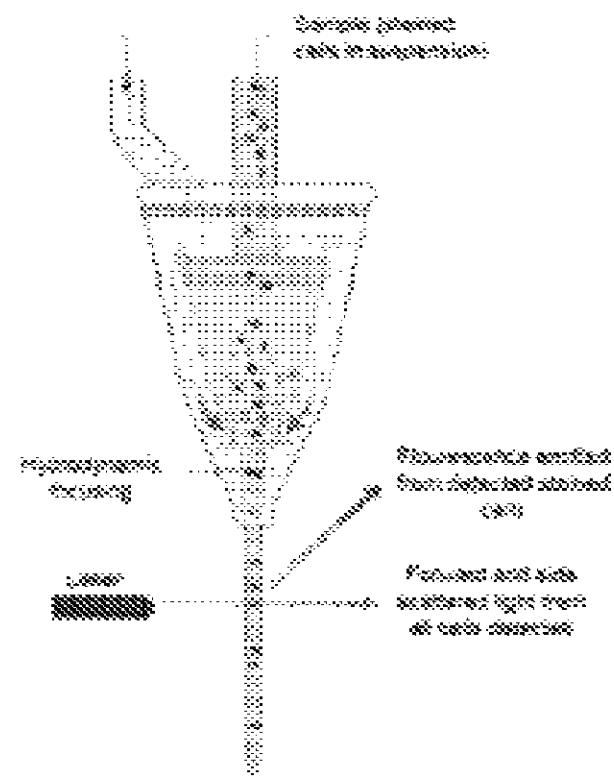
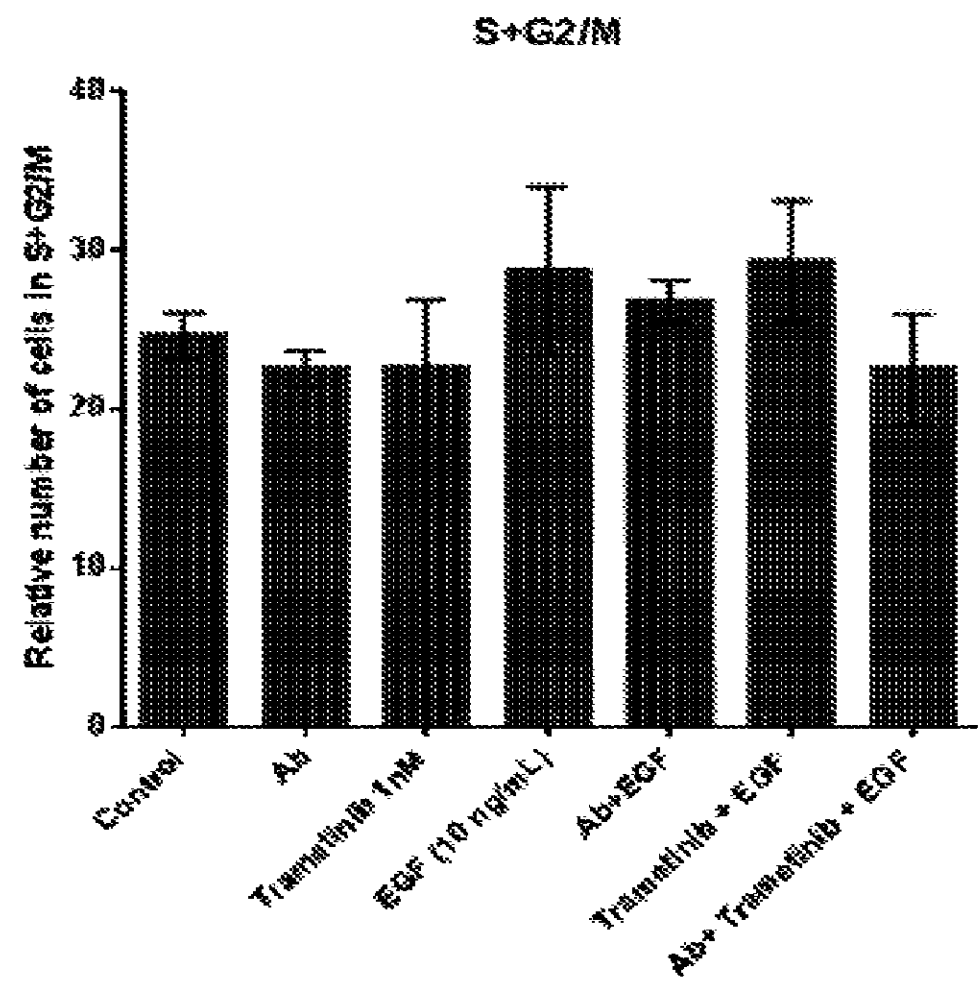


FIG. 30

Anti-EGF VacAbs in combination with trametinib in A549:
Cell cycle



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FIG. 31

Anti-EGF VacAbs in combination with trametinib in A549:
Apoptosis

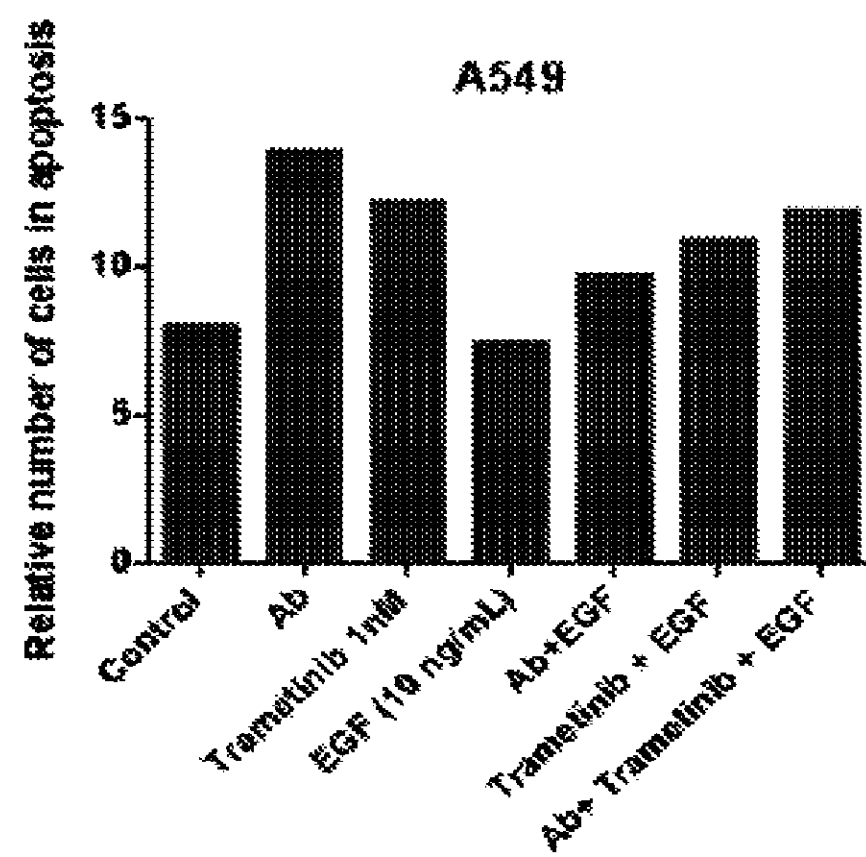


FIG. 32

Anti-EGF YacAbs in combination with trametinib in DLD1:
Cell cycle

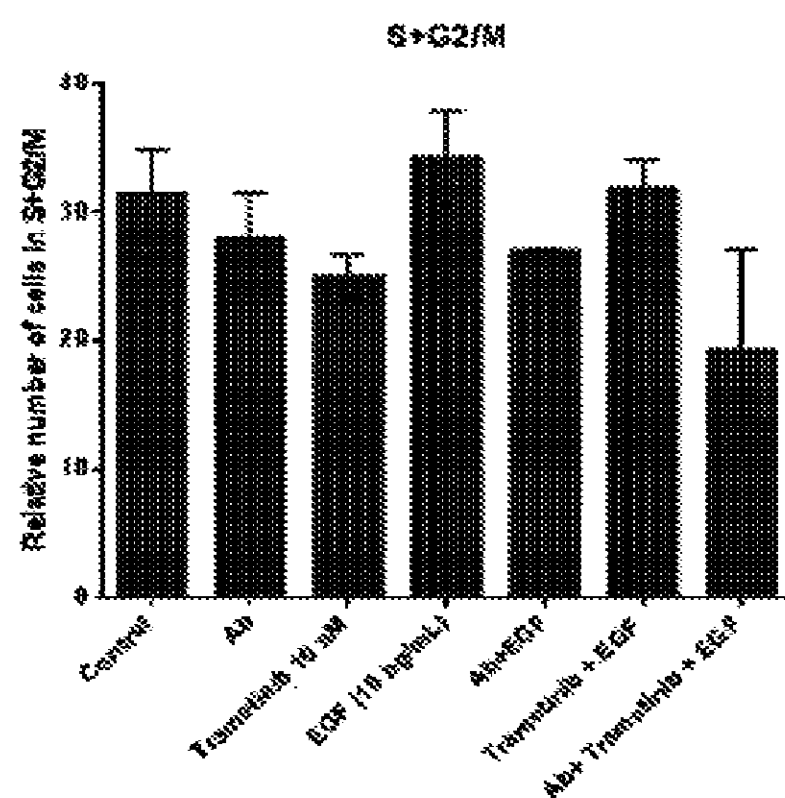


FIG. 33

Anti-EGF VacAbs in combination with trametinib in DLD1:
Apoptosis

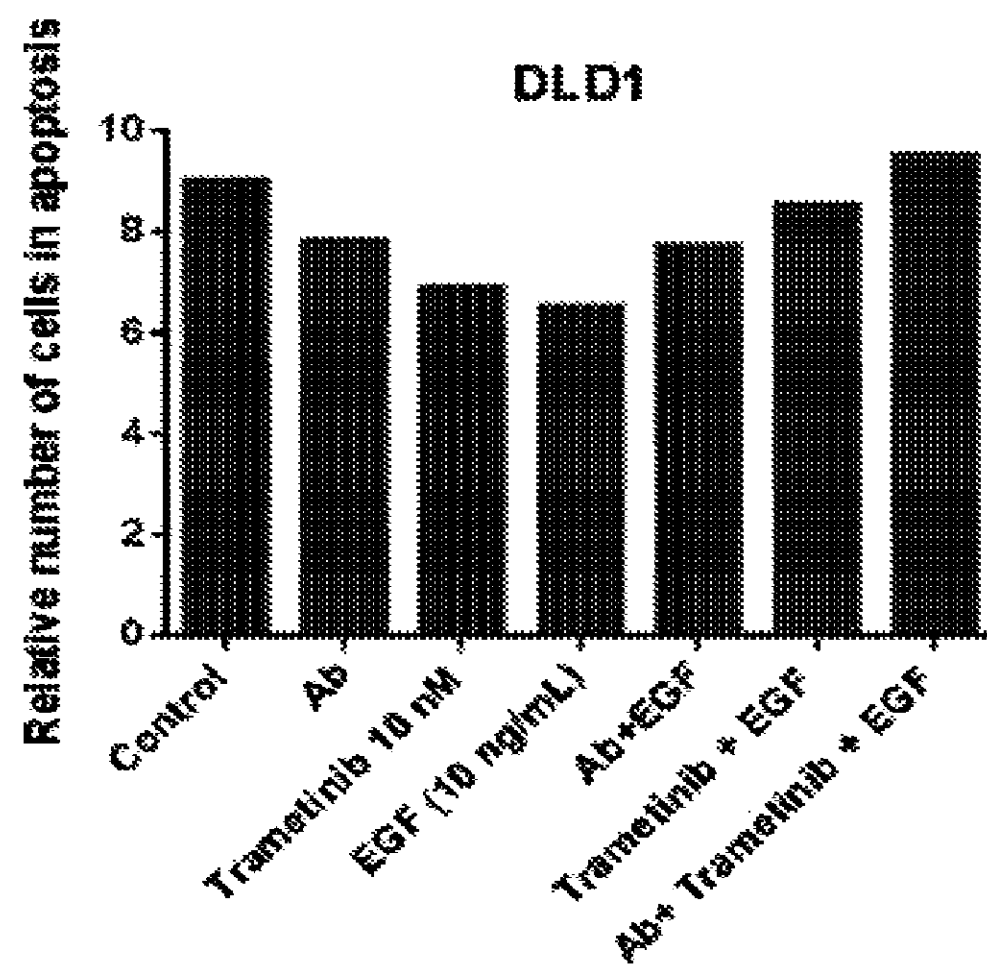


FIG. 34

Anti-EGF VacAbs in combination with brigatinib in H2228:
Cell cycle

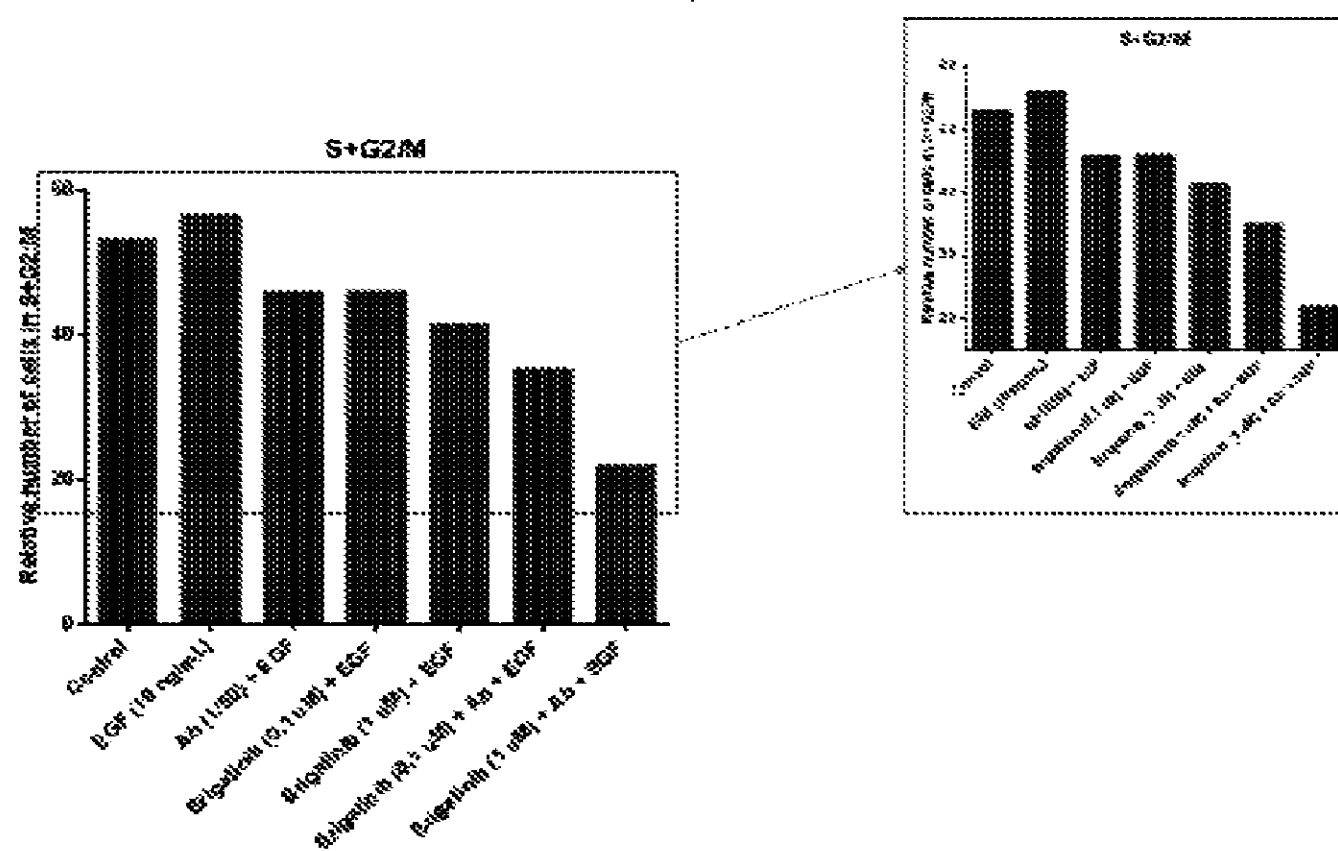


FIG. 35

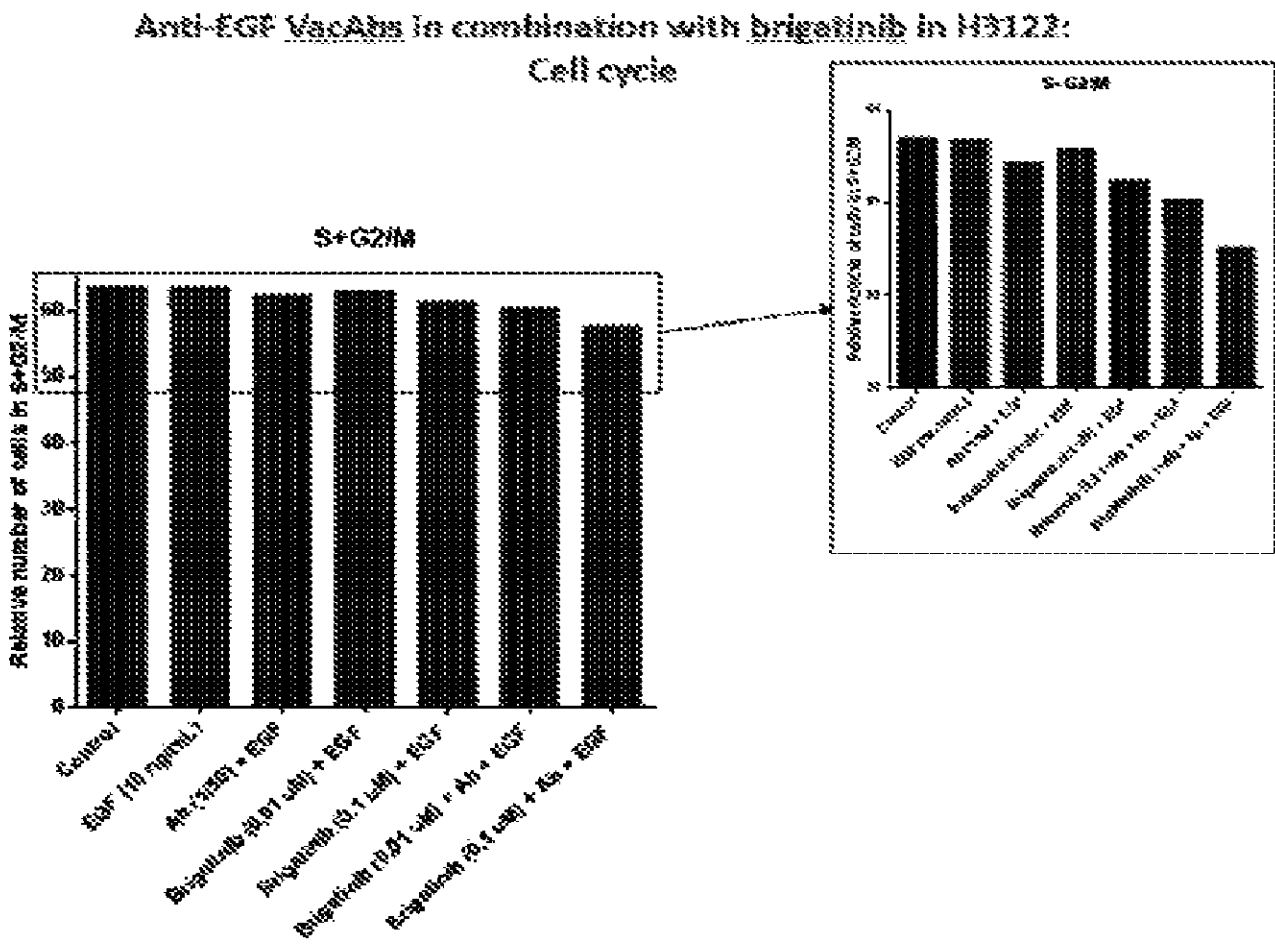


FIG. 36

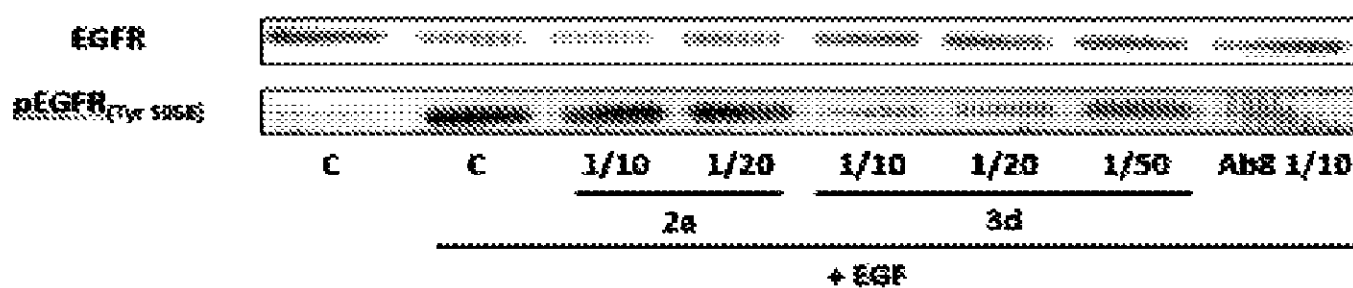
BV-NSCLC-002 western blots

Patient alteration	Cell line used	Cell lines main alteration
ALK translocated	H2228	EML4-ALK (v3; E6-A20)
KRAS mutant	A549	KRAS mutant (G12S)
ALK and KRAS wild type	SW900	ALK and KRAS wild type

