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(54) Title: COMPOSITIONS AND METHODS FOR TREATING PATIENTS WITH RTK MUTANT CELLS

(57) Abstract: Disclosed herein are compositions and methods for treating cancer patients who have been previously treated with one or more chemotherapeutic agents and have developed at least partial resistance to such chemotherapeutic agents. Also disclosed are methods for selecting compounds suitable for treatment of cancer in a patient who has become resistant to an inhibitor of a receptor tyrosine kinase (RTK).

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COMPOSITIONS AND METHODS FOR TREATING PATIENTS WITH RTK MUTANT CELLS

RELATED APPLICATIONS

[0001] The present application claims priority to U.S. Provisional Patent Application Serial No. 62/168,237, filed on May 29, 2015, and U.S. Provisional Patent Application Serial No. 62/309,900, filed on March 17, 2016. The contents of the above-referenced applications are hereby expressly incorporated by reference in their entireties.

INCORPORATION OF THE SEQUENCE LISTING

[0002] The material in the accompanying sequence listing is hereby incorporated by reference into this application. The accompanying sequence listing text file, named IGNYT.051WO_Sequence Listing, was created on May 5, 2016 and is 69 KB. The file can be assessed using Microsoft Word on a computer that uses Windows OS.

FIELD

[0003] The present disclosure relates to compositions and methods for treating cancer patients, for example cancer patients who have been previously treated with one or more chemotherapeutic agents and have developed at least partial resistance to the one or more chemotherapeutic agents.

BACKGROUND

[0004] The materials described in this section are not admitted to be prior art by inclusion in this section.

[0005] Cancer chemotherapy, particularly with a combination of anti-cancer agents, has increasingly become the treatment of choice for delocalized tumors that are untreatable by surgery or radiation. However, in many cases the cancers acquire resistance to these chosen chemotherapeutics and ultimately become refractory to treatment. As a result,

some patients relapse after even a short period of time, and do not respond to a second course of chemotherapy.

[0006] The underlying cause of progressive drug resistance is generally related to spontaneous genetic mutations which occur in all living cells, which mutations are inheritable and may be passed on to succeeding generations. In any cell population, including cancer cell populations, mutants that are resistant to any given drug occur at a frequency of somewhere between one in 10^5 and one in 10^8 cells. Although this is a very rare event, it can have a large impact on the outcome of chemotherapy.

[0007] Therefore, there is a need for the determining the underlying causes of such resistance so that suitable diagnostic tests can be developed and more effective treatments can be provided. Moreover, there is a need for new compounds that are able to treat patients that show cancer progression or relapse despite initial response to current tyrosine kinase inhibitors.

SUMMARY

[0008] This section provides a general summary of the disclosure, and is not comprehensive of its full scope or all of its features.

[0009] In one aspect, disclosed herein are methods for treating cancer in a patient, comprising (a) acquiring knowledge of the presence of one or more molecular alterations in a biological sample from the patient, wherein the one or more molecular alterations includes one or more mutations in one or more receptor tyrosine kinase polypeptides selected from TrkA, TrkB, TrkC, ALK and ROS1; (b) selecting a chemotherapeutic agent appropriate for the treatment of the cancer; and (c) administering a therapeutically effective amount of the selected chemotherapeutic agent to the patient.

[0010] Implementations of the methods disclosed herein can include one or more of the following features. In some embodiments, the one or more mutation includes one or more amino acid substitutions in a kinase catalytic domain of the one or more receptor tyrosine kinase polypeptides. In some embodiments, the one or more one amino acid substitutions is at a position corresponding to an amino acid residue selected from the amino acid residues identified in FIG. 1 and/or TABLE 1 as conserved residues, and combinations of any thereof. In some embodiments, the one or more amino acid substitutions is at a

position corresponding to an amino acid residue selected from V573, F589, G595 and G667 of the TrkA polypeptide of SEQ ID NO: 1; V619, F633, G639 and G709 of the TrkB polypeptide of SEQ ID NO: 3; V603, F618, G623 and G696 of the TrkC polypeptide of SEQ ID NO: 5; V1182, L1196, G1202 and G1269 of the ALK polypeptide of SEQ ID NO: 7; and L2012, L2026, G2032 and G2101 of the ROS1 polypeptide of SEQ ID NO: 9. In some embodiments, the one or more amino acid substitutions is at a position corresponding to amino acid residue V573 of the TrkA polypeptide of SEQ ID NO: 1. In some embodiments, the one or more amino acid substitutions is a Val-to-Met substitution at a position corresponding to amino acid residue V573 of the TrkA polypeptide (V573M). In some embodiments, the one or more amino acid substitutions is at a position corresponding to amino acid residue F589 of the TrkA polypeptide of SEQ ID NO: 1. In some embodiments, the one or more amino acid substitutions is a Phe-to-Leu substitution at a position corresponding to amino acid residue F589 of the TrkA polypeptide (F589L). In some embodiments, the one or more amino acid substitutions is at a position corresponding to amino acid residue G595 of the TrkA polypeptide of SEQ ID NO: 1. In some embodiments, the one or more amino acid substitutions is a Gly-to-Arg substitution at a position corresponding to amino acid residue G595 of the TrkA polypeptide (G595R). In some embodiments, the one or more amino acid substitutions is at a position corresponding to amino acid residue G667 of the TrkA polypeptide of SEQ ID NO: 1. In some embodiments, the one or more amino acid substitutions is a Gly-to-Cys substitution at a position corresponding to amino acid residue G667 of the TrkA polypeptide (G667C). In some embodiments, the one or more amino acid substitutions is a Gly-to-Ala substitution at a position corresponding to amino acid residue G667 of the TrkA polypeptide (G667A). In some embodiments, the one or more amino acid substitutions is a Gly-to-Ser substitution at a position corresponding to amino acid residue G667 of the TrkA polypeptide (G667S). In some embodiments, the one or more amino acid substitutions is at a position corresponding to amino acid residue V619 of the TrkB polypeptide of SEQ ID NO: 3. In some embodiments, the one or more amino acid substitutions is a Val-to-Met substitution at a position corresponding to amino acid residue V619 of the TrkB polypeptide (V619M). In some embodiments, the one or more amino acid substitutions is at a position corresponding to amino acid residue F633 of the TrkB polypeptide of SEQ ID NO: 3. In some

embodiments, the one or more amino acid substitutions is a Phe-to-Leu substitution at a position corresponding to amino acid residue F633 of the TrkB polypeptide (F633L). In some embodiments, the one or more amino acid substitutions is at a position corresponding to amino acid residue G639 of the TrkB polypeptide of SEQ ID NO: 3. In some embodiments, the one or more amino acid substitutions is a Gly-to-Arg substitution at a position corresponding to amino acid residue G639 of the TrkB polypeptide (G639R). In some embodiments, the one or more amino acid substitutions is at a position corresponding to amino acid residue G709 of the TrkB polypeptide of SEQ ID NO: 3. In some embodiments, the one or more amino acid substitutions is a Gly-to-Cys substitution at a position corresponding to amino acid residue G709 of the TrkB polypeptide (G709C). In some embodiments, the one or more amino acid substitutions is a Gly-to-Ala substitution at a position corresponding to amino acid residue G709 of the TrkB polypeptide (G709A). In some embodiments, the one or more amino acid substitutions is a Gly-to-Ser substitution at a position corresponding to amino acid residue G709 of the TrkB polypeptide (G709S). In some embodiments, the one or more amino acid substitutions is at a position corresponding to amino acid residue V603 of the TrkC polypeptide of SEQ ID NO: 5. In some embodiments, the one or more amino acid substitutions is a Val-to-Met substitution at a position corresponding to amino acid residue V603 of the TrkC polypeptide of SEQ ID NO: 5 (V603M). In some embodiments, the one or more amino acid substitutions is at a position corresponding to amino acid residue F617 of the TrkC polypeptide of SEQ ID NO: 5. In some embodiments, the one or more amino acid substitutions is a Phe-to-Leu substitution at a position corresponding to amino acid residue F617 of the TrkC polypeptide of SEQ ID NO: 5 (F617L). In some embodiments, the one or more amino acid substitutions is at a position corresponding to amino acid residue G623 of the TrkC polypeptide of SEQ ID NO: 5. In some embodiments, the one or more amino acid substitutions is a Gly-to-Arg substitution at a position corresponding to amino acid residue G623 of the TrkC polypeptide (G623R). In some embodiments, the one or more amino acid substitutions is at a position corresponding to amino acid residue G696 of the TrkC polypeptide of SEQ ID NO: 5. In some embodiments, the one or more amino acid substitutions is a Gly-to-Cys substitution at a position corresponding to amino acid residue G696 of the TrkC polypeptide (G696C). In some embodiments, the one or more amino acid substitutions is a Gly-to-Ala substitution at a

position corresponding to amino acid residue G696 of the TrkB polypeptide (G696A). In some embodiments, the one or more amino acid substitutions is a Gly-to-Ser substitution at a position corresponding to amino acid residue G696 of the TrkB polypeptide (G696S).