FIG. 37

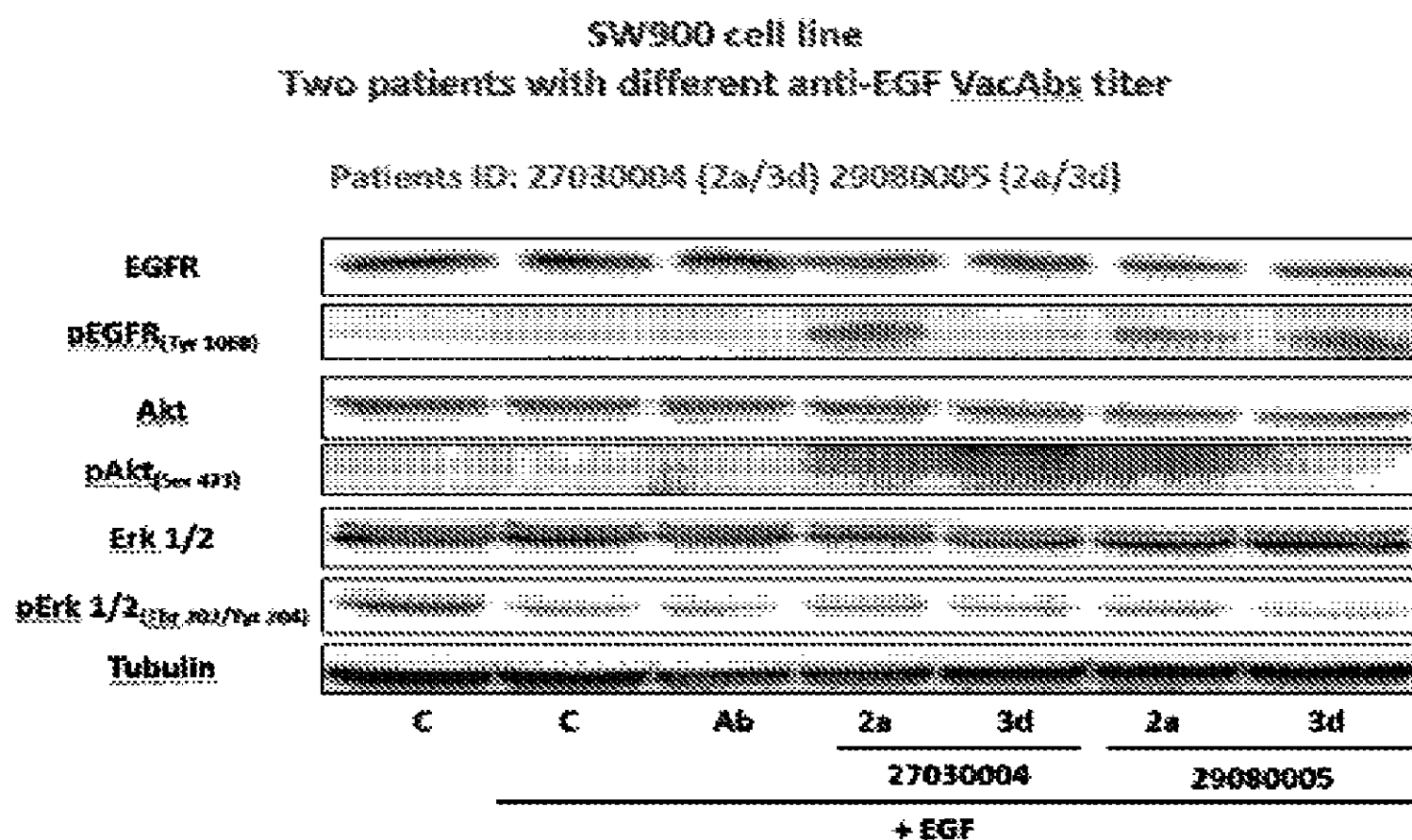
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SW900 cell line
 Dose – Response experiment of a patient with high anti-EGF VacAbs titer
 Patient ID: 22180004-2a / 22180004-3d



PatId	Cell line	IHC ALK	FISH ALK	KRAS TX	EGF 2a	EGF 3d	Titer (1/cox)
22180004	SW900 (wt)	NEG		ND	262,44	9,30	21000

FIG. 38



PatId	Cell line	IHC ALK	FISH ALK	KRAS TX	EGF 2a	EGF 3d	Titer (1/cox)
27030004	SW900 (wt)				296,27	31,86	8300
29080005	SW900 (wt)	NEG		ND	185,47	17,96	2300

FIG. 39

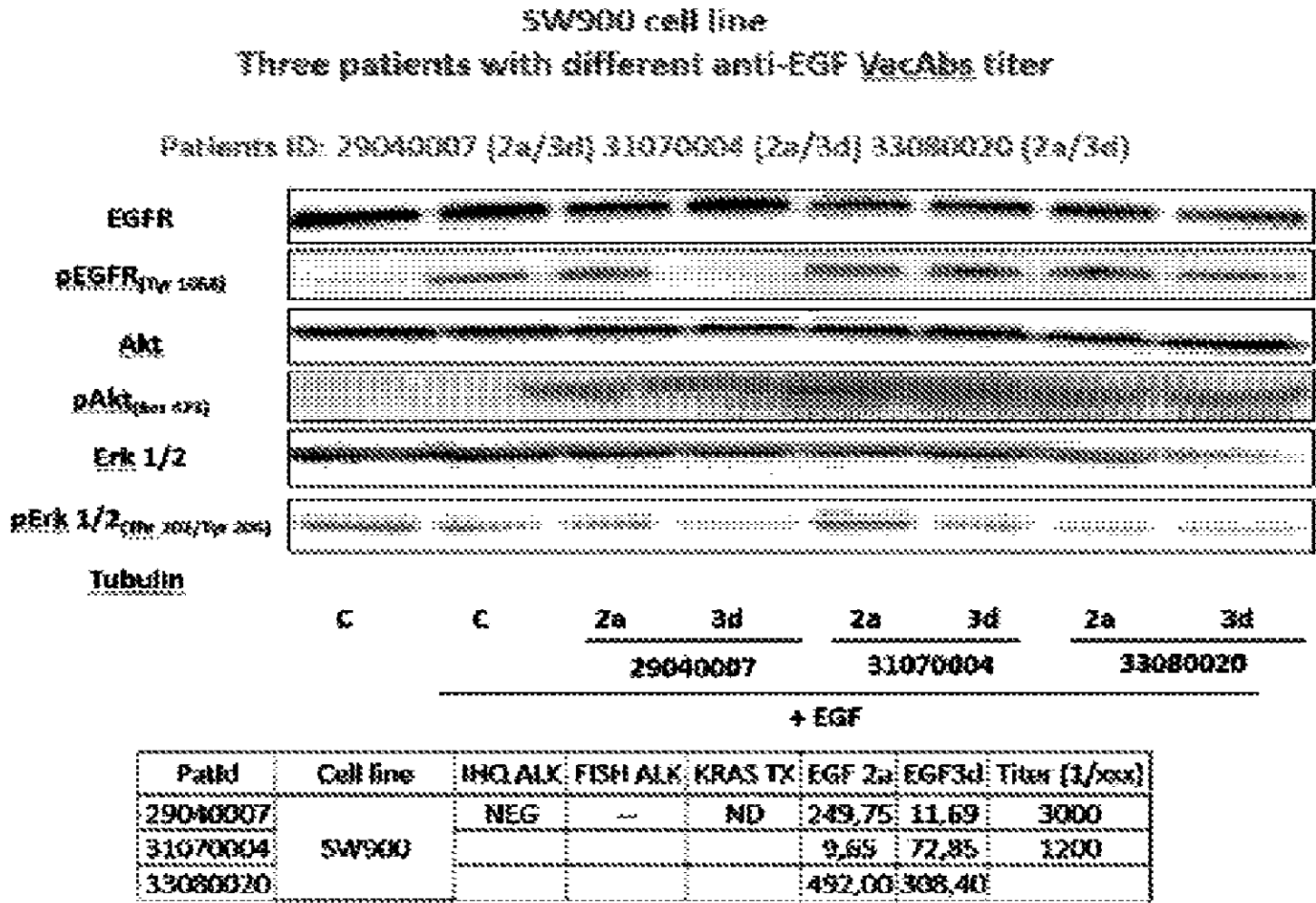


FIG. 40

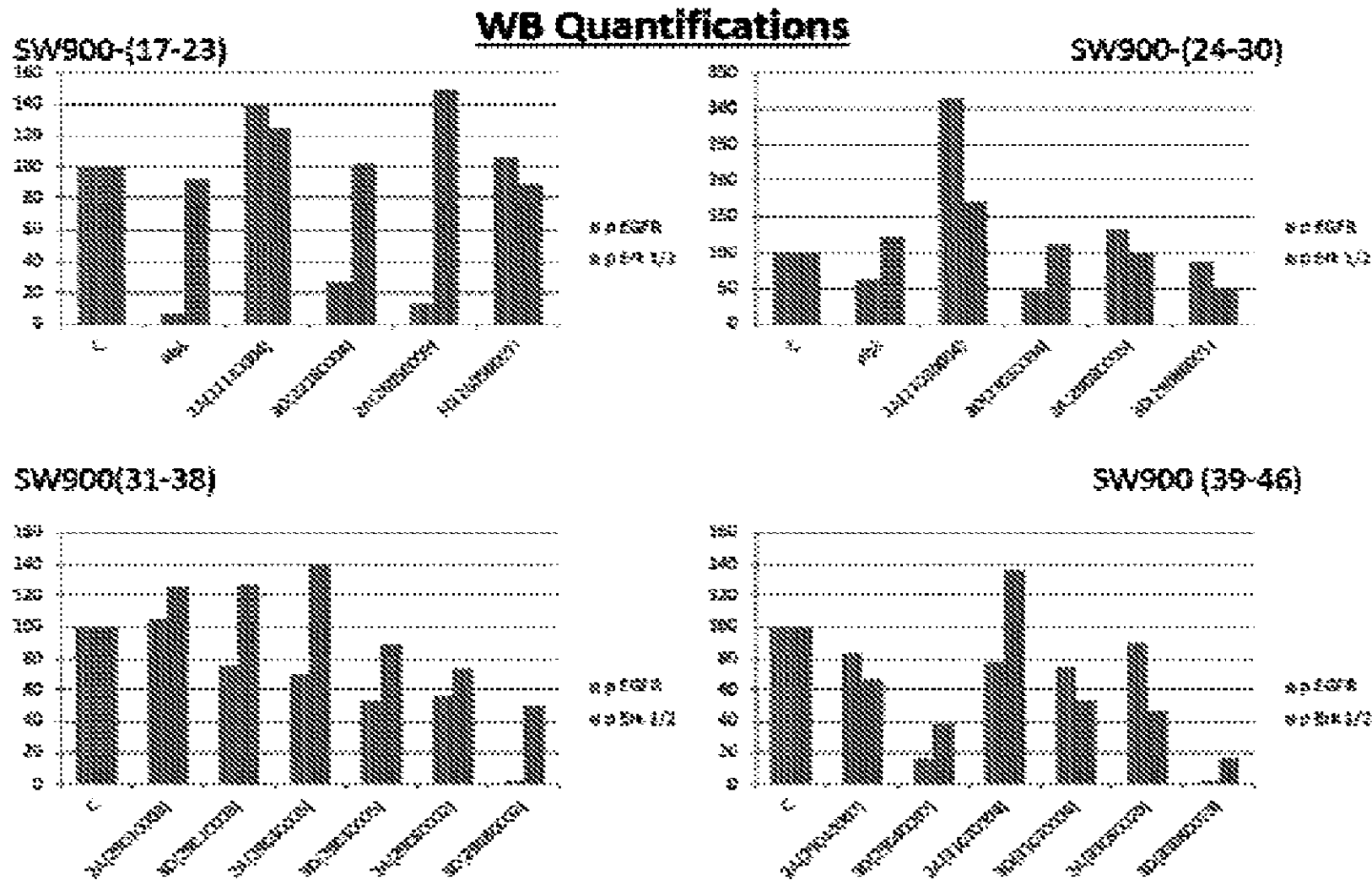


FIG. 41

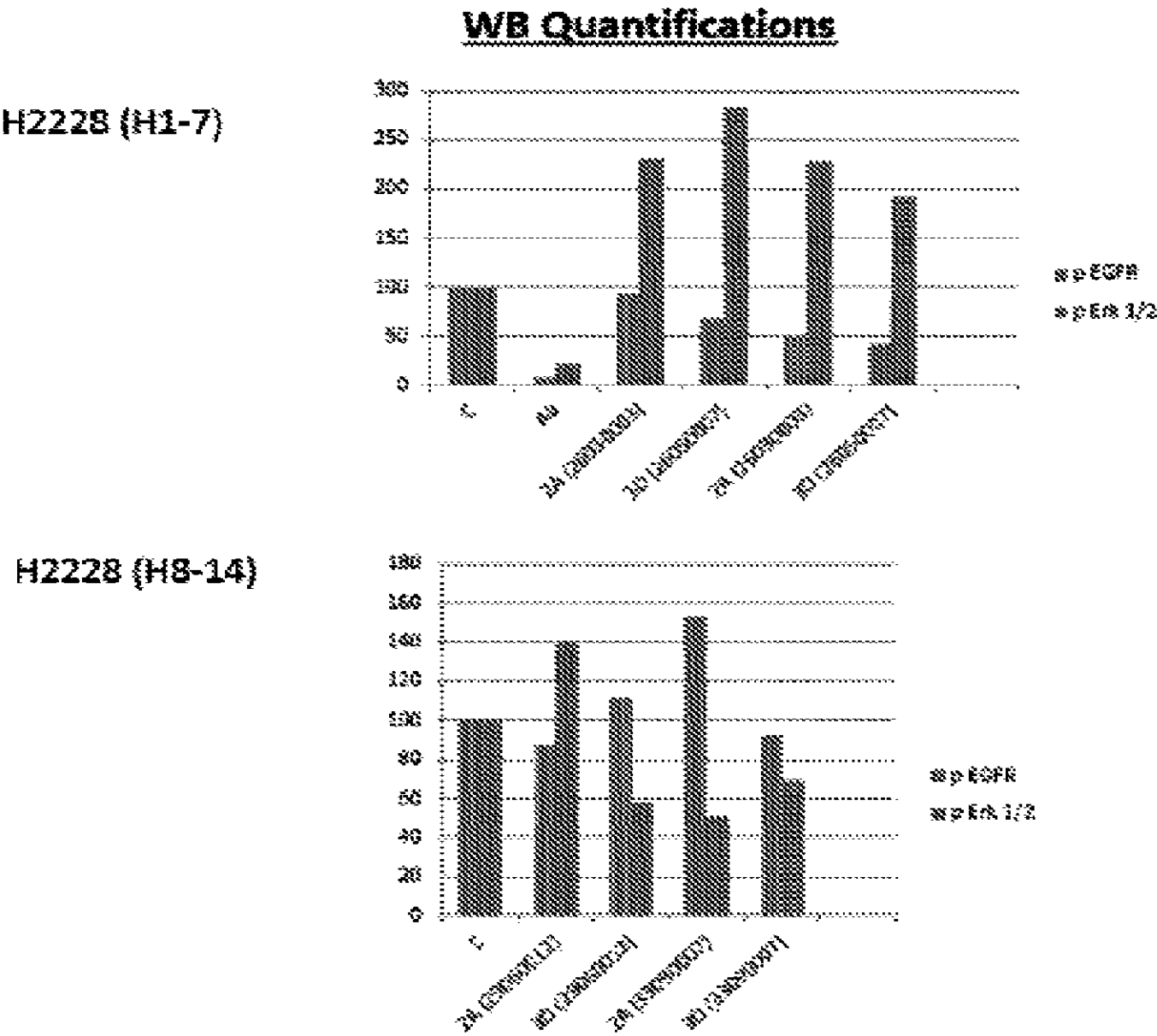


FIG. 42

CELL LINE	HISTOLOGY	RELEVANT ALTERATION	OTHER ALTERATION
OLD1	Colorectal ADC	KRAS mutant (p.G13D)	PIK3CA mutant (p.E545K)
LS174T	Colorectal ADC	KRAS mutant (p.G12C)	-----
HT29	Colorectal ADC	BRAF mutant (p.V600E)	-----
H508	Colorectal ADC	PIK3CA mutant (p.E545K)	BRAF mutant (p.G596R)

FIG. 43

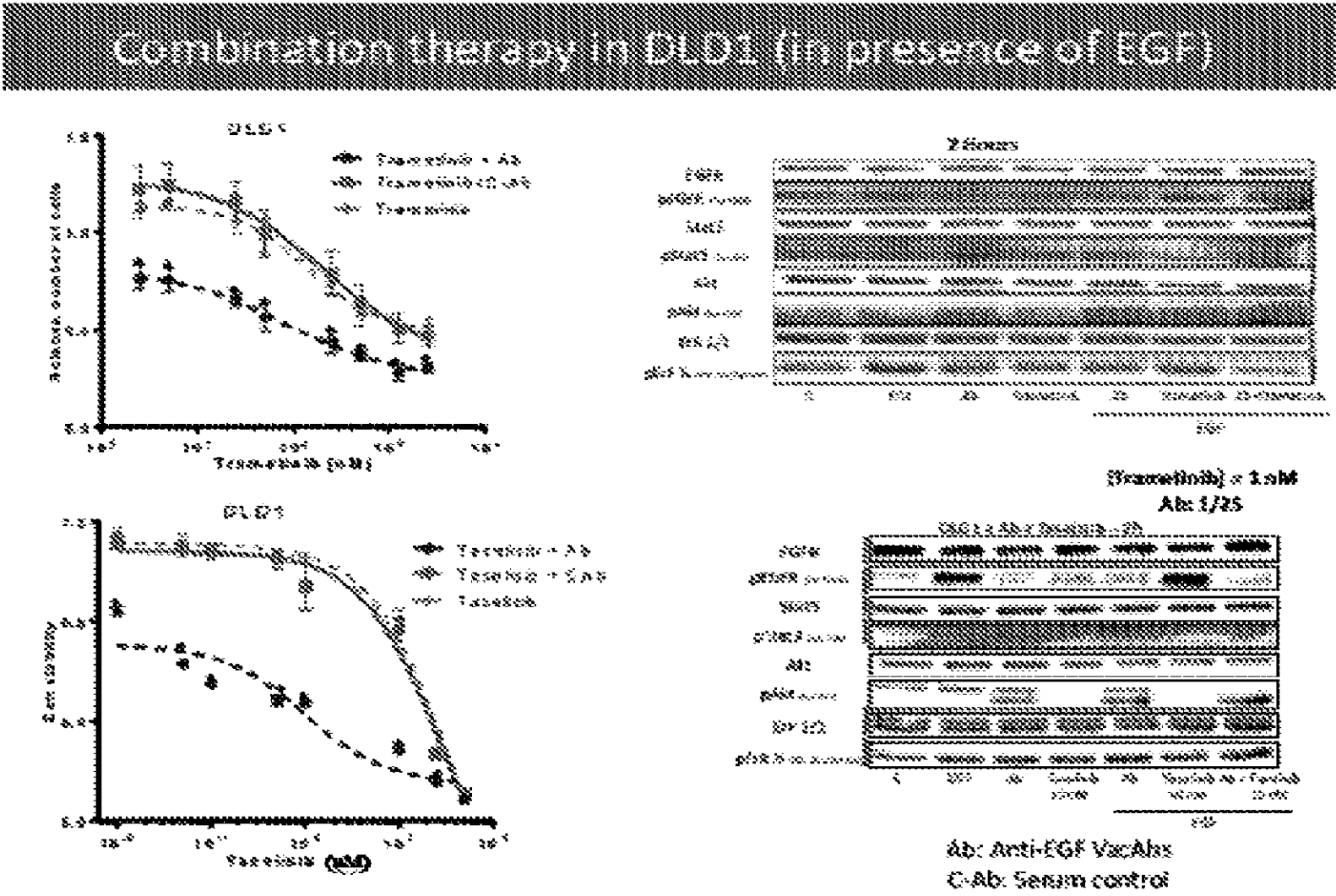


FIG. 46

Cell cycle effects of the combination (DLD1)

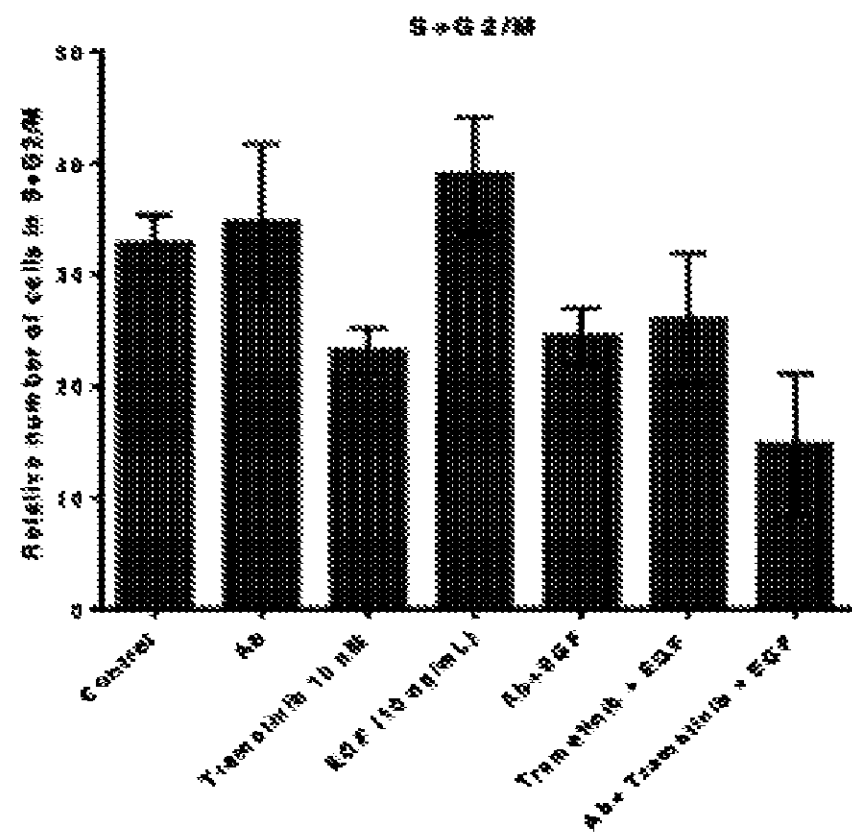


FIG. 47

Apoptotic effects of the combination (DLD1)

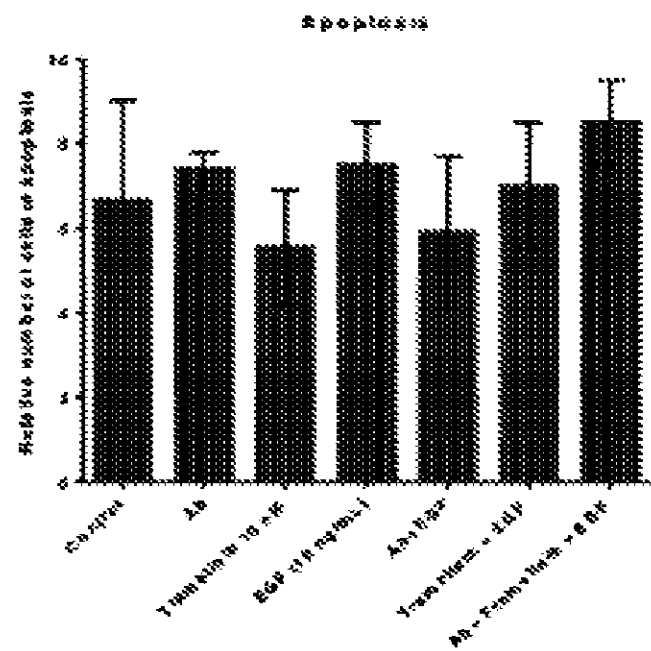


FIG. 48

Emergence of resistance to Trametinib (DLD1)

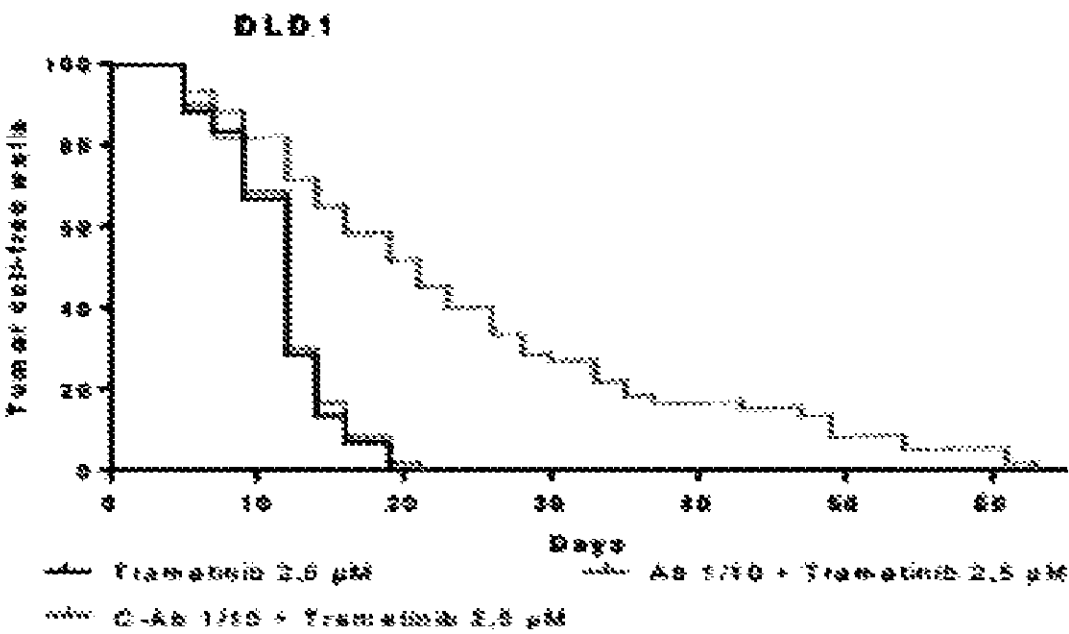


FIG. 49

Trametinib with/without EGF in LS174T

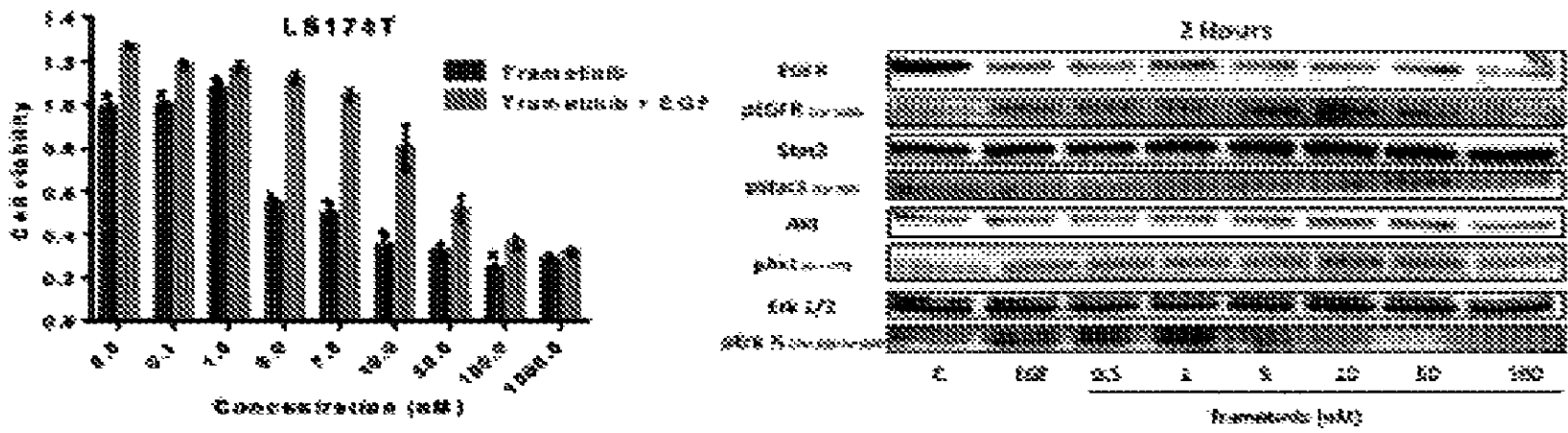


FIG. 50

Combination therapy in LS174T (in presence of EGF)

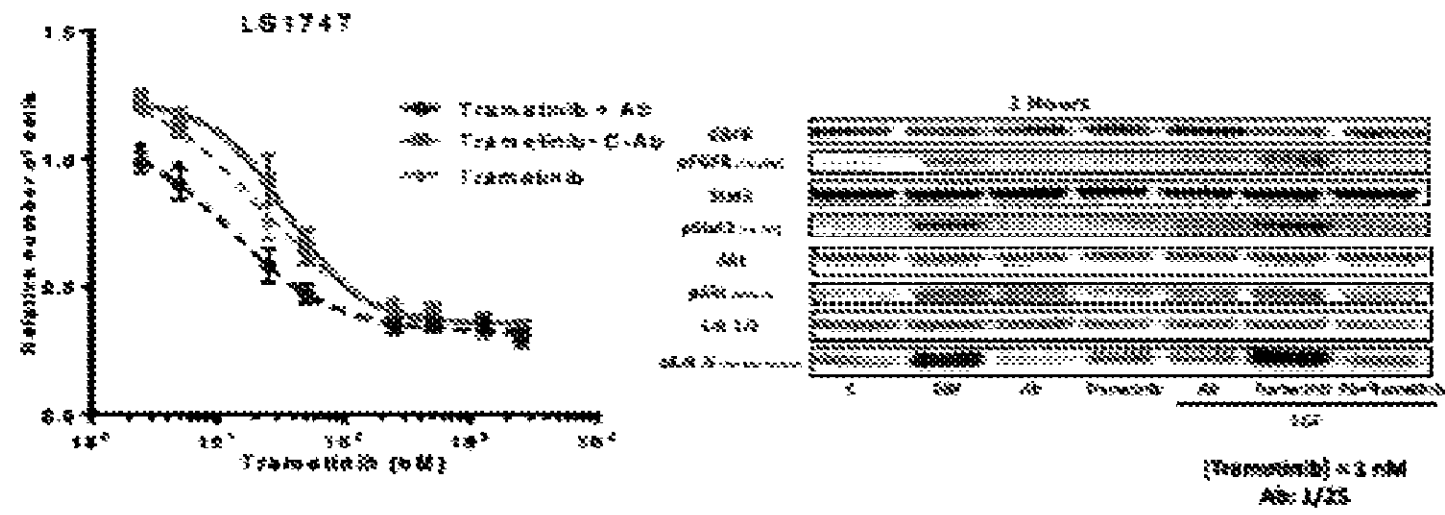


FIG. 51

Inhibitory effects of the combination (LS174T)

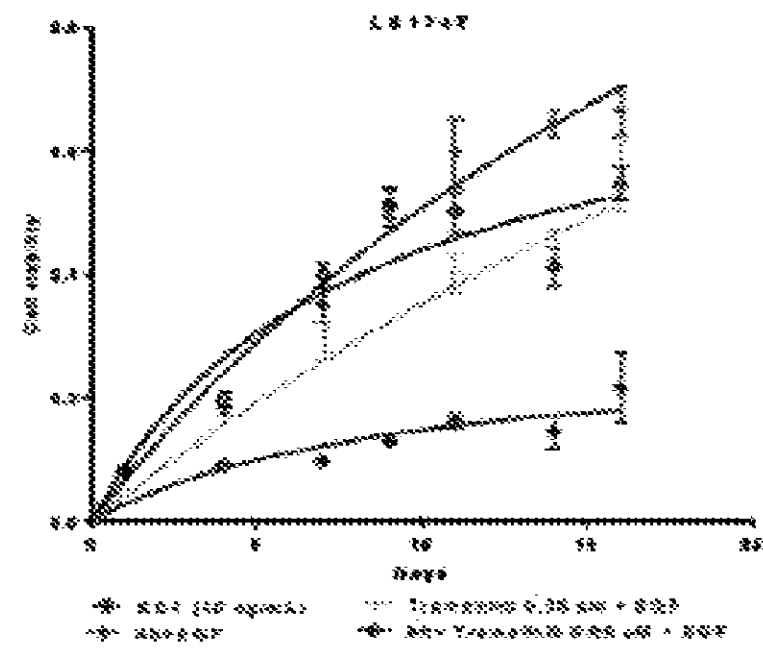


FIG. 52

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Emergence of resistance to Trametinib (LS174T)