[0011] In some embodiments, the patient has been previously treated with one or more receptor tyrosine kinase inhibitors and has developed at least partial resistance to the one or more receptor tyrosine kinase inhibitors described herein.

[0012] In some embodiments, the selected chemotherapeutic agent is selected from the group consisting of entrectinib, NVP-TAE684, rebastinib, Compound 2, and any pharmaceutically acceptable salt thereof.

[0013] In some embodiments, the cancer is selected from anaplastic large-cell lymphoma (ALCL), colorectal cancer (CRC), cholangiocarcinoma, gastric, glioblastomas (GBM), leiomyosarcoma, melanoma, non-small cell lung cancer (NSCLC), squamous cell lung cancer, neuroblastoma (NB), ovarian cancer, pancreatic cancer, prostate cancer, medullary thyroid cancer, breast cancer, and papillary thyroid cancer, or any combination thereof. In some embodiments, the biological sample from the patient includes sputum, bronchoalveolar lavage, pleural effusion, tissue, whole blood, serum, plasma, buccal scrape, saliva, cerebrospinal fluid, urine, stool, circulating tumor cells, circulating nucleic acids, bone marrow, or any combination thereof.

[0014] In some embodiments, the knowledge of the presence of the one or more molecular alterations is acquired from an analytical assay selected from nucleic acid sequencing, polypeptide sequencing, restriction digestion, capillary electrophoresis, nucleic acid amplification-based assays, nucleic acid hybridization assay, comparative genomic hybridization, real-time PCR, quantitative reverse transcription PCR (qRT-PCR), PCR-RFLP assay, HPLC, mass-spectrometric genotyping, fluorescent in-situ hybridization (FISH), next generation sequencing (NGS), and a kinase activity assay, or any combination thereof. In some embodiments, the analytical assay is an electrophoretic mobility assay in which a nucleic acid sequence encoding the mutation is detected by amplifying the nucleic acid region corresponding to the mutation in the receptor tyrosine kinase gene and comparing the electrophoretic mobility of the amplified nucleic acid to the electrophoretic mobility of the corresponding region in a wild-type receptor tyrosine kinase gene. In some embodiments, the analytical assay is an allele-specific polymerase chain reaction or next-generation

sequencing. In some embodiments, the analytical assay is a nucleic acid hybridization assay comprising contacting nucleic acids from the biological sample with a nucleic acid probe comprising a nucleic acid sequence complementary to a nucleic acid sequence encoding the one or more mutations and further including a detectable label.

[0015] In some embodiments, the knowledge of the presence of the one or more molecular alterations is acquired from an antibody-based assay selected from ELISA, immunohistochemistry, western blotting, mass spectrometry, flow cytometry, protein-microarray, immunofluorescence, and a multiplex detection assay, or any combination thereof. In some embodiments, the antibody-based assay includes one or more antibodies that specifically bind to one or more of TrkA, TrkB, TrkC, ALK, and ROS1 polypeptides.

[0016] In some embodiments, the selected chemotherapeutic agent, or a pharmaceutically acceptable salt thereof, is administered as a single therapeutic agent or in combination with one or more additional therapeutic agents.

[0017] In one aspect, some embodiments disclosed herein relate to methods for treating cancer in a patient, comprising (a) identifying a patient having one or more mutations at an amino acid position selected from V573, F589, G595 and G667 of the TrkA polypeptide of SEQ ID NO:1; V619, F633, G639 and G709 of the TrkB polypeptide of SEQ ID NO:3; V603, F617, G623 and G696 of the TrkC polypeptide of SEQ ID NO:5; V1182, L1196, G1202 and 1269 of the ALK polypeptide of SEQ ID NO:7; and L2012, L2026, G2032 and 2101 of the ROS1 polypeptide of SEQ ID NO:9; (b) selecting a chemotherapeutic agent that is appropriate for treating said patient having said one or more mutations; and (c) administering a therapeutically effective amount of the selected chemotherapeutic agent to the patient.

[0018] In one aspect, some embodiments disclosed herein relate to methods for selecting a patient having cancer who is predicted to have an increased risk of unresponsiveness to treatment with a therapeutic regimen, comprising (a) acquiring knowledge of the presence of one or more mutations in a biological sample from said patient, wherein the one or more mutations is at an amino acid position selected from V573, F589, G595 and G667 of the TrkA polypeptide of SEQ ID NO:1; V619, F633, G639 and G709 of the TrkB polypeptide of SEQ ID NO:3; V603, F617, G623 and G696 of the TrkC polypeptide of SEQ ID NO:5; V1182, L1196, G1202 and 1269 of the ALK polypeptide of

SEQ ID NO:7; and L2012, L2026, G2032 and 2101 of the ROS1 polypeptide of SEQ ID NO:9; and (b) selecting the patient as predicted to have an increased risk of unresponsiveness to treatment with a therapeutic regimen if one or more said mutations is detected in the biological sample, or selecting the patient as predicted to not have an increased risk of unresponsiveness to treatment with a therapeutic regimen if none of said one or more mutations is detected in the biological sample, wherein the therapeutic regimen includes administering to said selected patient a therapeutically effective amount of one or more chemotherapeutic agents. In some embodiments, the one or more chemotherapeutic agents is entrectinib, rebastinib, NVP-TAE684, staurosporine, or Compound 2, or a pharmaceutically acceptable salt thereof. In some embodiments, the methods further include treating the patient selected as having an increased risk of unresponsiveness to treatment with the therapeutic regimen. In some embodiments, the treating includes administering to the patient a therapeutic agent that is appropriate for treating a patient having one or more of the mutations. In some embodiments, the treating includes administering to said patient a therapeutic agent that is effective against multiple receptor tyrosine kinases.

[0019] In one aspect, some embodiments disclosed herein relate to methods for identifying a compound suitable for treatment of cancer in a patient who has become resistant to an inhibitor of a receptor tyrosine kinase resulting from one or more mutations in the receptor tyrosine kinase, comprising (a) acquiring knowledge of the presence of one or more mutations in a biological sample from said patient, wherein the one or more mutations is at an amino acid position selected from V573, F589, G595 and G667 of the TrkA polypeptide of SEQ ID NO:1; V619, F633, G639 and G709 of the TrkB polypeptide of SEQ ID NO:3; V603, F617, G623 and G696 of the TrkC polypeptide of SEQ ID NO:5; V1182, L1196, G1202 and 1269 of the ALK polypeptide of SEQ ID NO:7; and L2012, L2026, G2032 and 2101 of the ROS1 polypeptide of SEQ ID NO:9; (b) determining the ability of the compound to inhibit the receptor tyrosine kinase having one or more of the mutations; and (c) identifying a compound as suitable for treatment of the patient if the compound inhibits the receptor tyrosine kinase having one or more of the mutations.

[0020] In one aspect, some embodiments disclosed herein relate to methods for selecting a treatment regimen for a patient having cancer, comprising (a) acquiring knowledge of the presence of one or more mutations in a biological sample from the patient,

wherein the one or more mutations is at an amino acid position selected from V573, F589, G595 and G667 of the TrkA polypeptide of SEQ ID NO:1; V619, F633, G639 and G709 of the TrkB polypeptide of SEQ ID NO:3; V603, F617, G623 and G696 of the TrkC polypeptide of SEQ ID NO:5; V1182, L1196, G1202 and 1269 of the ALK polypeptide of SEQ ID NO:7; and L2012, L2026, G2032 and 2101 of the ROS1 polypeptide of SEQ ID NO:9; and (b) selecting an appropriate treatment regimen for the patient based on whether one or more of the mutations is present is the biological sample.

[0021] In one aspect, some embodiments disclosed herein relate to methods for predicting the outcome of a treatment regimen for a patient having cancer, comprising (a) acquiring knowledge of the presence of one or more mutations in a biological sample from the patient, wherein the one or more mutations is at an amino acid position selected from V573, F589, G595 and G667 of the TrkA polypeptide of SEQ ID NO:1; V619, F633, G639 and G709 of the TrkB polypeptide of SEQ ID NO:3; V603, F617, G623 and G696 of the TrkC polypeptide of SEQ ID NO:5; V1182, L1196, G1202 and 1269 of the ALK polypeptide of SEQ ID NO:7; and L2012, L2026, G2032 and 2101 of the ROS1 polypeptide of SEQ ID NO:9, wherein the presence of one or more of the mutations in the biological sample is indicative of an increased unresponsiveness in the patient to the treatment regimen.