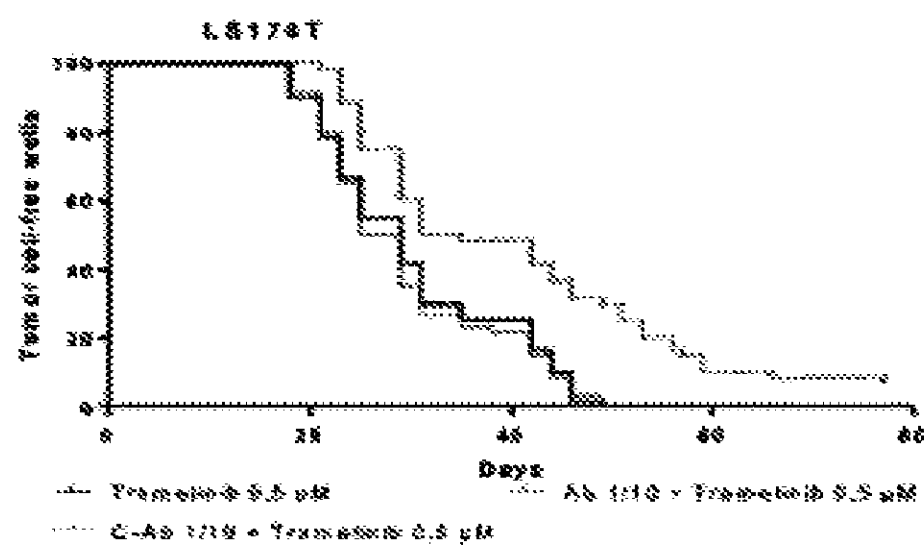


FIG. 53

Trametinib with/without EGF in HT29

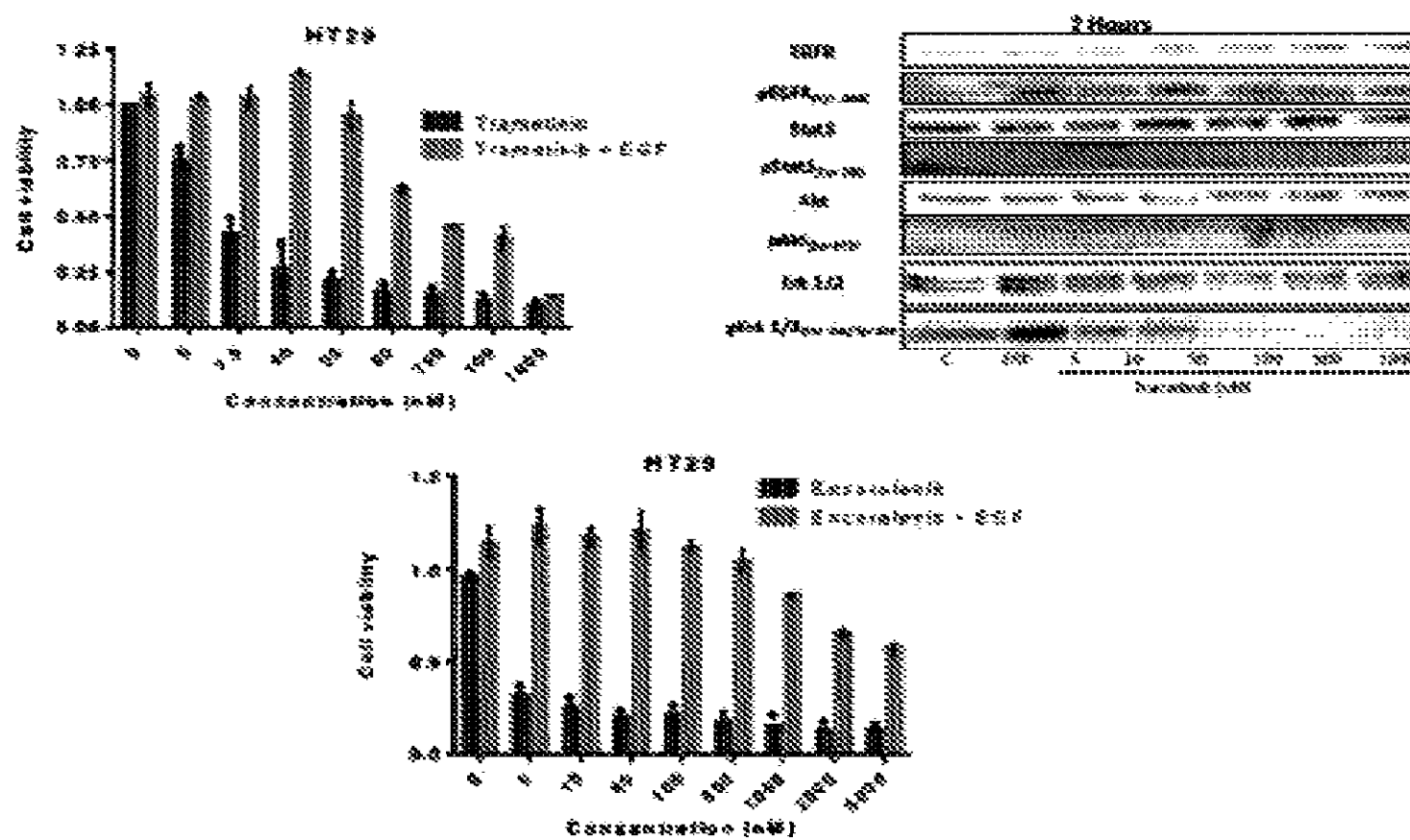


FIG. 54

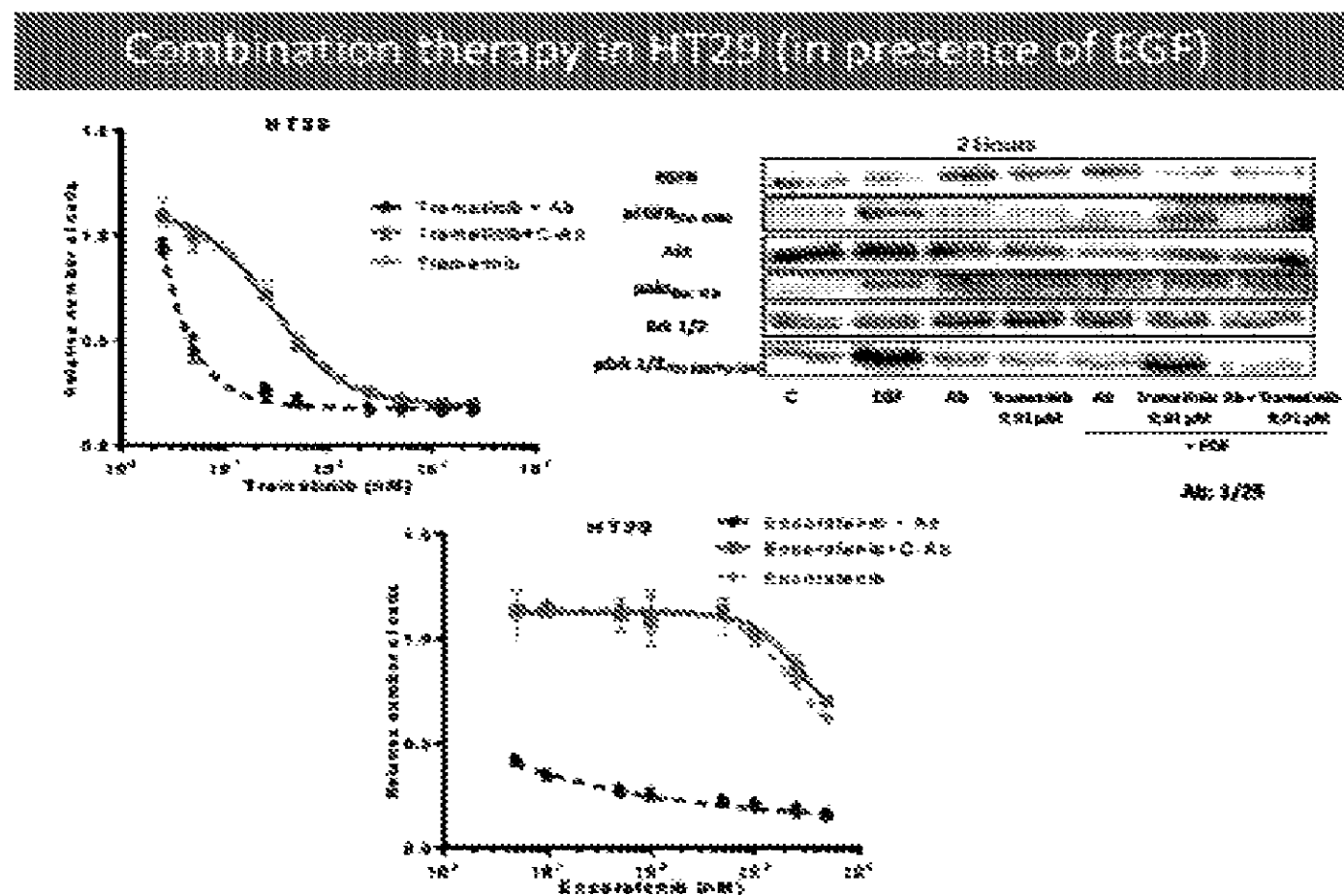


FIG. 55

Inhibitory effects of the combination (HT29)

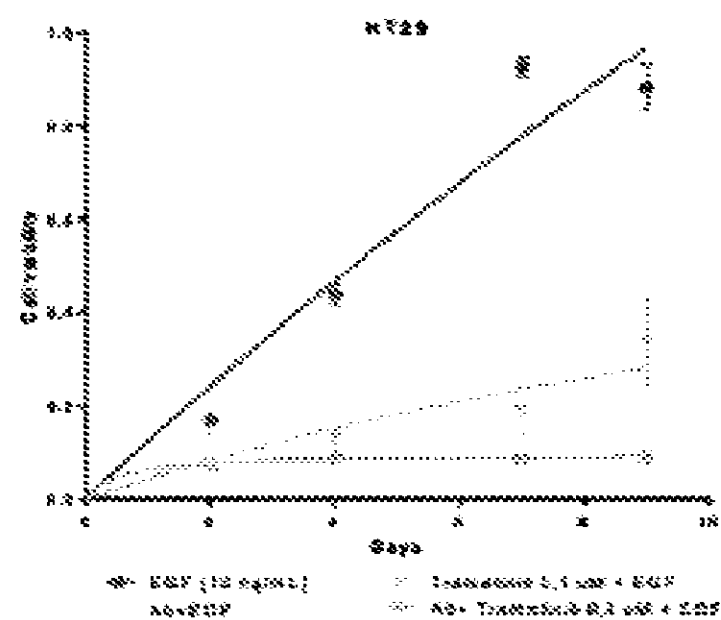


FIG. 56

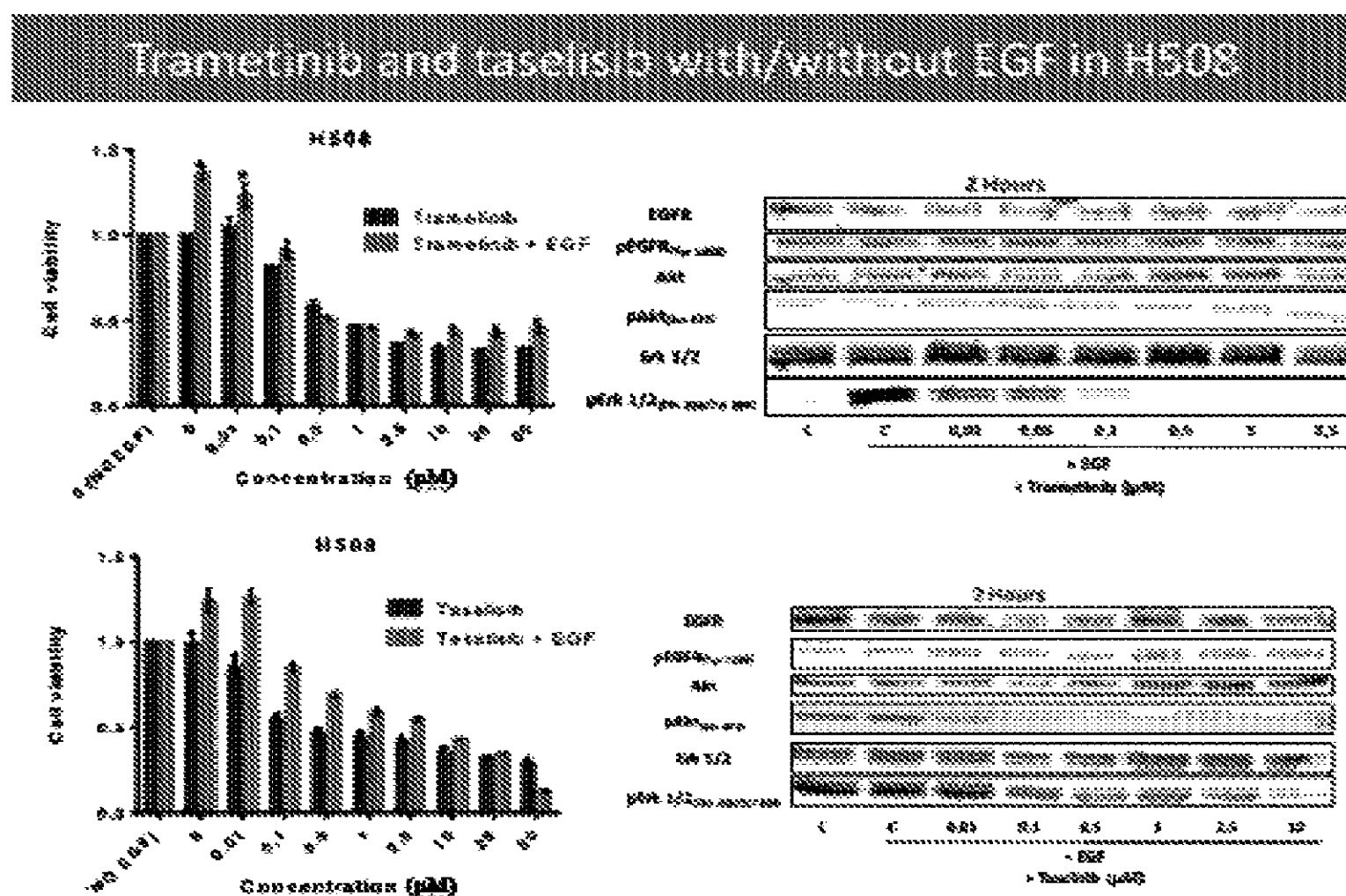


FIG. 57

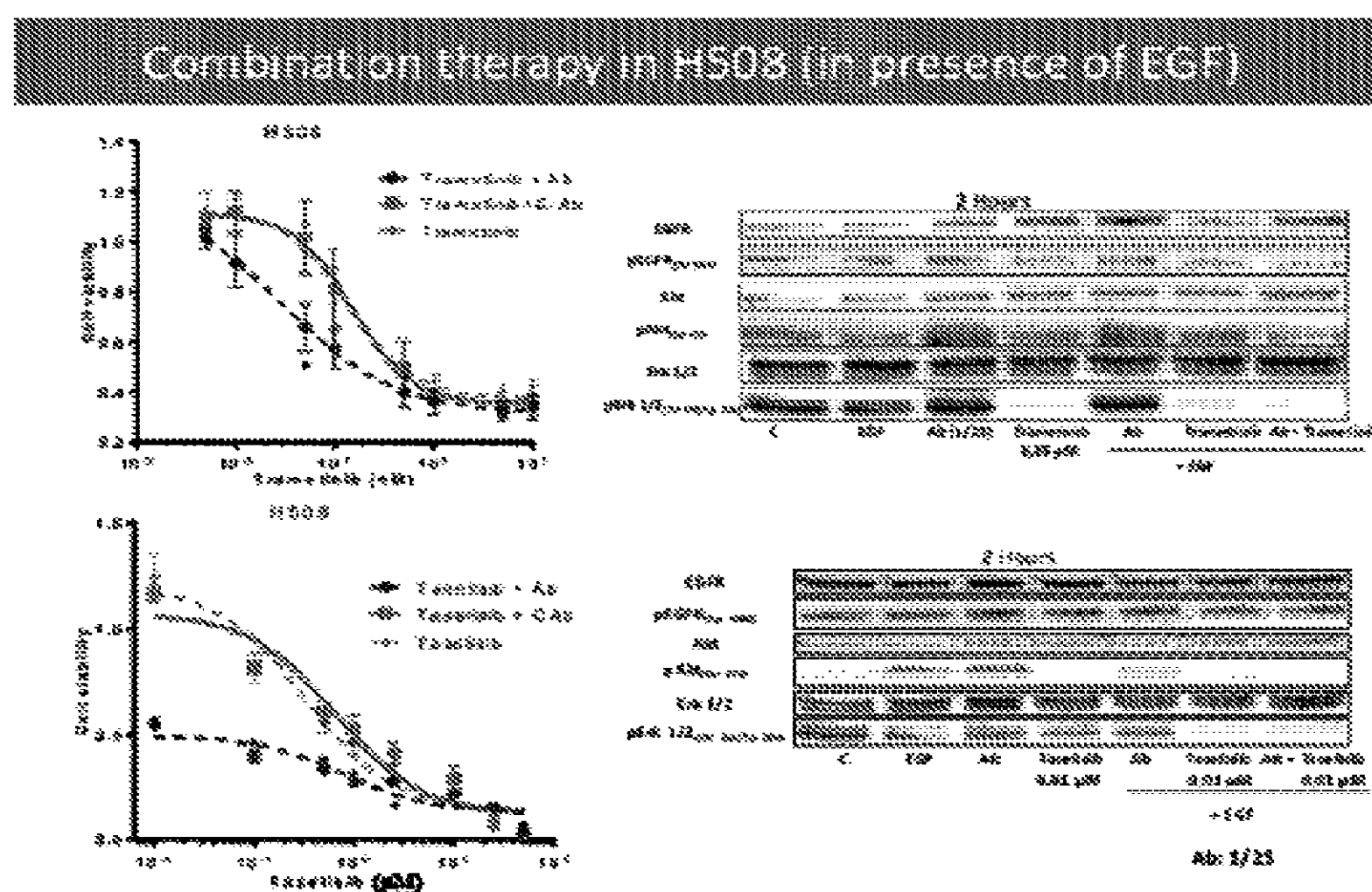


FIG. 58

Combination therapy in H508 (in presence of EGF)

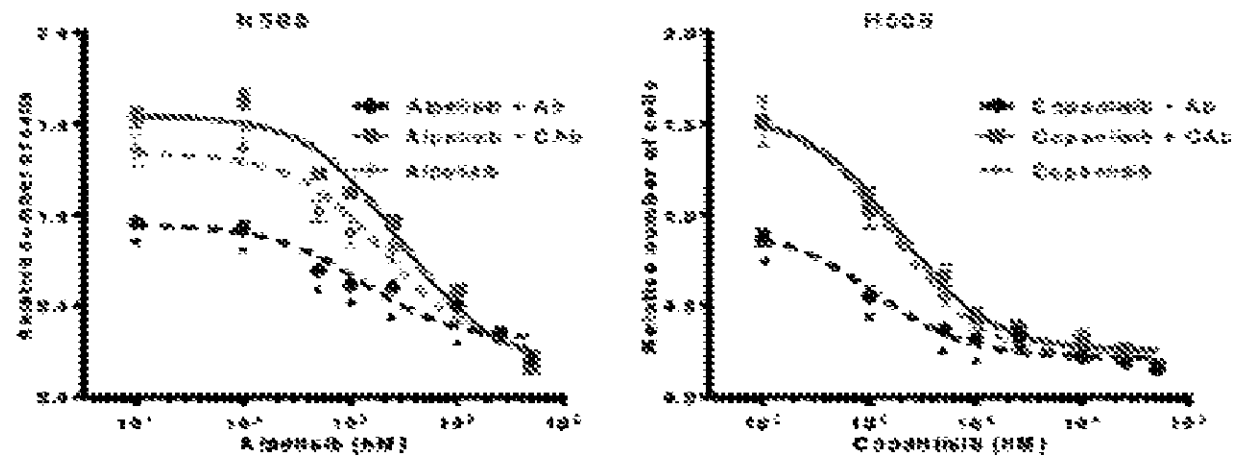


FIG. 59

Cell cycle effects of the combination (H508)

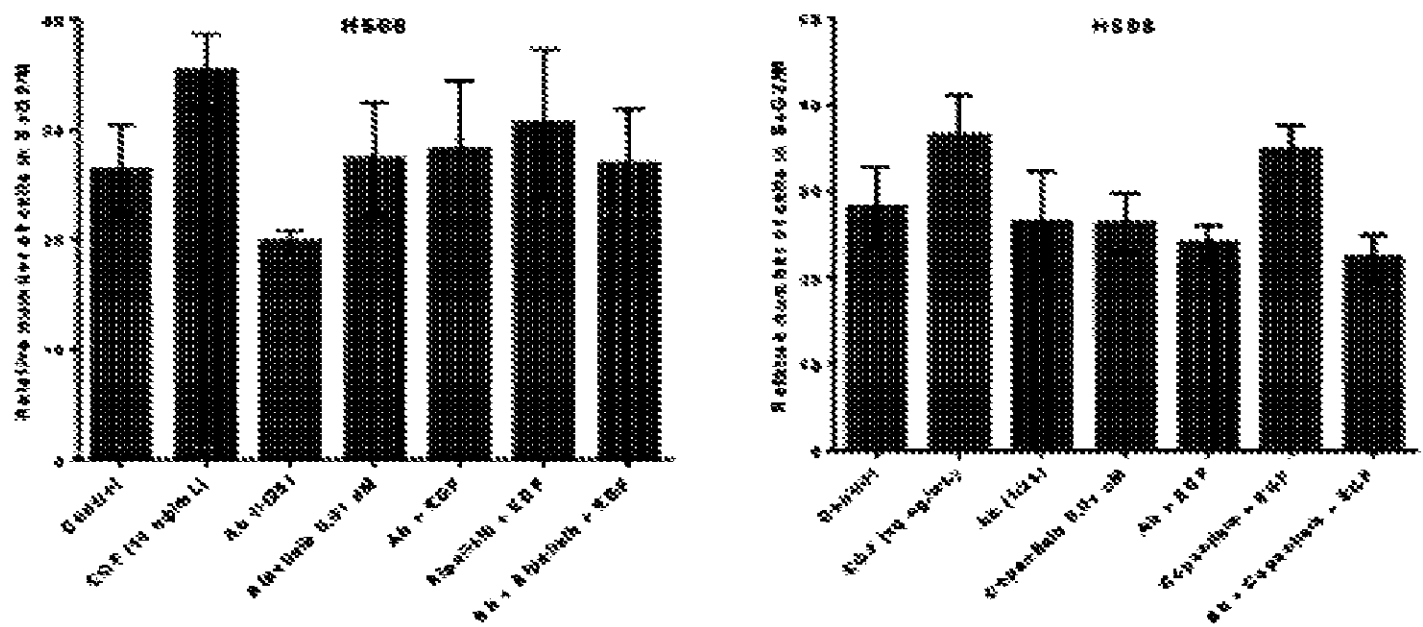


FIG. 60

inhibitory effects of the combination (H508)

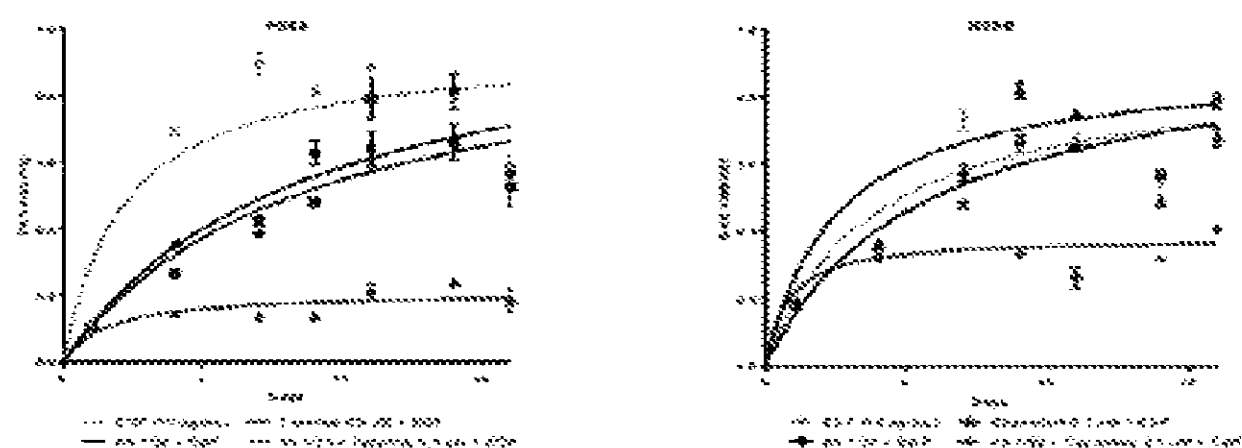


FIG. 61

Emergence of resistance to Taselisib (H508)

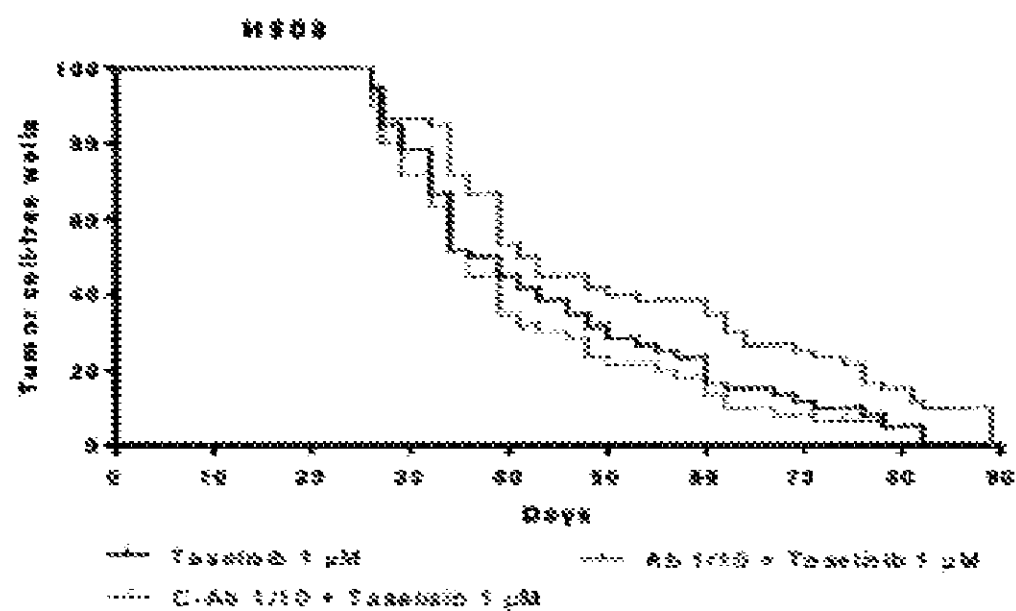


FIG. 62

CELL LINE	HISTOLOGY	RELEVANT ALTERATION
HCC15	NSCLC (squamous carcinoma)	<i>NRAS</i> mutant (p.Q61K)
HT29	Colorectal ADC	<i>BRAF</i> mutant (p.V600E)