[0022] In one aspect, some embodiments disclosed herein relate to methods for treating a patient having a cancer tumor, comprising (a) determining the presence of a nucleic acid encoding a mutated Trk protein in a tumor sample from said patient, wherein said mutated Trk protein mutation comprises at least one mutation at an amino acid position selected from V573, F589, G595 and G667 of the TrkA polypeptide of SEQ ID NO:1; V619, F633, G639 and G709 of the TrkB polypeptide of SEQ ID NO:3; V603, F617, G623 and G696 of the TrkC polypeptide of SEQ ID NO:5; V1182, L1196, G1202 and 1269 of the ALK polypeptide of SEQ ID NO:7; and L2012, L2026, G2032 and 2101 of the ROS1 polypeptide of SEQ ID NO:9; (b) selecting a Trk inhibitor appropriate for the treatment of said tumor; and (c) administering said Trk inhibitor to the patient.

[0023] In one aspect, some embodiments disclosed herein relate to methods for treating a patient having a cancer tumor, wherein the cancer tumor contains a mutated Trk gene, and wherein the mutated Trk gene within the cancer tumor shows resistance or acquired resistance to treatment with Trk inhibitors. The method includes administering a

therapeutically effective amount of a Trk inhibitor that is active against a polypeptide encoded by the mutated Trk gene to a patient in need thereof, optionally in combination with radiotherapy, radio-immunotherapy and/or tumor resection by surgery.

[0024] In one aspect, some embodiments disclosed herein relate to methods for treating cancer in a patient comprising the steps of (a) selecting a patient with cancer having a Trk mutation; and (b) administering to the patient an inhibitor that is active against one or more of said Trk mutations.

[0025] In one aspect, some embodiments disclosed herein relate to methods for treating a patient having a cancer tumor, comprising (a) determining the presence of one or more mutations in the DNA sequence encoding a Trk protein in a tumor sample from the patient, the one or more mutations is at a position corresponding to an amino acid residue selected from V573, F589, G595 and G667 of the TrkA polypeptide of SEQ ID NO:1; V619, F633, G639 and G709 of the TrkB polypeptide of SEQ ID NO:3; V603, F617, G623 and G696 of the TrkC polypeptide of SEQ ID NO:5; V1182, L1196, G1202 and 1269 of the ALK polypeptide of SEQ ID NO:7; and L2012, L2026, G2032 and 2101 of the ROS1 polypeptide of SEQ ID NO:9; (b) selecting a Trk inhibitor appropriate for the treatment of the tumor; and (c) administering the Trk inhibitor to the patient.

[0026] In one aspect, some embodiments disclosed herein relate to methods for treating a cancer in a patient bearing a Trk mutation, wherein said subject has become resistant to at least one Trk inhibitor, comprising administering to said patient an effective amount of one or more inhibitors effective against multiple receptor tyrosine kinases.

[0027] The foregoing summary is illustrative only and is not intended to be in any way limiting. In addition to the illustrative aspects, alternatives, and features described above, further aspects, alternatives, objects and features of the disclosure will become fully apparent from the drawings and the following detailed description and the claims.

[0027a] Definitions of the specific embodiments of the invention as claimed herein follow.

[0027b] According to a first embodiment of the invention, there is provided a method for treating a patient having a cancer tumor, comprising

a) determining the presence of a nucleic acid encoding a mutated Trk protein in a tumor sample from said patient, wherein said mutated Trk protein comprises at least one mutation at an amino acid position selected from:

i. V573, F589, G595 and G667 of the TrkA polypeptide set forth in SEQ ID NO: 1;

ii. V619, F633, G639 and G709 of the TrkB polypeptide set forth in SEQ ID NO: 3; and

iii. V603, F617, G623 and G696 of the TrkC polypeptide set forth in SEQ ID NO: 5; and

b) administering to said patient a Trk-inhibiting compound.

[0027c] According to a second embodiment of the invention, there is provided use of a Trk-inhibiting compound in the manufacture of a medicament for the treatment of a patient having a cancer tumor, wherein said tumor is determined to contain a nucleic acid encoding a mutated Trk protein, and wherein said mutated Trk protein comprises at least one mutation at an amino acid position selected from:

(i) V573, F589, G595 and G667 of the TrkA polypeptide set forth in SEQ ID NO: 1;

(ii) V619, F633, G639 and G709 of the TrkB polypeptide set forth in SEQ ID NO: 3; and

(iii) V603, F617, G623 and G696 of the TrkC polypeptide set forth in SEQ ID NO: 5.

BRIEF DESCRIPTION OF THE DRAWINGS

[0028] FIG. 1 is an alignment of the kinase domains from human receptor tyrosine kinases TrkA (NCBI Accession No. NP_002520.2; SEQ ID NO: 1), TrkB (NCBI Accession No. NP_006171.2; SEQ ID NO: 3), TrkC (NCBI Accession No. NP_001012338.1; SEQ ID NO: 5), ALK (NCBI Accession No. NM_004304.4; SEQ ID NO:

[Text continues on page 10.]

7), and ROS (NCBI Accession No. NP_002935.2; SEQ ID NO: 9). The sequence alignment of FIG. 1 was generated using the program CLUSTAL 2.1 with default settings. The amino acid numbering of each aligned sequence is with reference to the full-length polypeptide sequence indicated by the corresponding SEQ ID NO. In the alignment figure shown herein, a dash in an aligned sequence represents a gap, *i.e.*, a lack of amino acid at that position. As discussed in detail below, several conserved amino acid residues and polypeptide motifs with high degree of conservation have been identified from this sequence comparison analysis. The amino acid residues corresponding to the kinase domain of each aligned sequence are indicated between parentheses. Asterisks identify identical and conserved amino acids among the aligned sequences. Boxed letters identify the amino acid residues within the aligned sequences that correspond to the conserved V573, F589, G595, and G667 residues of TrkA.

[0029] FIG. 2 is a brief description of some of the cell lines used in the experiments described at the Examples section.

[0030] FIG. 3 is a schematic illustration of a strategy for generating inhibitor-resistant cell lines and the subsequent characterization.

[0031] FIG. 4 illustrates an exemplary scheme for the selection of entrectinib-resistant KM12 cells.

[0032] FIG. 5 is a graphical summary of the results obtained from growth inhibition studies described in the Examples section herein where KM12 cells of Set A grown in media containing 0-30 nM entrectinib for 3 days upshifted.

[0033] FIG. 6 is a graphical summary of the results obtained from growth inhibition studies described in the Examples section herein where KM12 cell grown in media containing increasing concentrations of entrectinib for 4 weeks.

[0034] FIG. 7 is the sequencing results showing that the KM12 cell pools of Set B described at Example 4 were found to possess two point mutations at position G595 (G595R) and G667 (G677C) in the TrkA kinase domain.

[0035] FIG. 8 illustrates an exemplary scheme for the selection of entrectinib-resistant BaF3-tel/trkA cells.

[0036] FIG. 9 illustrates the establishment of BaF3-tel/trkA cell pools that developed resistance to 10 nM entrectinib after 2-week selection.

[0037] FIG. 10 is a graphical illustration of the reduced sensitivity of the KM12 cells of Set B to entrectinib, as described in detail at Example 4.

[0038] FIGs. 11A and 11B are graphical illustration of the results obtained from growth inhibition studies showing that the 10nM entrectinib-resistant Baf3-trkA (A) cell pools displayed >100 fold higher IC50 compared to parental cells.

[0039] FIGs. 12A – 12E show that withdrawing entrectinib from the 10nM-resistant Baf3-trkA cell pools did not affect the resistance phenotype. Also shown in this figure is some exemplary inhibitory activity of RTK inhibitors in these cells.

[0040] FIG. 13 is a summary of the results from the 1st RT-PCR and sequencing analysis of the kinase domain of TrkA, as described at Examples 4 and 5.

[0041] FIG. 14 shows that a G ⇒A substitution in the TrkA kinase domain (Exon 14) in entrectinib-10nM resistant-BaF3-tel/trkA cells. As a control, the Figure also shows that the TrkA sequence of the 100-nM entrectinib treated KM12 cell pools of Set A possessed wild-type sequence. The G ⇒A single base substitution is indicated (encircled).