DRUG	ACTIVITY
Trametinib	MEK inhibitor
Encorafenib	BRAF inhibitor

FIG. 63

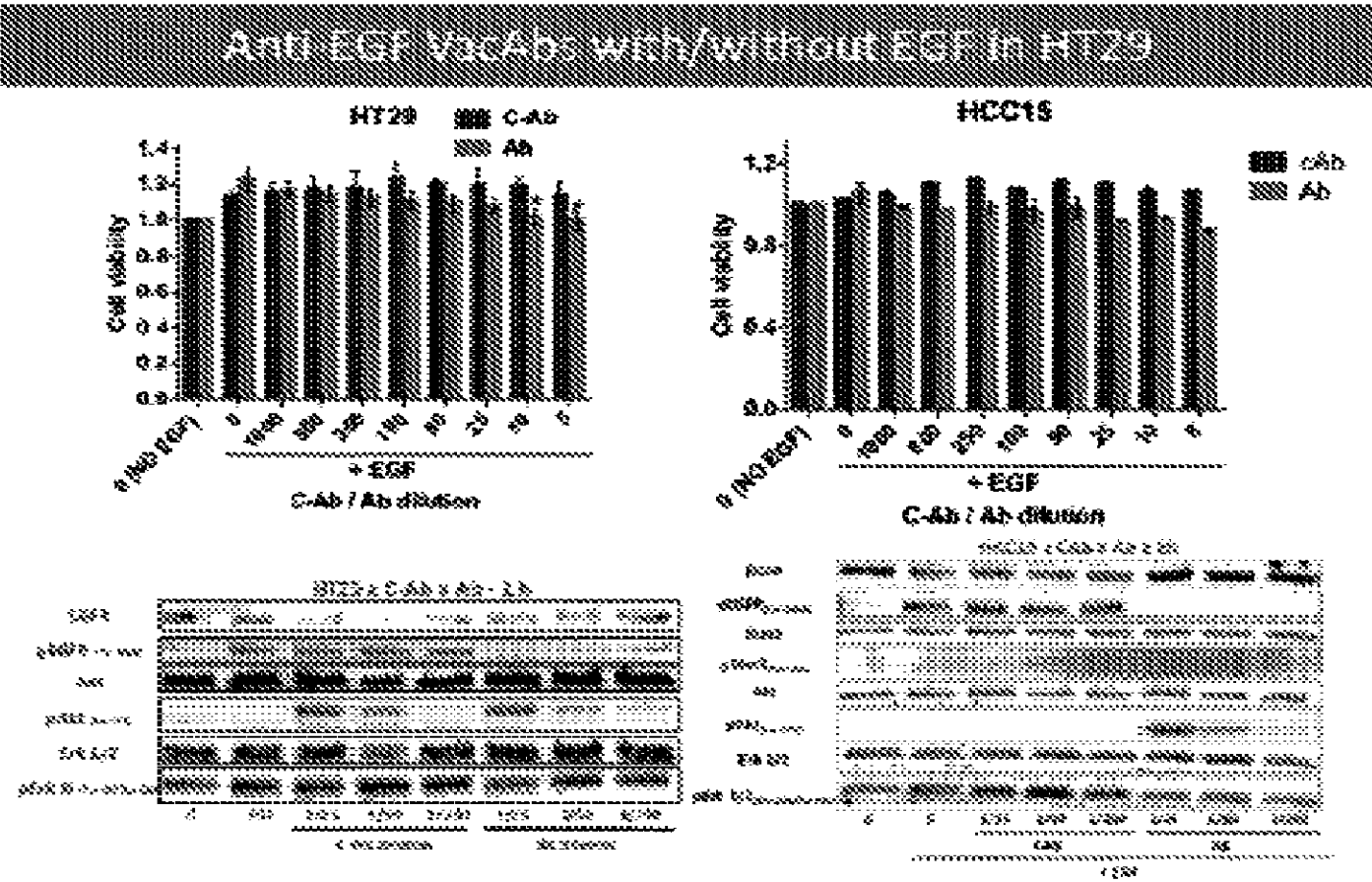


FIG. 64

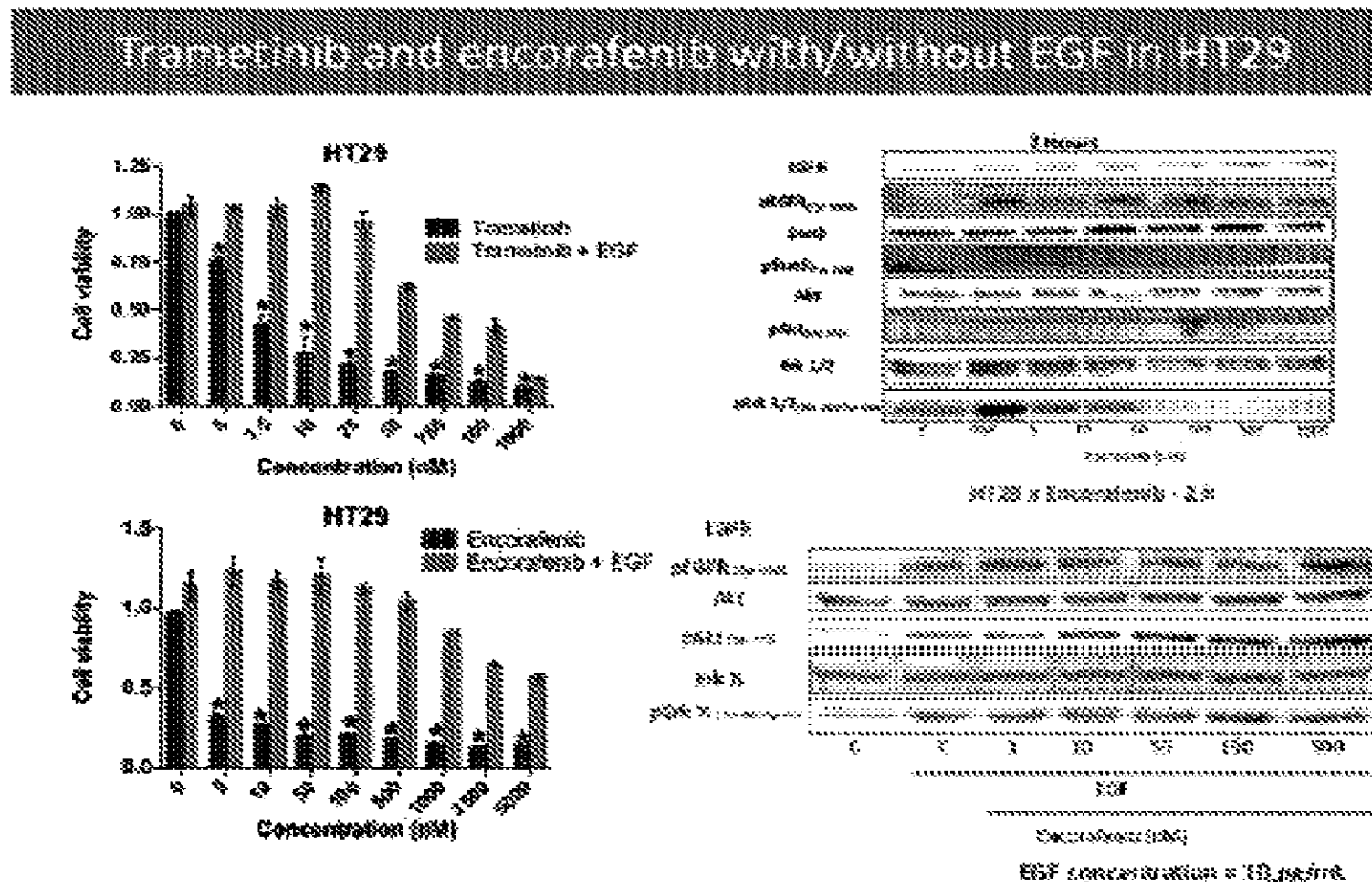


FIG. 65

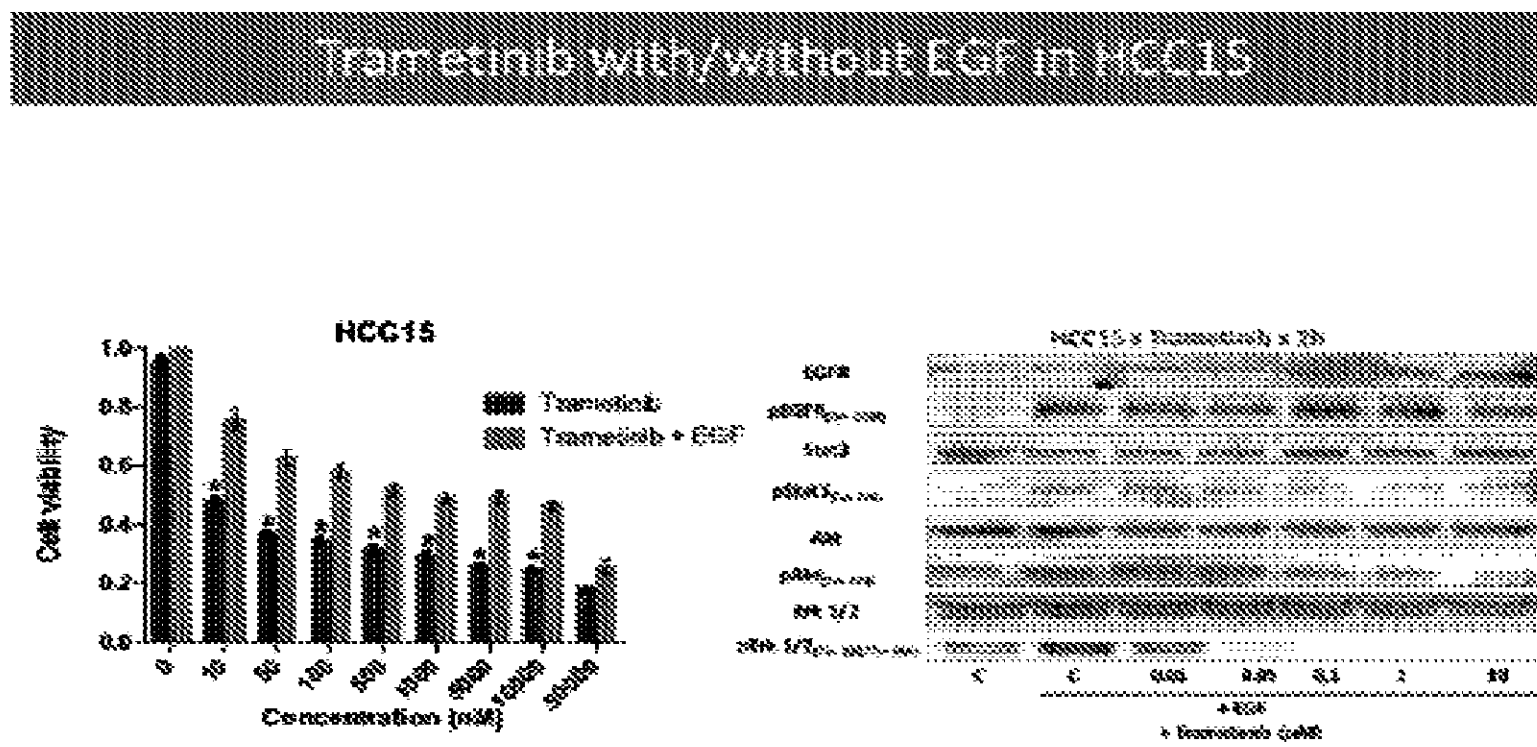


FIG. 66

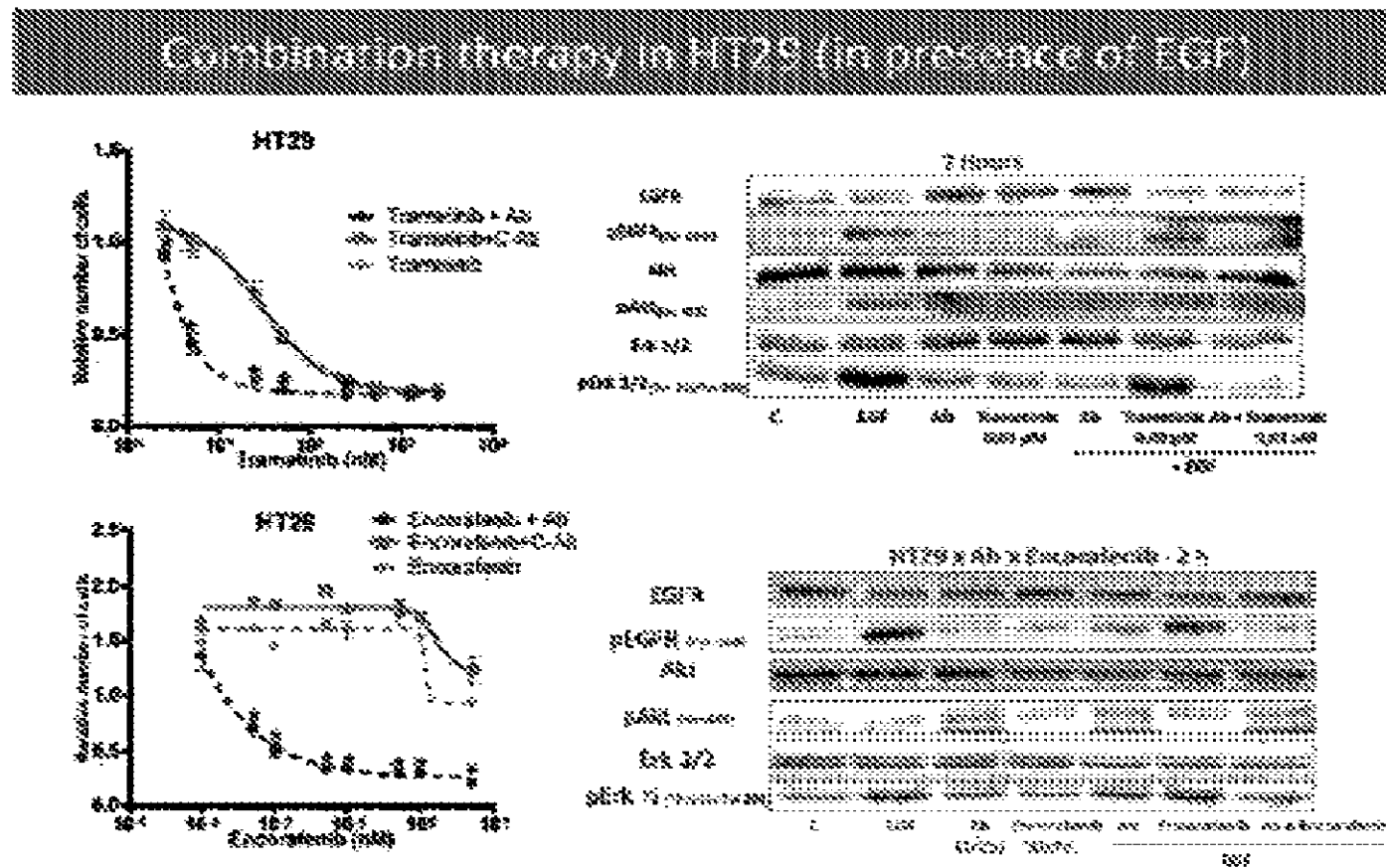


FIG. 67

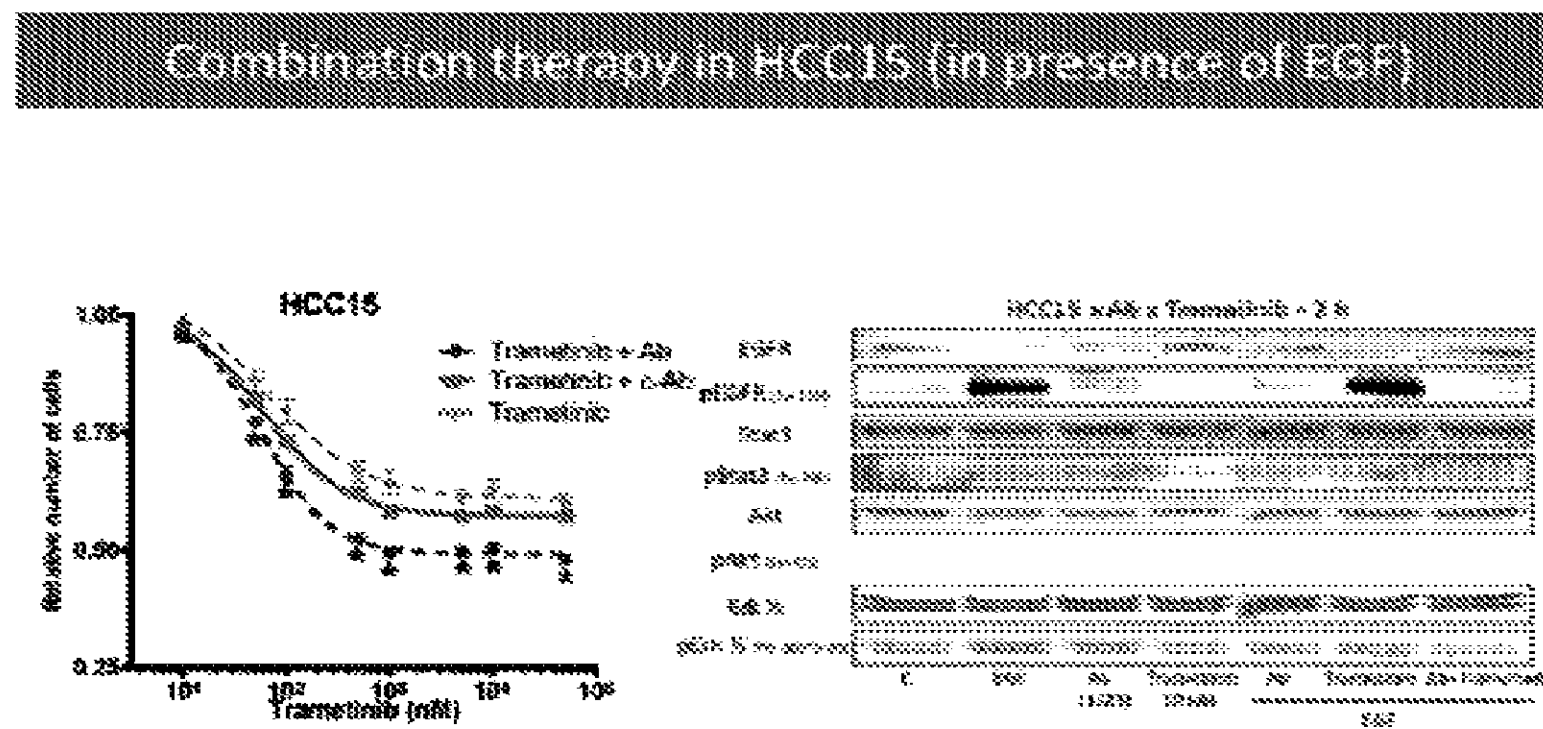


FIG. 68

Inhibitory effects of the combinations at 10 ng/mL EGF (HT29)

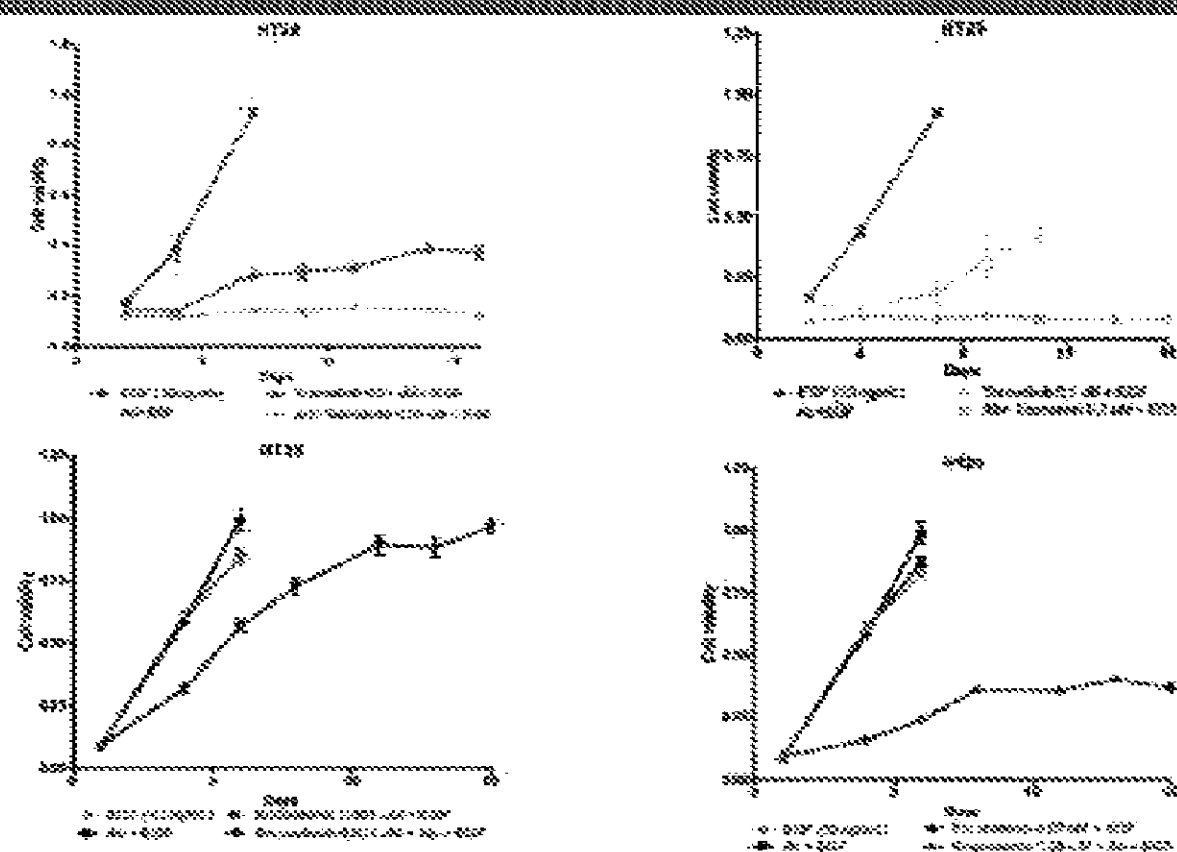


FIG. 69

Inhibitory effects of the combination in presence of physiological concentrations of hEGF (in HT29 cells)

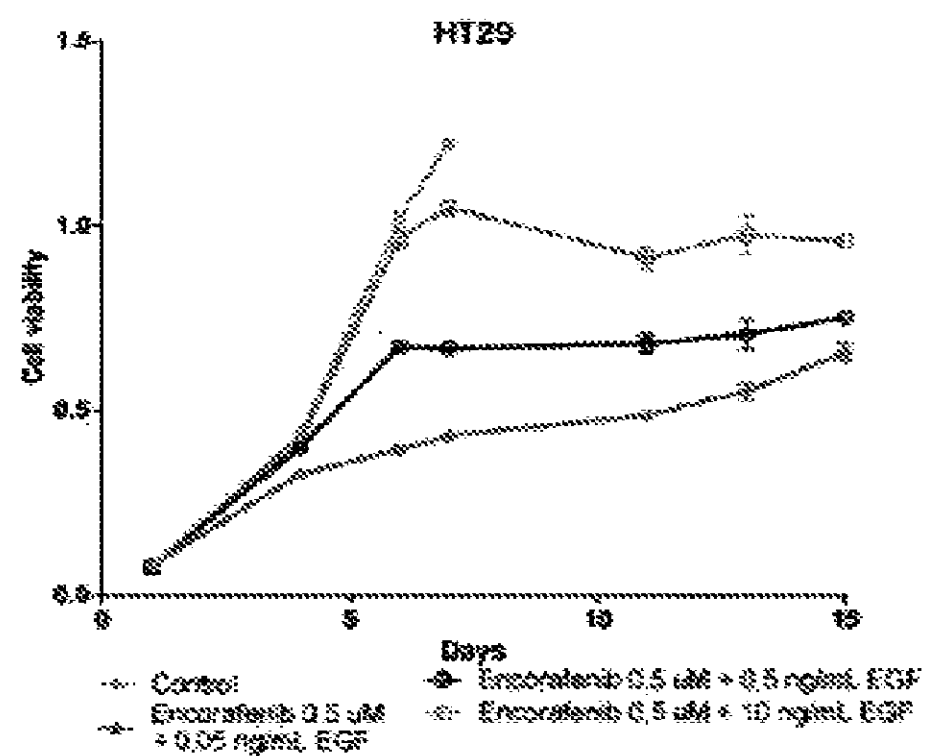


FIG. 70

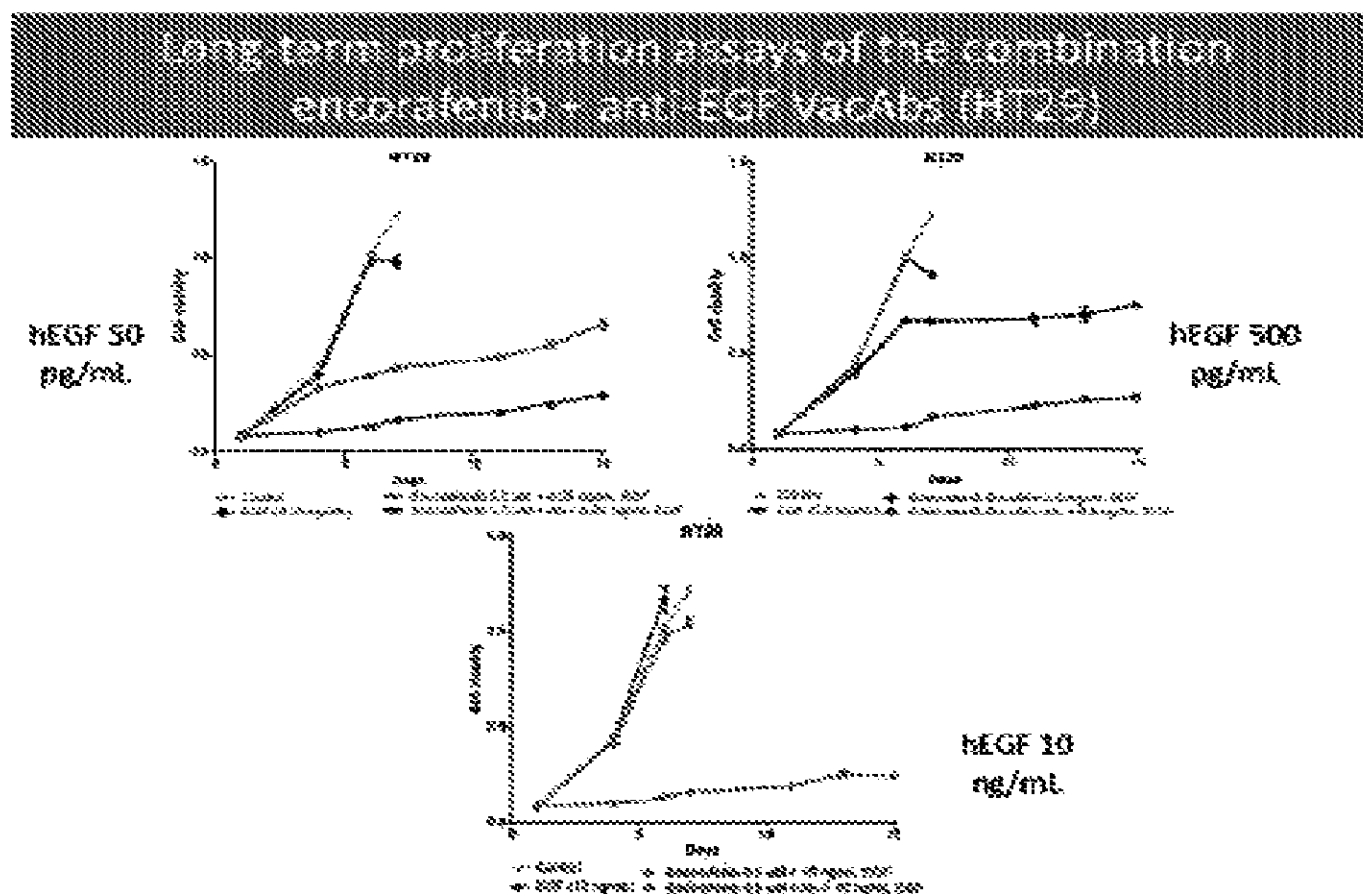


FIG. 71

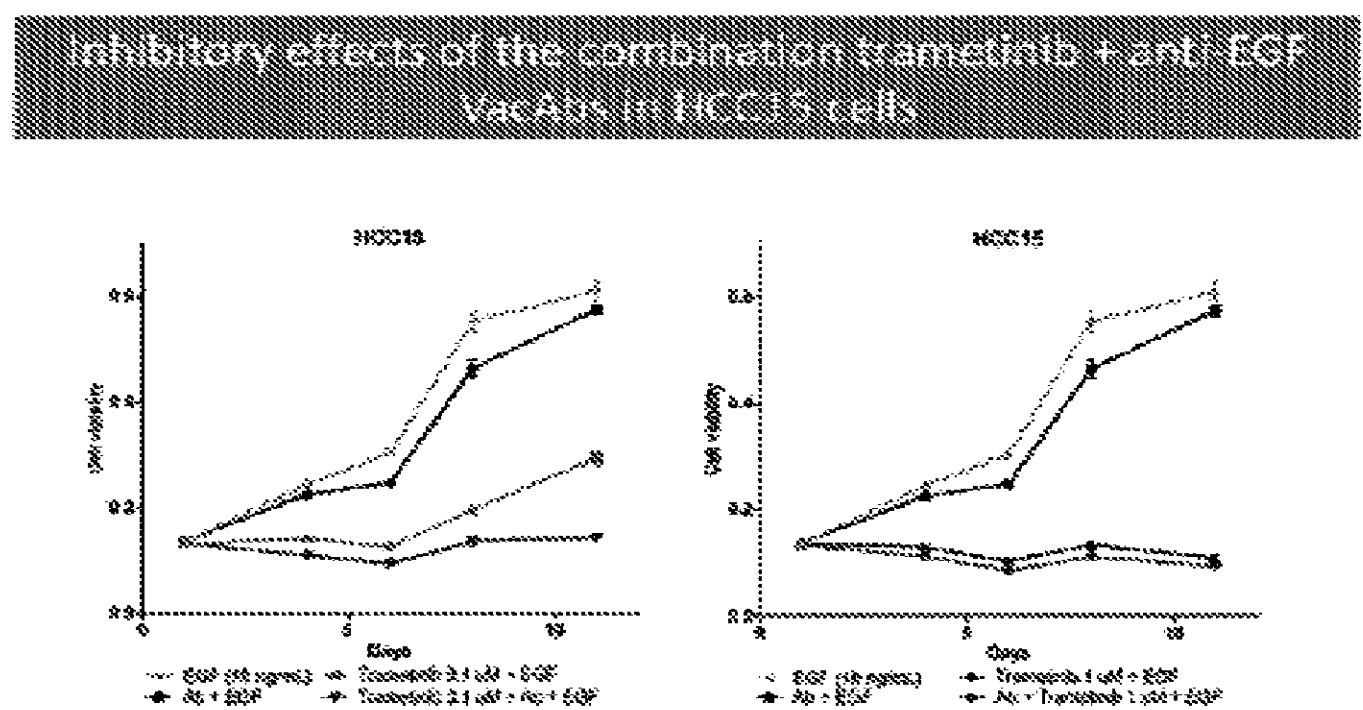


FIG. 72

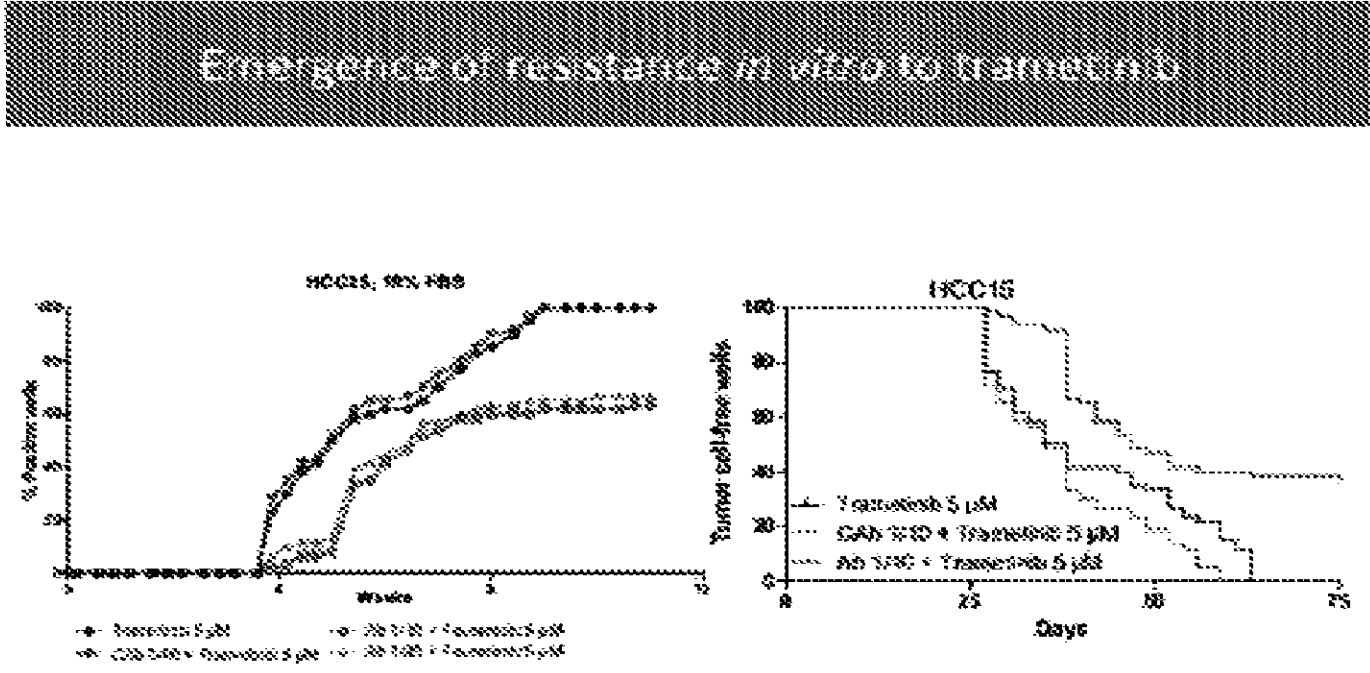


FIG. 73

CELL LINE	HISTOLOGY	RELEVANT ALTERATION	OTHER ALTERATION
H150B	Colonial AdC	PI3KCA mutant (p.E545K)	BRAC1 mutant (p.G556R)