[0042] FIG. 15 is a summary of a sequence analysis experiment, which confirmed the presence of G595R mutation in entrectinib-resistant BaF3-tel/trkA-10nMA cells. The G ⇒A single base substitution is indicated (encircled).

[0043] FIG. 16 shows a tridimensional modeling of the TrkA kinase domain illustrating that the G595 and G667C substitutions in the TrkA protein interferes with entrectinib binding to the ATP pocket of Trk polypeptide.

[0044] FIG. 17 shows a tridimensional modeling of the ALK kinase domain illustrating that the G1202 substitution interferes with entrectinib binding to the ATP pocket of ALK polypeptide, which is similar to the G595R and G2032R substitutions in TrkA and ROS1, respectively.

[0045] FIG. 18 is a summary of the results from the 2nd RT-PCR and sequencing analysis of the kinase domain of TrkA in KM12 and BaF3-tel/TrkA cell lines, as described at Examples 4 and 5.

[0046] FIG. 19 is a summary of the results obtained from a sequence analysis experiment, which identified an additional G667C mutation in Exon 15 the in KM12 set B and BaF3-tel/trkA-12nM entrectinib-resistant pools.

[0047] FIG. 20 is a sequencing chromatogram illustrating that DNA samples from KM12 cell pools show clean sequencing data for both G595R and G667C mutations, suggesting the pools derived from clonal cells. The G →T single base substitution is indicated (encircled).

[0048] FIG. 21 is a sequencing chromatogram illustrating that DNA samples from entrectinib-12 nM entrectinib-resistant BaF3-tel/trkA cells contain a mixture of G and T for G667C mutation.

[0049] FIG. 22 illustrates an exemplary scheme for subcloning of BaF3-tel/trkA-12nMA2 and 12nMB3 pools.

[0050] FIG. 23 is a summary of the results obtained from the sequencing analysis of twelve isolated clones derived from the subcloning experiment described at Figure 22 above.

[0051] FIG. 24 illustrates an exemplary screening protocol and cells lines used in the experiments described in the Examples section.

[0052] FIG. 25 is a summary of the IC50 values of a number of chemical compounds that were tested against 7 cell lines including entrectinib-resistant BaF3-tel/trkA cells, as described in detail at Example 6.

[0053] FIG. 26 shows the biochemical IC50s of a list of candidate compounds against a number of kinases.

[0054] FIG. 27 illustrates another screening protocol and cells lines used in the experiments described in the Examples section.

[0055] FIG. 28 a summary of the IC50 values of a number of chemical compounds that were tested against entrectinib-resistant BaF3-Tel/TrkA cells, as described in detail at Example 6.

[0056] FIG. 29 is a general scheme of the study of the effect of entrectinib on RTKs and Proteins in the down-stream signal transduction pathway.

[0057] FIG. 30 is a summary of the results obtained from the characterization of entrectinib-resistant mutant cell line 10nM BaF3-tel/trkA-containing G595R by using Western Blot analysis.

[0058] FIG. 31 is a summary of the results obtained from experiments comparing the phosphorylation level of TrkA and down-stream signal molecules in BaF3-Tel-TrkA and BaF3-Tel-TrkA-10nMA(G595R) cell lines by Western Blot analysis.

[0059] FIG. 32 illustrates a general scheme for the identification of point mutations as the primary resistance mechanism in entrectinib-resistant KM12 cell pools of Set B that is resistant to entrectinib.

[0060] FIG. 33 provides an overview of the cellular IC50 determination procedure described in further detail in Examples section.

[0061] FIG. 34 is a graphical illustration of the growth inhibition of BaF3-TPM3-TrkA cells and BaF3-TPM3-TrkA_G595R mutant cells by entrectinib.

[0062] FIG. 35 depicts an exemplary experimental design for Western Blot analysis of BaF3-fusion Trks cells treated with Entrectinib.

[0063] FIG. 36 is a summary of the results obtained from Western Blot analyses that were performed on BaF3-TPM3-TrkA cells and BaF3-TPM3-TrkA-G595R mutant cells.

[0064] FIG. 37 summarizes the results obtained from Western Blot analyses that were performed on BaF3-TPM3-TrkA cells and BaF3-TPM3-TrkA-G595R mutant cells.

DETAILED DESCRIPTION OF THE DISCLOSURE

[0065] In the following detailed description, reference is made to the accompanying drawings, which form a part hereof. In the drawings, similar symbols typically identify similar components, unless context dictates otherwise. The illustrative alternatives described in the detailed description, drawings, and claims are not meant to be limiting. Other alternatives may be used, and other changes may be made, without departing from the spirit or scope of the subject matter presented here. It will be readily understood that the aspects, as generally described herein, and illustrated in the Figures, can be arranged, substituted, combined, and designed in a wide variety of different configurations, all of which are explicitly contemplated and make part of this disclosure.

[0066] Unless otherwise defined, all terms of art, notations and other scientific terms or terminology used herein are intended to have the meanings commonly understood by those of skill in the art to which this disclosure pertains. In some cases, terms with commonly understood meanings are defined herein for clarity and/or for ready reference, and

the inclusion of such definitions herein should not necessarily be construed to represent a substantial difference over what is generally understood in the art. Many of the techniques and procedures described or referenced herein are well understood and commonly employed using conventional methodology by those skilled in the art.

Some Definitions

[0067] The singular form "a", "an", and "the" include plural references unless the context clearly dictates otherwise. For example, the term "a cell" includes one or more cells, comprising mixtures thereof. "A and/or B" is used herein to include all of the following alternatives: "A", "B", "A or B", and "A and B".

[0068] "About" means either within plus or minus 10% of the provided value, or rounded to the nearest significant figure, in all cases inclusive of the provided value. Where ranges are provided, they are inclusive of the boundary values.

[0069] The terms "administration" and "administering", as used herein, refer to the delivery of a bioactive composition or formulation by an administration route comprising, but not limited to, oral, intravenous, intra-arterial, intramuscular, intraperitoneal, subcutaneous, intramuscular, and topical administration, or combinations thereof.

[0070] As used herein, anaplastic lymphoma kinase (ALK) refers to ALK tyrosine kinase receptor or CD246 (cluster of differentiation 246), for example a human enzyme encoded by the ALK gene and has the UniProt identified ALK_HUMAN.

[0071] As used herein, the term "antibody" refers to an immunoglobulin that specifically binds to, and is thereby defined as complementary with, a particular spatial and polar organization of another molecule. The antibody can be monoclonal or polyclonal and can be prepared by techniques that are well known in the art, such as immunization of a host and collection of sera (polyclonal), or by preparing continuous hybrid cell lines and collecting the secreted protein (monoclonal), or by cloning and expressing nucleotide sequences or mutagenized versions thereof coding at least for the amino acid sequences required for specific binding of natural antibodies. Antibodies may include a complete immunoglobulin or fragment thereof, which immunoglobulins include the various classes and isotypes, such as IgA, IgD, IgE, IgG1, IgG2a, IgG2b and IgG3, IgM, *etc.* Fragments thereof may include Fab, Fv and F(ab′)2, Fab′, and the like. In addition, aggregates,

polymers, and conjugates of immunoglobulins or their fragments can be used where appropriate so long as binding affinity for a particular target is maintained.

[0072] The terms “monoclonal antibody,” “mAb” and “MAB” refer to an antibody that is an immunoglobulin produced by a single clone of lymphocytes which recognizes only a single epitope on an antigen. For example, a monoclonal antibody useful for the methods disclosed herein displays a single binding specificity and affinity for a particular epitope of one or more tyrosine kinases.

[0073] The term "polyclonal antibody" as used herein refers to a composition of different antibody molecules which is capable of binding to or reacting with several different specific antigenic determinants on the same or on different antigens. The variability in antigen specificity of a polyclonal antibody is located in the variable regions of the individual antibodies constituting the polyclonal antibody, in particular in the complementarity determining regions (CDRs). Preferably, the polyclonal antibody is prepared by immunization of an animal with the target tyrosine kinases or portions thereof. Alternatively, the polyclonal antibody may be prepared by mixing multiple monoclonal antibodies having desired specificity to a target tyrosine kinase.

[0074] The term "biological sample," as used herein, encompasses a variety of sample types obtained from an organism. In some embodiments, a biological sample can be used in a diagnostic or monitoring assay. The biological sample may be obtained or derived from a healthy tissue, a diseased tissue or a tissue suspected of being diseased tissue. The biological sample may be a sample obtained from a biopsy taken, for example, during a surgical procedure. The biological sample may be collected via means of fine needle aspiration, scraping or washing a cavity to collects cells or tissue therefrom. The biological sample may be of a tumor such as, for example, solid and hematopoietic tumors as well as of neighboring healthy tissue. The biological sample may be a smear of individual cells or a tissue section. The term encompasses blood, blood components comprising plasma and other liquid samples of biological origin, solid tissue samples, such as a biopsy specimen or tissue cultures or cells derived therefrom and the progeny thereof. The term encompasses samples that have been manipulated in any way after their procurement, such as by treatment with reagents, solubilization, or enrichment for certain components. The term encompasses clinical samples, and also includes cells in cell culture, cell supernatants, cell lysates, cell

extracts, cell homogenates, subcellular components comprising synthesized proteins, serum, plasma, bodily and other biological fluids, and tissue samples. The biological sample can contain compounds that are not naturally intermixed with the cell or tissue in nature such as preservatives, anticoagulants, buffers, fixatives, nutrients, antibiotics or the like. In some embodiments, the sample biological is preserved as a frozen sample or as formaldehyde- or paraformaldehyde-fixed paraffin-embedded (FFPE) tissue preparation. For example, the biological sample can be embedded in a matrix, e.g., an FFPE block or a frozen sample.

[0075] The term "cancer" or "tumor" is used interchangeably herein. These terms refer to the presence of cells possessing characteristics typical of cancer-causing cells, such as uncontrolled proliferation, immortality, metastatic potential, rapid growth and proliferation rate, and certain characteristic morphological features. Cancer cells are often in the form of a tumor, but such cells can exist alone within an animal, or can be a non-tumorigenic cancer cell, such as a leukemia cell. These terms include a solid tumor, a soft tissue tumor, or a metastatic lesion. As used herein, the term "cancer" includes premalignant, as well as malignant cancers. In some embodiments, the cancer is a solid tumor, a soft tissue tumor, or a metastatic lesion.

[0076] The term "chemotherapeutic agent" and “therapeutic agent”, which are used interchangeably herein, refers to a chemical substance, such as a cytotoxic or cytostatic agent, that is used to treat a condition, particularly cancer. In some embodiments, the chemotherapeutic agents include AZ-23, BMS-754807, bosutinib, cabozantinib, ceritinib, crizotinib, entrectinib, foretinib, GNF 5837, GW441756, imatinib mesylate, K252a, LOXO-101, MGCD516, nilotinib hydrochloride monohydrate, NVP-TAE684, PF-06463922, rebastinib, staurosporine, sorafenib tosylate, sunitinib malate, and TSR-011, and any pharmaceutically acceptable salts thereof.

[0077] As used herein the terms “combination” and “in combination with” mean the administration of a therapeutic agent described herein together with at least one additional pharmaceutical or medicinal agent (*e.g.*, an anti-cancer agent), either sequentially or simultaneously. For example, the term encompasses dosing simultaneously, or within minutes or hours of each other, or on the same day, or on alternating days, or dosing the therapeutic agent described herein on a daily basis, or multiple days per week, or weekly basis, for example, while administering another compound such as a chemotherapeutic agent

on the same day or alternating days or weeks or on a periodic basis during a time simultaneous therewith or concurrent therewith, or at least a part of the time during which the therapeutic agent described herein is dosed.

[0078] As used herein, “contact” in reference to specificity or specific binding means two molecules are close enough so that short range non-covalent chemical interactions, such as Van der Waal forces, hydrogen bonding, hydrophobic interactions, and the like, dominate the interaction of the molecule.

[0079] The term “cell line” as used herein refers to one or more generations of cells which are derived from a clonal cell. The term “clone,” or “clonal cell,” refers to a single cell which is expanded to produce an isolated population of phenotypically similar cells (*i.e.* a “clonal cell population”).

[0080] As used herein, the term "expression" refers to the process of converting genetic information of a polynucleotide into RNA through transcription, which is typically catalyzed by an enzyme, RNA polymerase, and, where the RNA encodes a polypeptide, into protein, through translation of mRNA on ribosomes to produce the encoded protein.

[0081] The term "immunohistochemistry", as used herein, refers to the process of localizing antigens (*e.g.* proteins) in biological samples, cells and/or cells of a tissue section exploiting the principle of antibodies binding specifically to antigens. Immunohistochemical staining is widely used in the diagnosis of abnormal cells such as those found in cancerous tumors. Specific molecular markers are characteristic of particular cellular events, such as cell proliferation or cell death. Visualizing an antibody-antigen interaction can be accomplished in a number of ways. In the most common instance, an antibody is conjugated to an enzyme, such as peroxidase, that can catalyze a color-producing reaction. Alternatively, the antibody can also be tagged to a fluorophore thus employing the principles of immunofluorescence. Immunohistochemistry can also be used to evaluate tumor content in the sample on which qPCR is carried out in order to account for the fact that qPCR result will be influenced by the amount of tumor tissue present.

[0082] As used herein, the term “one or more molecular alterations” means any variation in the genetic or protein sequence in or more cells of a patient as compared to the corresponding wild-type genes or proteins. One or more molecular alterations include, but are not limited to, genetic mutations, gene amplifications, splice variants, deletions,

insertions/deletions, gene rearrangements, single-nucleotide variations (SNVs), insertions, and aberrant RNA/protein expression.

[0083] A "multiplexed assay," as used herein, refers to an assay in which multiple assay reactions, *e.g.* simultaneous assays of multiple target biomarkers, are carried out in a single reaction chamber and/or and analyzed in a single separation and detection format. "Multiplex identification", as used herein, refers to the simultaneous identification of one or more target biomarkers in a single mixture. For example, a two-plex assay refers to the simultaneous identification, in a single reaction mixture, of two different target biomarkers.

[0084] The terms "nucleic acid molecule" and "polynucleotide" are used interchangeably herein, and refer to RNA and DNA molecules or mixture or hybrid thereof. In some embodiments, nucleic acid molecules comprise cDNA, genomic DNA, synthetic DNA, and DNA or RNA molecules containing nucleic acid analogs. Nucleic acid molecules can have any three-dimensional structure. A nucleic acid molecule can be double-stranded or single-stranded (*e.g.*, a sense strand or an antisense strand). Non-limiting examples of nucleic acid molecules include genes, gene fragments, exons, introns, messenger RNA (mRNA), transfer RNA, ribosomal RNA, siRNA, micro-RNA, tracrRNAs, crRNAs, guide RNAs, ribozymes, cDNA, recombinant polynucleotides, branched polynucleotides, nucleic acid probes and nucleic acid primers. A nucleic acid molecule may contain unconventional or modified nucleotides. The terms "polynucleotide sequence" and "nucleic acid sequence" as used herein interchangeably refer to the sequence of a polynucleotide molecule. The nomenclature for nucleotide bases as set forth in 37 CFR §1.822 is used herein.

[0085] As used herein, "ROS1" refers to ROS1 receptor tyrosine-protein kinase, for example the ROS1 receptor tyrosine-protein kinase having the UniProt designation ROS1_HUMAN.

[0086] "Selectively binds" is used herein to refer to the situation in which one member of a specific intra- or inter-species binding pair will not show any significant binding to molecules other than its specific intra- or inter-species binding partner (*e.g.*, an affinity of about 50-fold less or more preferably 100-fold less), which means that only minimal cross-reactivity occurs.

[0087] "Specific", as used herein in reference to the binding of two molecules or a molecule and a complex of molecules, refers to the specific recognition of one for the other

and the formation of a stable complex, as compared to substantially less recognition of other molecules and the lack of formation of stable complexes with such other molecules. Preferably, "specific," in reference to binding, means that to the extent that a molecule forms complexes with other molecules or complexes, it forms at least fifty percent of the complexes with the molecule or complex for which it has specificity. Generally, the molecules or complexes have areas on their surfaces or in cavities giving rise to specific recognition between the two binding moieties. Exemplary of specific binding are antibody-antigen interactions, enzyme-substrate interactions, polynucleotide hybridizations and/or formation of duplexes, cellular receptor-ligand interactions, and so forth.