DRUG	ACTIVITY
Alpelisib	PI3KCA inhibitor
Copanlisib	PI3KCA inhibitor

FIG. 74

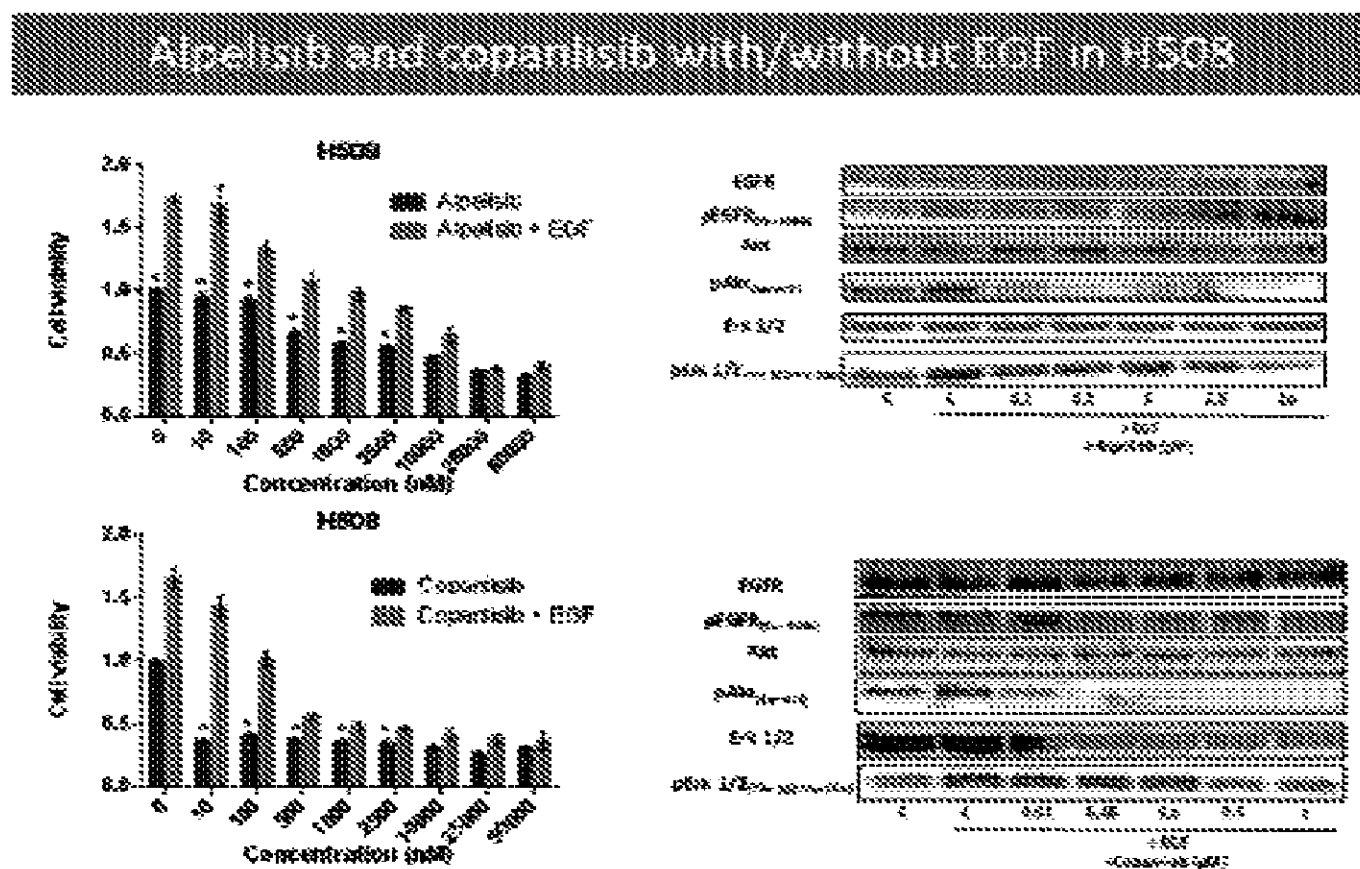


FIG. 75

Combination therapy in H508 (in presence of EGF)

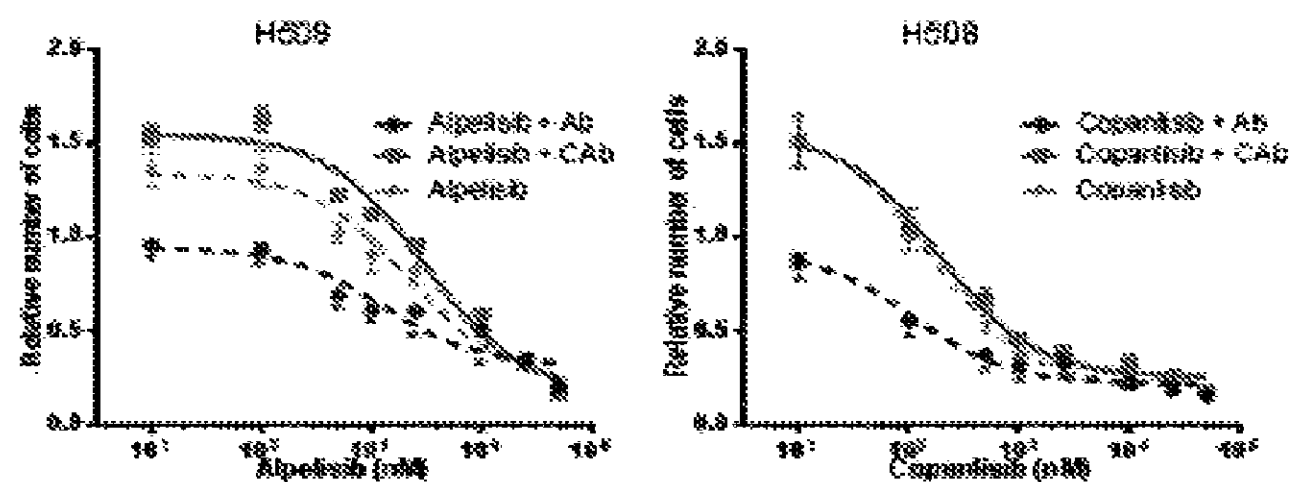


FIG. 76

Inhibitory effects of the combination (H508)

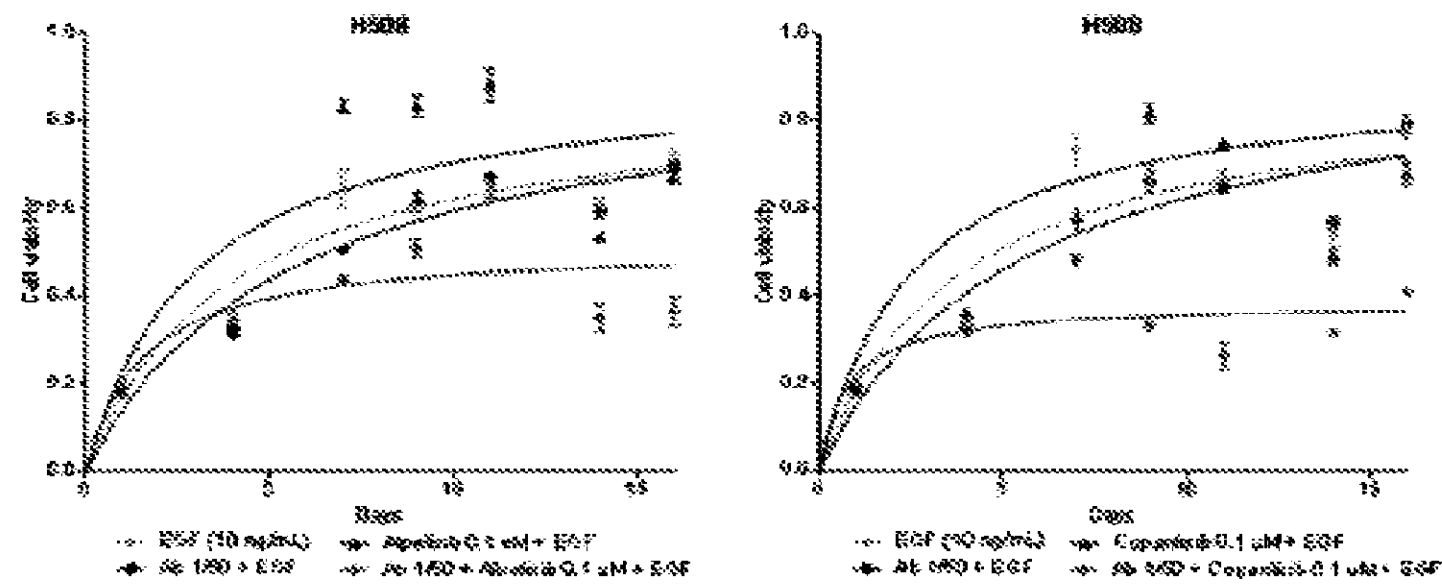


FIG. 77

CELL LINE	HISTOLOGY	RELEVANT ALTERATION
LC-L/ac	Lung ADC	RET translocated (+CD65-RET)

DRUG	ACTIVITY
BLU667	RET inhibitor

FIG. 78

BLU667 with/without EGF in LC-2/ad

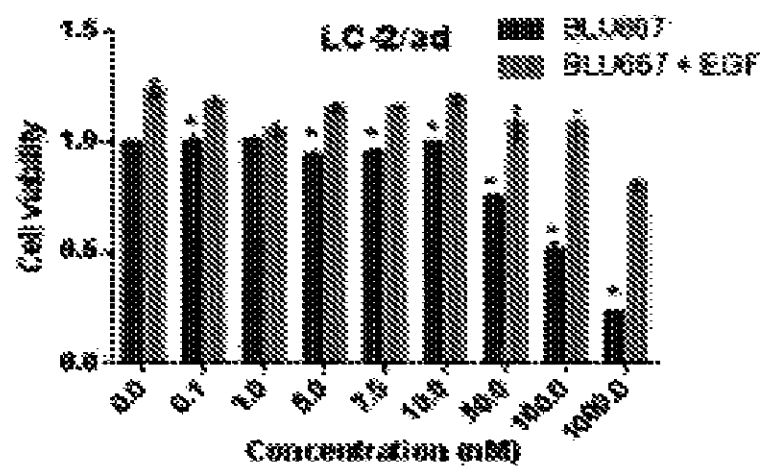


FIG. 79

Combination therapy in LC-2/ad (in presence of EGF)

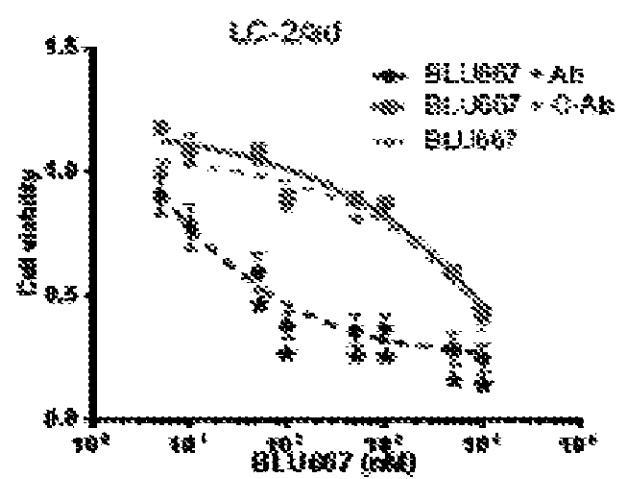


FIG. 80

inhibitory effects of the combination (LC-2/ad)

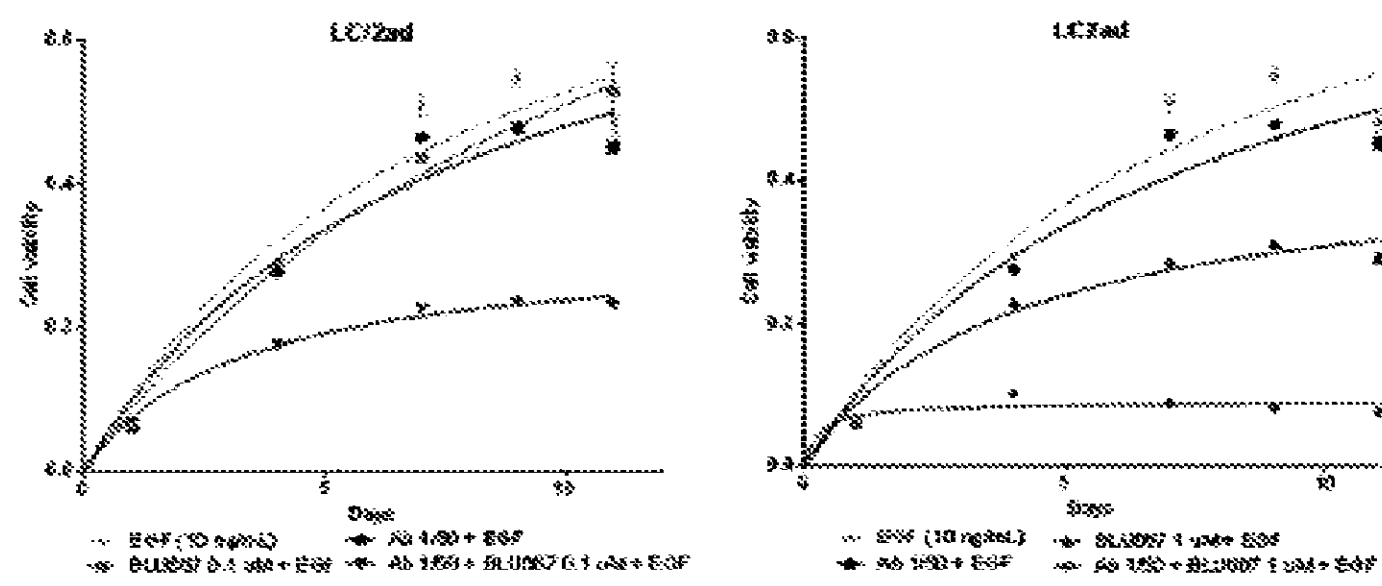


FIG. 81

CELL LINE	HISTOLOGY	RELEVANT ALTERATION
EBC-1	Squamous Cell Lung Carcinoma	MET amplification
Hs746T	Gastric Adenocarcinoma	MET amplification MET exon 14 deletion