[0088] As used herein, the term "tropomyosin receptor kinase" refers to any members of the family of tropomyosin receptor kinases (Trks) that are activated by peptide hormones of the neurotrophin family. Examples of tropomyosin receptor kinase include, but are not limited to, TrkA, TrkB, and TrkC. As used herein, the term "TrkA" refers to the wild-type tropomyosin receptor kinase A having the UniProt identifier NTRK1_HUMAN. As used herein, the term "TrkB" refers to the wild-type tropomyosin receptor kinase B having the UniProt identifier NTRK2_HUMAN. As used herein, the term "TrkC" refers to the wild-type tropomyosin receptor kinase C having the UniProt identifier NTRK3_HUMAN. TrkA, TrkB and TrkC are also referred to by those of skill in the art as Trk1, Trk2 and Trk3, respectively. A reference to TrkA is a reference to Trk1. A reference to TrkB is a reference to Trk2. A reference to TrkC is a reference to Trk3.

[0089] As will be understood by one having ordinary skill in the art, for any and all purposes, such as in terms of providing a written description, all ranges disclosed herein also encompass any and all possible sub-ranges and combinations of sub-ranges thereof. Any listed range can be easily recognized as sufficiently describing and enabling the same range being broken down into at least equal halves, thirds, quarters, fifths, tenths, *etc.* As a non-limiting example, each range discussed herein can be readily broken down into a lower third, middle third and upper third, *etc.* As will also be understood by one skilled in the art all language such as "up to," "at least," "greater than," "less than," and the like include the number recited and refer to ranges which can be subsequently broken down into sub-ranges as discussed above. Finally, as will be understood by one skilled in the art, a range includes each individual member. Thus, for example, a group having 1-3 articles refers to groups

having 1, 2, or 3 articles. Similarly, a group having 1-5 articles refers to groups having 1, 2, 3, 4, or 5 articles, and so forth.

[0090] Headings, *e.g.*, (a), (b), (i) *etc.*, are presented merely for ease of reading the specification and claims. The use of headings in the specification or claims does not require the steps or elements be performed in alphabetical or numerical order or the order in which they are presented.

Receptor Tyrosine Kinases and Diseases Associated with Their Activities

[0091] Neurotrophins control many aspects of neuronal survival and differentiation in the vertebrate nervous system by binding and signaling through the trk family of receptor tyrosine kinases (RTK). Gene families encoding RTKs with fundamental roles in nervous system have been shown to be highly conserved throughout evolution (Gad *et al., J. Neurobiol.* Jul;60(1):12-20, 2004). Examples of the receptor tyrosine kinase include, but are not limited to, epidermal growth factor receptor family (EGFR), platelet-derived growth factor receptor (PDGFR) family, vascular endothelial growth factor receptor (VEGFR) family, nerve growth factor receptor (NGFR) family, fibroblast growth factor receptor family (FGFR) insulin receptor family, ephrin receptor family, Met family, and Ror family. Each family may comprise one or more family member that possesses characteristic structural and/or functional similarities.

[0092] Human Trk family proteins are receptor tyrosine kinases composed of three family members, TrkA, TrkB and TrkC. These proteins bind with high affinity to, and mediate the signal transduction induced by the neurotrophin family of ligands whose prototype members are Nerve Growth Factor (NGF), Brain-Derived Neurotrophic Factor (BDNF) and Neurotrophin 3-5 (NT 3-5). In addition, a co-receptor lacking enzymatic activity, p75, has been identified which binds all neurotrophins (NTs) with low affinity and regulates neurotrophin signaling. A critical role of the Trks and their ligands during the development of the central and peripheral nervous systems have been established through gene disruption studies in mice. In particular, TrkA-NGF interaction was shown as a requirement for the survival of certain peripheral neuron populations involved in mediating pain signaling. It has been shown that increased expression of TrkA also correlates with an increased level of pain in the case of pancreatic cancer (Zhu, *et al.*, Journal of Clinical

Oncology, 17:2419-2428 (1999)). Increased expression of NGF and TrkA was also observed in human osteoarthritis chondrocytes (Iannone *et al.*, *Rheumatology* 41:1413-1418 (2002)).

[0093] Although the amino acid sequences of various NTRK polypeptides differ in length, the relative positions of residues subject to the molecular alterations and mutations in accordance with the methods of the present invention are conserved (see, *e.g.*, Gad *et al.*, *J. Neurobiol.* Jul;60(1):12-20, 2004; and TABLE 1 and FIG. 1). The molecular alterations and mutations described in the present disclosure in terms of amino acid positions correspond to the amino acid residue numbers of the human TrkA polypeptide (SEQ ID NO: 1). For examples, residue 639 of the human TrkB (disclosed herein as SEQ ID NO: 3) corresponds to residue 595 of the human TrkA polypeptide (SEQ ID NO: 1), which corresponds to residue 623 of the human TrkC polypeptide (SEQ ID NO: 5), residue 1202 of the human ALK polypeptide (SEQ ID NO: 7), and residue 2032 of the human ROS1 polypeptide (SEQ ID NO: 9). As another example, residue 709 of the human TrkB (disclosed herein as SEQ ID NO: 3) corresponds to residue 667 of the human TrkA polypeptide (SEQ ID NO: 1), which corresponds to residue 696 of the human TrkC polypeptide (SEQ ID NO: 5), residue 1269 of the human ALK polypeptide (SEQ ID NO: 7), and residue 2101 of the human ROS1 polypeptide (SEQ ID NO: 9). As yet another example, residue 619 of the human TrkB (disclosed herein as SEQ ID NO: 3) corresponds to residue 573 of the human TrkA polypeptide (SEQ ID NO: 1), which corresponds to residue 603 of the human TrkC polypeptide (SEQ ID NO: 5), residue 1182 of the human ALK polypeptide (SEQ ID NO: 7), and residue 2012 of the human ROS1 polypeptide (SEQ ID NO: 9). Non-limiting examples of conserved residues, motif, domains, and regions of correspondence relevant to the one or more molecular alterations in the TrkA polypeptide sequence disclosed herein are set forth in FIG. 1 and TABLE 1. Based on such correspondence, the corresponding conserved positions in the NRTK sequences not specifically disclosed herein can be readily determined by one of skill in the art.

TABLE 1: Concordant positions of exemplary conserved amino acid residues in human TrkA, TrkB, TrkC, ALK and ROS1 polypeptides. Throughout the present disclosure, the TrkA polypeptide is commonly used as reference sequence in comparative sequence

analysis because structural features and residues important for the kinase activity and physiological function of this polypeptide has been most extensively characterized.

TrkA (SEQ ID NO: 1)	TrkB (SEQ ID NO: 3)	TrkC (SEQ ID NO:5)	ALK (SEQ ID NO: 7)	ROS1 (SEQ ID NO: 9)
V573	V619	V603	V1182	L2012
F589	F633	F617	L1196	L2026
E590	E634	E618	E1197	E2027
M592	M636	M620	M1199	M2029
G595	G639	G623	G1202	G2032
D596	D640	D624	D1203	D2033
L597	L641	L625	L1204	L2034
K665	K707	K694	K61267	K2019
I666	I708	I695	I1268	I2100
G667	G709	G696	G1269	G2101
D668	D710	D697	D1270	D2102
F669	F711	F698	F1271	F2103
G670	G712	G699	G1272	G2104

[0094] Accordingly, in some embodiments of the methods disclosed herein, the one or more mutations in a receptor tyrosine kinase polypeptide sequence include one or more amino acid deletions, insertions, or substitutions at one or more of the positions corresponding to conserved amino acid residues: V573, F589, E590, M592, G595, D596, L597, K665, I666, G667, D668, F669, and G670, or combinations thereof, of the polypeptide of SEQ ID NO: 1. In some embodiments, the one or more mutations in a receptor tyrosine kinase polypeptide sequence can include one or more amino acid deletions, insertions, or substitutions at one or more of the positions corresponding to conserved amino acid residues: V619, F633, E634, M636, G639, D640, L641, K707, I708, G709, D710, F711, G712, and combinations of any thereof, of the polypeptide of SEQ ID NO: 3. In some embodiments, the one or more mutations in a receptor tyrosine kinase polypeptide sequence include one or more amino acid deletions, insertions, or substitutions at one or more of the positions corresponding to conserved amino acid residues: V603, F617, E618, M620, G623, D624, L625, K694, I695, G696, D697, F698, G699, and combinations of any thereof, of the polypeptide of SEQ ID NO: 5. In some embodiments, the one or more mutations in a receptor tyrosine kinase polypeptide sequence include one or more amino acid deletions, insertions, or substitutions at one or more of the positions corresponding to conserved amino

acid residues: V1182, L1196, E1197, M1199, G1202, D1203, L1204, K61267, I1268, G1269, D1270, F1271, G1272, and combinations of any thereof, of the polypeptide of SEQ ID NO: 7. In some embodiments, the one or more mutations in a receptor tyrosine kinase polypeptide sequence include one or more amino acid deletions, insertions, or substitutions at one or more of the positions corresponding to conserved amino acid residues: L2012, L2026, E2027, M2029, G2032, D2033, L2034, K2019, I2100, G2101, D2102, F2103, G2104, and combinations of any thereof, of the polypeptide of SEQ ID NO: 9.

[0095] In some embodiments of the methods disclosed herein, the one or more mutations in a receptor tyrosine kinase polypeptide sequence include one or more amino acid deletions, insertions, or substitutions at one or more of the positions corresponding to conserved amino acid residues V573, F589, G595, G667, and combination thereof, of the polypeptide of SEQ ID NO: 1. In some embodiments, the one or more mutations in a receptor tyrosine kinase polypeptide sequence include one or more amino acid deletions, insertions, or substitutions at one or more of the positions corresponding to conserved amino acid residues V619, F633, G639, G709, and a combination thereof, of the polypeptide of ID NO: 3. In some embodiments, the one or more mutations in a receptor tyrosine kinase polypeptide sequence include one or more amino acid deletions, insertions, or substitutions at one or more of the positions corresponding to conserved amino acid residues V603, F617, G623, G696, and a combination thereof, of the polypeptide of SEQ ID NO: 5. In some embodiments, the one or more mutations in a receptor tyrosine kinase polypeptide sequence include one or more amino acid deletions, insertions, or substitutions at one or more of the positions corresponding to conserved amino acid residues V1182, L1196, G1202, G1269, and a combination thereof, of the polypeptide of SEQ ID NO: 7. In some embodiments, the one or more mutations in a receptor tyrosine kinase polypeptide sequence include one or more amino acid deletions, insertions, or substitutions at one or more of the positions corresponding to conserved amino acid residues L2012, L2026, G2032, G2101, and a combination thereof, of the polypeptide of SEQ ID NO: 9.

[0096] In some embodiments of the methods disclosed herein, the one or more mutations in a receptor tyrosine kinase polypeptide sequence include an amino acid deletion, insertion, or substitution at a position corresponding to the conserved amino acid residue V573 of the polypeptide of SEQ ID NO: 1. In some embodiments, the one or more mutations

in a receptor tyrosine kinase polypeptide sequence include an amino acid deletion, insertion, or substitution at a position corresponding to a Val-to-Met substitution V573M of the polypeptide of SEQ ID NO: 1. In some embodiments of the methods disclosed herein, the one or more mutations in a receptor tyrosine kinase polypeptide sequence include an amino acid deletion, insertion, or substitution at a position corresponding to the conserved amino acid residue F589 of the polypeptide of SEQ ID NO: 1. In some embodiments, the one or more mutations in a receptor tyrosine kinase polypeptide sequence include an amino acid deletion, insertion, or substitution at a position corresponding to a Phe-to-Leu substitution F589L of the polypeptide of SEQ ID NO: 1. In some embodiments of the methods disclosed herein, the one or more mutations in a receptor tyrosine kinase polypeptide sequence include an amino acid deletion, insertion, or substitution at a position corresponding to the conserved amino acid residue G595 of the polypeptide of SEQ ID NO: 1. In some embodiments, the one or more mutations in a receptor tyrosine kinase polypeptide sequence include an amino acid deletion, insertion, or substitution at a position corresponding to a Gly-to-Arg substitution G595R of the polypeptide of SEQ ID NO: 1. In some embodiments, the one or more mutations in a receptor tyrosine kinase polypeptide sequence include an amino acid deletion, insertion, or substitution at a position corresponding to the conserved amino acid residue G667 of the polypeptide of SEQ ID NO: 1. In some embodiments, the one or more mutations in a receptor tyrosine kinase polypeptide sequence include an amino acid deletion, insertion, or substitution at a position corresponding to a Gly-to-Cys substitution G667C of the polypeptide of SEQ ID NO: 1. In some embodiments, the one or more mutations in a receptor tyrosine kinase polypeptide sequence include an amino acid deletion, insertion, or substitution at a position corresponding to a Gly-to-Ala substitution G667A of the polypeptide of SEQ ID NO: 1. In some embodiments, the one or more mutations in a receptor tyrosine kinase polypeptide sequence include an amino acid deletion, insertion, or substitution at a position corresponding to a Gly-to-Ser substitution G667S of the polypeptide of SEQ ID NO: 1.

[0097] In some embodiments of the methods disclosed herein, the one or more mutations in a receptor tyrosine kinase polypeptide sequence include an amino acid deletion, insertion, or substitution at a position corresponding to the conserved amino acid residue V619 of the polypeptide of SEQ ID NO: 3. In some embodiments, the one or more mutations

in a receptor tyrosine kinase polypeptide sequence include an amino acid deletion, insertion, or substitution at a position corresponding to a Val-to-Met substitution V619M of the polypeptide of SEQ ID NO: 3. In some embodiments of the methods disclosed herein, the one or more mutations in a receptor tyrosine kinase polypeptide sequence include an amino acid deletion, insertion, or substitution at a position corresponding to the conserved amino acid residue F633 of the polypeptide of SEQ ID NO: 3. In some embodiments, the one or more mutations in a receptor tyrosine kinase polypeptide sequence include an amino acid deletion, insertion, or substitution at a position corresponding to a Phe-to-Leu substitution F633L of the polypeptide of SEQ ID NO: 3.In some embodiments of the methods disclosed herein, the one or more mutations in a receptor tyrosine kinase polypeptide sequence include an amino acid deletion, insertion, or substitution at a position corresponding to the conserved amino acid residue G639 of the polypeptide of SEQ ID NO: 3. In some embodiments, the one or more mutations in a receptor tyrosine kinase polypeptide sequence include an amino acid deletion, insertion, or substitution at a position corresponding to a Gly-to-Arg substitution G639R of the polypeptide of SEQ ID NO: 3. In some embodiments, the one or more mutations in a receptor tyrosine kinase polypeptide sequence include an amino acid deletion, insertion, or substitution at a position corresponding to the conserved amino acid residue G709 of the polypeptide of SEQ ID NO: 3. In some embodiments, the one or more mutations in a receptor tyrosine kinase polypeptide sequence include an amino acid deletion, insertion, or substitution at a position corresponding to a Gly-to-Cys substitution G709C of the polypeptide of SEQ ID NO: 3. In some embodiments, the one or more mutations in a receptor tyrosine kinase polypeptide sequence include an amino acid deletion, insertion, or substitution at a position corresponding to a Gly-to-Ala substitution G709A of the polypeptide of SEQ ID NO: 3. In some embodiments, the one or more mutations in a receptor tyrosine kinase polypeptide sequence include an amino acid deletion, insertion, or substitution at a position corresponding to a Gly-to-Ser substitution G709S of the polypeptide of SEQ ID NO: 3.

[0098] In some embodiments of the methods disclosed herein, the one or more mutations in a receptor tyrosine kinase polypeptide sequence include an amino acid deletion, insertion, or substitution at a position corresponding to the conserved amino acid residue V603 of the polypeptide of SEQ ID NO: 5. In some embodiments, the one or more mutations

in a receptor tyrosine kinase polypeptide sequence include an amino acid deletion, insertion, or substitution at a position corresponding to a Val-to-Met substitution V603M of the polypeptide of SEQ ID NO: 5. In some embodiments of the methods disclosed herein, the one or more mutations in a receptor tyrosine kinase polypeptide sequence include an amino acid deletion, insertion, or substitution at a position corresponding to the conserved amino acid residue F617 of the polypeptide of SEQ ID NO: 5. In some embodiments, the one or more mutations in a receptor tyrosine kinase polypeptide sequence include an amino acid deletion, insertion, or substitution at a position corresponding to a Phe-to-Leu substitution F617L of the polypeptide of SEQ ID NO: 5. In some embodiments of the methods disclosed herein, the one or more mutations in a receptor tyrosine kinase polypeptide sequence include an amino acid deletion, insertion, or substitution at a position corresponding to the conserved amino acid residue G623 of the polypeptide of SEQ ID NO: 5. In some embodiments, the one or more mutations in a receptor tyrosine kinase polypeptide sequence include an amino acid deletion, insertion, or substitutions at a position corresponding to a Gly-to-Arg substitution G623R of the polypeptide of SEQ ID NO: 5. In some embodiments, the one or more mutations in a receptor tyrosine kinase polypeptide sequence include an amino acid deletion, insertion, or substitution at a position corresponding to the conserved amino acid residue G696 of the polypeptide of SEQ ID NO: 5. In some embodiments, the one or more mutations in a receptor tyrosine kinase polypeptide sequence include an amino acid deletion, insertion, or substitution at a position corresponding to a Gly-to-Cys substitution G696C of the polypeptide of SEQ ID NO: 5. In some embodiments, the one or more mutations in a receptor tyrosine kinase polypeptide sequence include an amino acid deletion, insertion, or substitution at a position corresponding to a Gly-to-Ala substitution G696A of the polypeptide of SEQ ID NO: 5. In some embodiments, the one or more mutations in a receptor tyrosine kinase polypeptide sequence include an amino acid deletion, insertion, or substitution at a position corresponding to a Gly-to-Ser substitution G696S of the polypeptide of SEQ ID NO: 5.