DRUG	ACTIVITY
Capmatinib	MET inhibitor
Tepotinib	MET inhibitor

FIG. 82

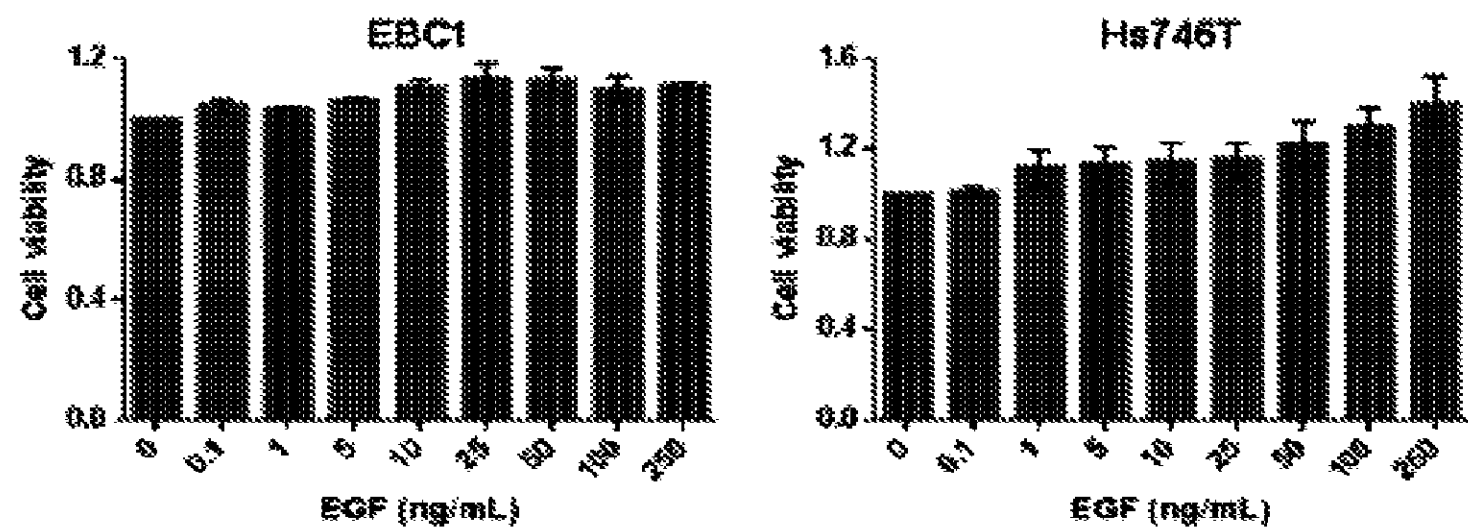


FIG. 83

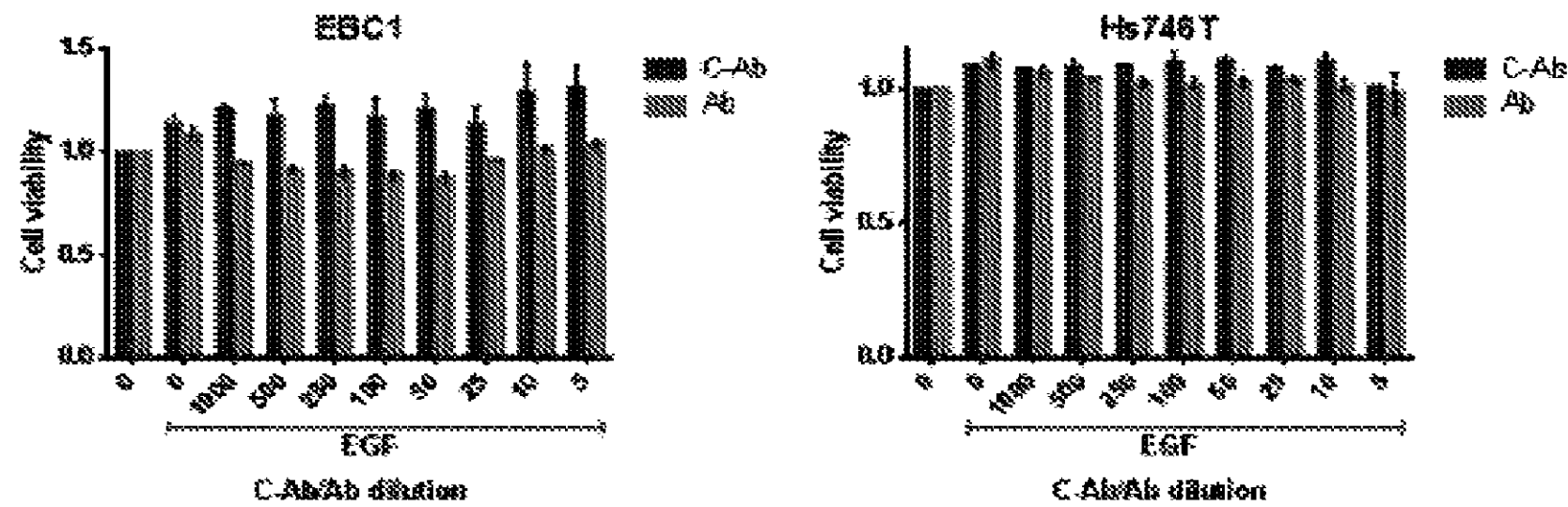


FIG. 84

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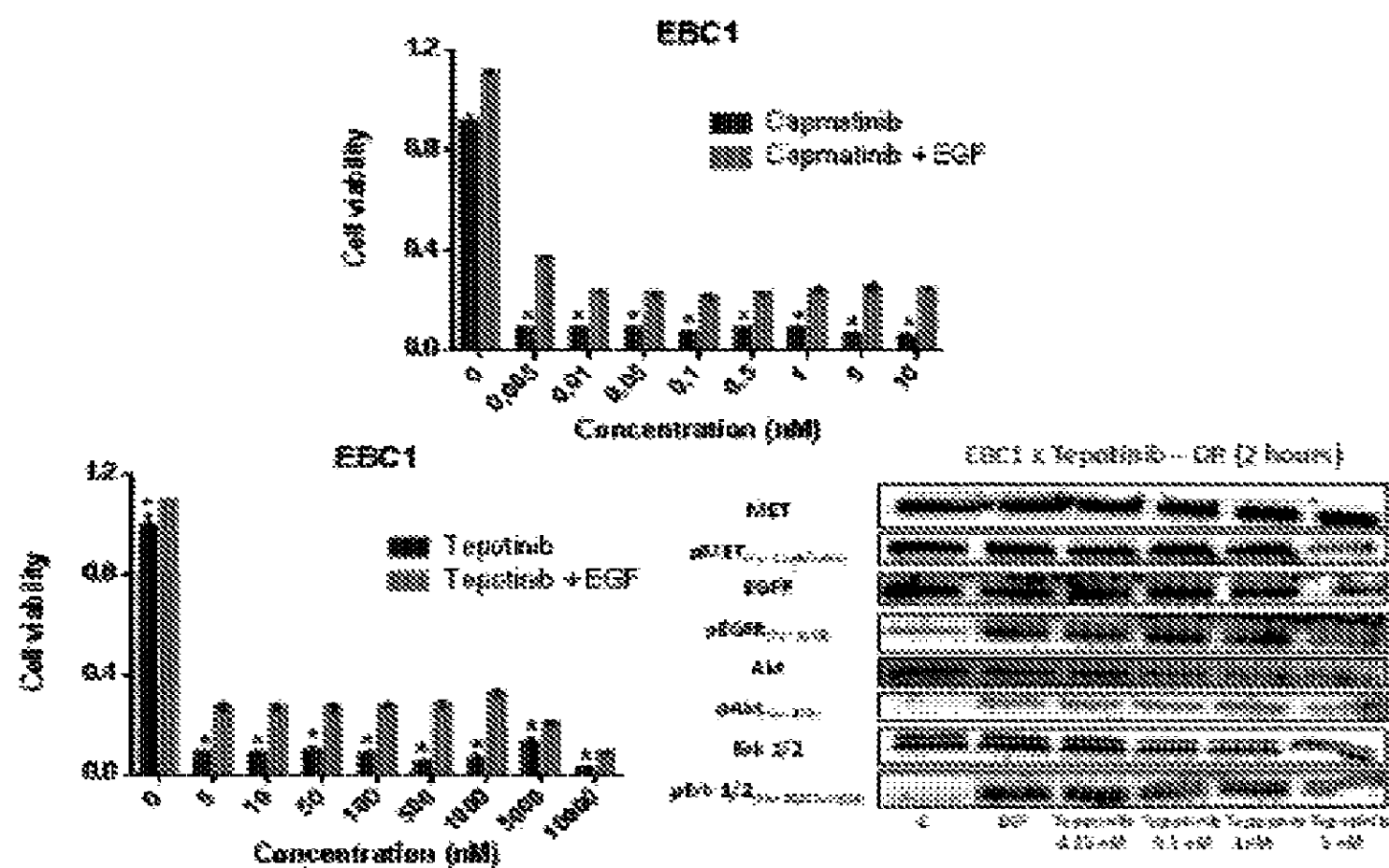


FIG. 85

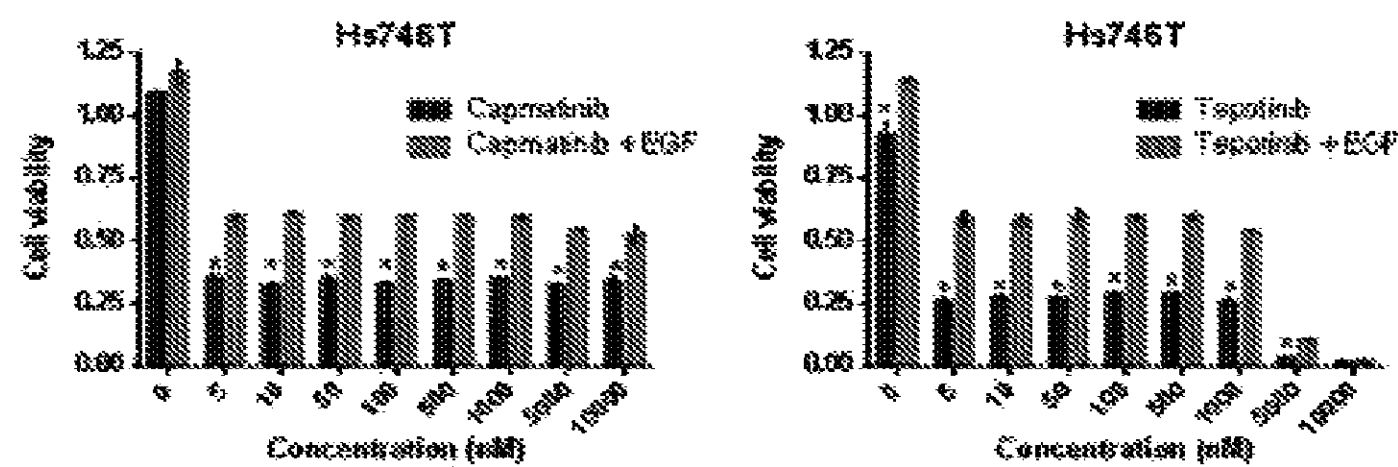


FIG. 86

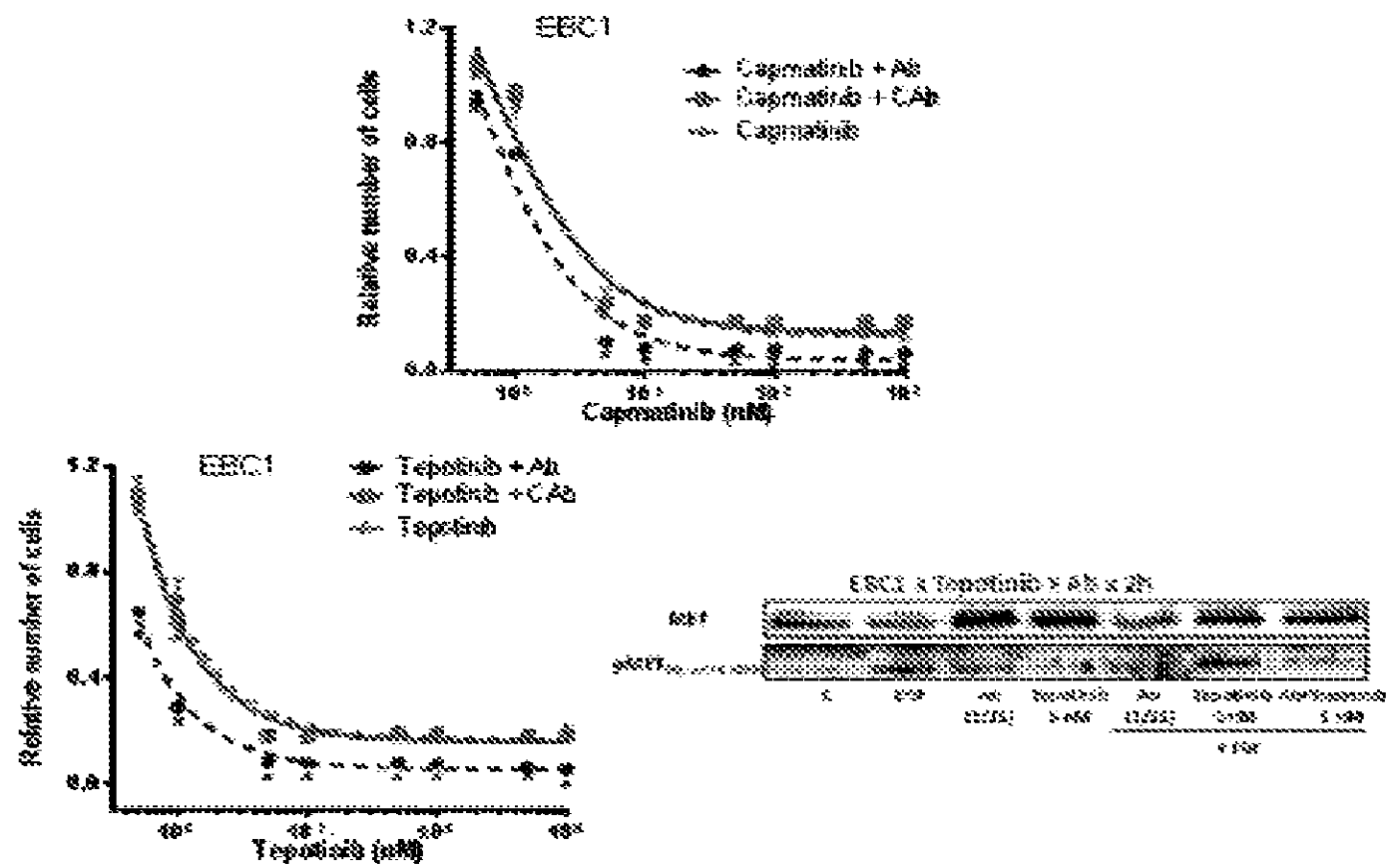


FIG. 87

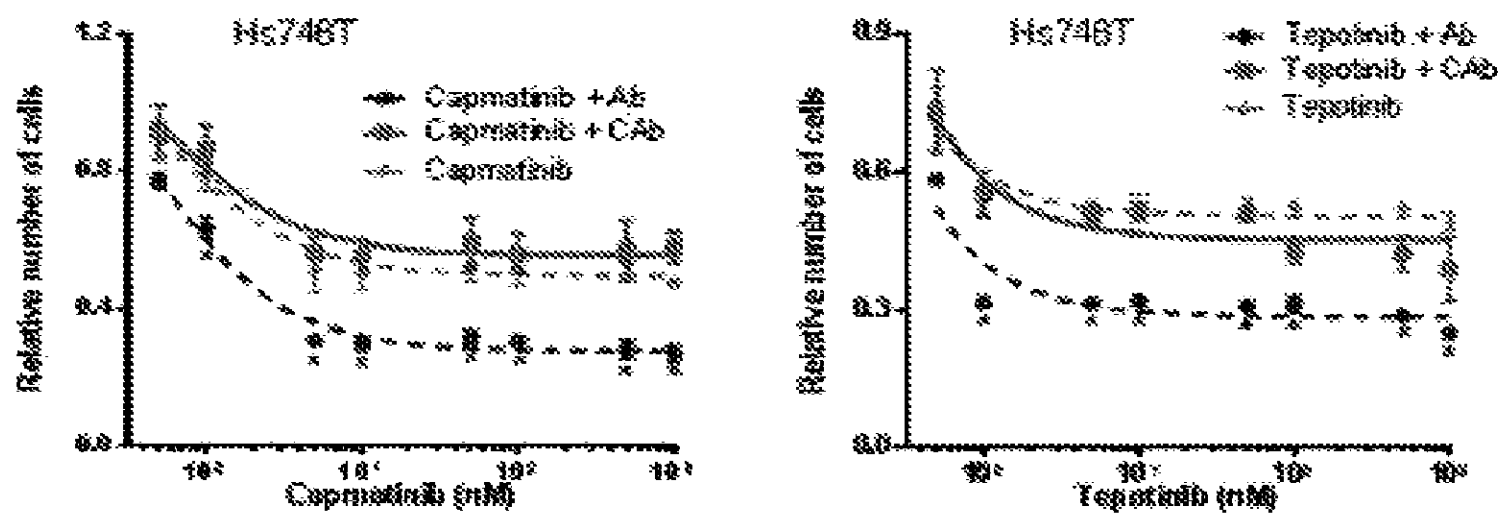


FIG. 88

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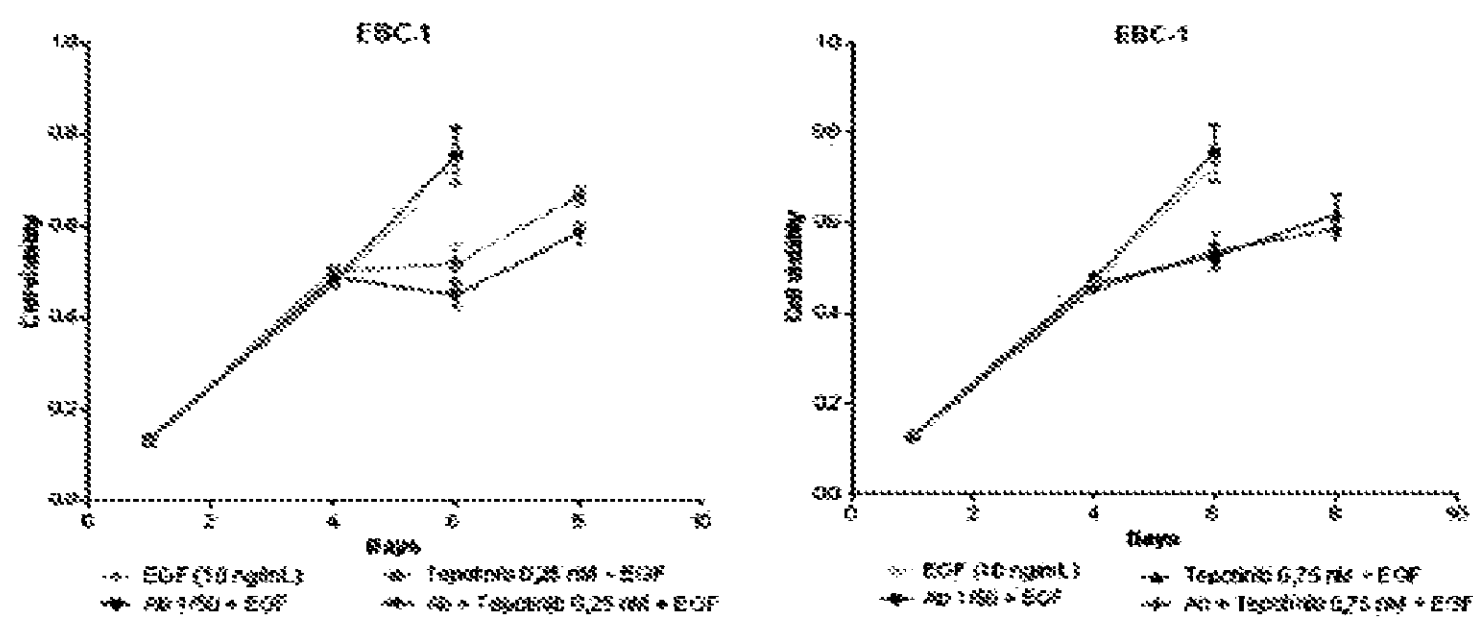


FIG. 89

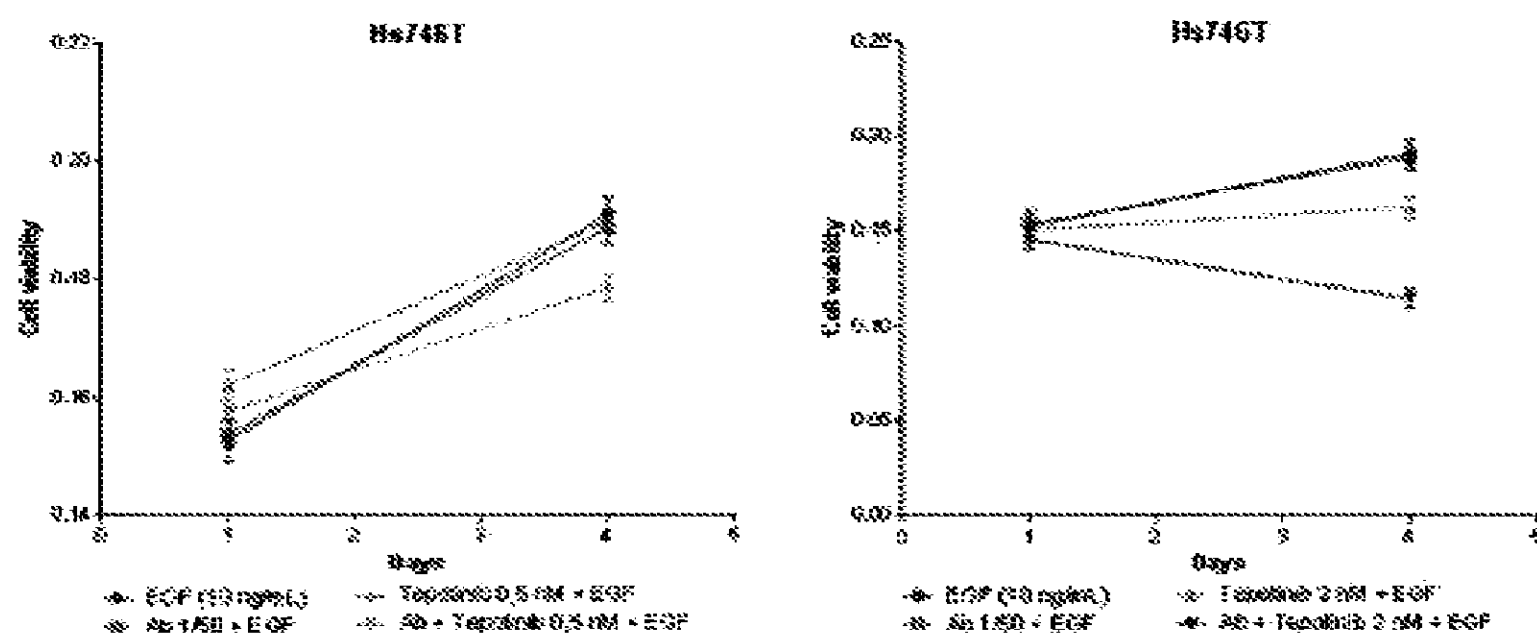


FIG. 90

4818-7501-1537.2