[0099] In some embodiments of the methods disclosed herein, the one or more mutations in a receptor tyrosine kinase polypeptide sequence include an amino acid deletion, insertion, or substitution at a position corresponding to the conserved amino acid residue V1182 of the polypeptide of SEQ ID NO: 7. In some embodiments, the one or more

mutations in a receptor tyrosine kinase polypeptide sequence include an amino acid deletion, insertion, or substitution at a position corresponding to a Val-to-Met substitution V1182M of the polypeptide of SEQ ID NO: 7. In some embodiments of the methods disclosed herein, the one or more mutations in a receptor tyrosine kinase polypeptide sequence include an amino acid deletion, insertion, or substitution at a position corresponding to the conserved amino acid residue L1196 of the polypeptide of SEQ ID NO: 7. In some embodiments of the methods disclosed herein, the one or more mutations in a receptor tyrosine kinase polypeptide sequence include an amino acid deletion, insertion, or substitution at a position corresponding to the conserved amino acid residue G1202 of the polypeptide of SEQ ID NO: 7. In some embodiments, the one or more mutations in a receptor tyrosine kinase polypeptide sequence include an amino acid deletion, insertion, or substitution at a position corresponding to a Gly-to-Arg substitution G1202R of the polypeptide of SEQ ID NO: 7. In some embodiments, the one or more mutations in a receptor tyrosine kinase polypeptide sequence include an amino acid deletion, insertion, or substitution at a position corresponding to the conserved amino acid residue G1269 of the polypeptide of SEQ ID NO: 7. In some embodiments, the one or more mutations in a receptor tyrosine kinase polypeptide sequence include an amino acid deletion, insertion, or substitution at a position corresponding to a Gly-to-Cys substitution G1269C of the polypeptide of SEQ ID NO: 7. In some embodiments, the one or more mutations in a receptor tyrosine kinase polypeptide sequence include an amino acid deletion, insertion, or substitution at a position corresponding to a Gly-to-Ala substitution G1269A of the polypeptide of SEQ ID NO: 7. In some embodiments, the one or more mutations in a receptor tyrosine kinase polypeptide sequence include an amino acid deletion, insertion, or substitution at a position corresponding to a Gly-to-Ser substitution G1269S of the polypeptide of SEQ ID NO: 7.

[0100] In some embodiments of the methods disclosed herein, the one or more mutations in a receptor tyrosine kinase polypeptide sequence include an amino acid deletion, insertion, or substitution at a position corresponding to the conserved amino acid residue L2012 of the polypeptide of SEQ ID NO: 9. In some embodiments, the one or more mutations in a receptor tyrosine kinase polypeptide sequence include an amino acid deletion, insertion, or substitution at a position corresponding to a Leu-to-Met substitution L2012M of the polypeptide of SEQ ID NO: 9. In some embodiments of the methods disclosed herein, the

one or more mutations in a receptor tyrosine kinase polypeptide sequence include an amino acid deletion, insertion, or substitution at a position corresponding to the conserved amino acid residue L2026 of the polypeptide of SEQ ID NO: 9. In some embodiments of the methods disclosed herein, the one or more mutations in a receptor tyrosine kinase polypeptide sequence include an amino acid deletion, insertion, or substitution at a position corresponding to the conserved amino acid residue G2032 of the polypeptide of SEQ ID NO: 9. In some embodiments, the one or more mutations in a receptor tyrosine kinase polypeptide sequence include an amino acid deletion, insertion, or substitution at a position corresponding to a Gly-to-Arg substitution G2032R of the polypeptide of SEQ ID NO: 9. In some embodiments, of the one or more mutations in a receptor tyrosine kinase polypeptide sequence include an amino acid deletion, insertion, or substitution at a position corresponding to the conserved amino acid residue G2101 of the polypeptide of SEQ ID NO: 9. In some embodiments, the one or more mutations in a receptor tyrosine kinase polypeptide sequence include an amino acid deletion, insertion, or substitution at a position corresponding to a Gly-to-Cys substitution G2101C of the polypeptide of SEQ ID NO: 9. In some embodiments, the one or more mutations in a receptor tyrosine kinase polypeptide sequence include an amino acid deletion, insertion, or substitution at a position corresponding to a Gly-to-Ala substitution G2101A of the polypeptide of SEQ ID NO: 9. In some embodiments, the one or more mutations in a receptor tyrosine kinase polypeptide sequence include an amino acid deletion, insertion, or substitution at a position corresponding to a Gly-to-Ser substitution G2101S of the polypeptide of SEQ ID NO: 9.

[0101] With respect to a nucleotide-based assay, degeneracy of the genetic code provides the possibility to substitute at least one base of the protein encoding sequence of a gene with a different base without affecting the amino acid sequence of the polypeptide produced from the mutated gene to be changed. Hence, the polynucleotide sequence of the probes, primers used in the methods disclosed herein may also have any base sequence that has been changed from any polynucleotide sequence described herein by substitution in accordance with degeneracy of the genetic code. References describing codon usage are readily available to one of ordinary skill in the art.

[0102] It is further contemplated that polynucleotide and polypeptide sequences of a receptor tyrosine kinase disclosed herein may be altered by various methods, and that

these alterations may result in polynucleotide and polypeptide sequences having one or more mutations different than the sequences disclosed herein. As such, any of the polynucleotide and polypeptide sequences disclosed herein may be altered in various ways comprising amino acid substitutions, deletions, truncations, and insertions of one or more amino acids of the polypeptide sequences set forth in the Sequence Listing, comprising up to about 2, about 3, about 4, about 5, about 6, about 7, about 8, about 9, about 10, about 15, about 20, about 25, about 30, about 35, about 40, about 45, about 50, about 55, about 60, about 65, about 70, about 75, about 80, about 85, about 90, about 100, about 105, about 110, about 115, about 120, about 125, about 130 or more amino acid substitutions, deletions or insertions. Methods for such manipulations are generally known in the art.

[0103] Accordingly, other possible molecular alterations and mutations will be apparent to those skilled in the art based on the amino acid mutations in the kinase domain of the NRTK polypeptides that have been reported herein to confer resistance to one or more of the therapeutic agents described herein.

Methods for selecting/treating cancer patient and methods for identifying compounds suitable for the treatment of cancer

[0104] In one aspect, the present disclosure provides methods for treating cancer in patient, comprising (a) acquiring knowledge of the presence of one or more molecular alterations in a biological sample from the patient, wherein the one or more molecular alterations includes one or more mutations in one or more receptor tyrosine kinase polypeptides, wherein one or more receptor tyrosine kinase polypeptides is selected from TrkA, TrkB, TrkC, ALK and ROS1; (b) selecting a chemotherapeutic agent appropriate for the treatment of the cancer; and (c) administering a therapeutically effective amount of the selected chemotherapeutic agent to the patient.

[0105] In another aspect, some embodiments disclosed herein relate to methods for selecting a treatment regimen for a patient having cancer, comprising (a) acquiring knowledge of the presence of one or more mutations in a biological sample from the patient, wherein the one or more mutations is at an amino acid position selected from V573, F589, G595 and G667 of the TrkA polypeptide of SEQ ID NO:1; V619, F633, G639 and G709 of the TrkB polypeptide of SEQ ID NO:3; V603, F617, G623 and G696 of the TrkC

polypeptide of SEQ ID NO:5; V1182, L1196, G1202 and 1269 of the ALK polypeptide of SEQ ID NO:7; and L2012, L2026, G2032 and 2101 of the ROS1 polypeptide of SEQ ID NO:9; and (b) selecting an appropriate treatment regimen for the patient based on whether one or more of the mutations is present is the biological sample.

[0106] In yet another aspect, some embodiments disclosed herein relate to methods for predicting the outcome of a treatment regimen for a patient having cancer, comprising (a) acquiring knowledge of the presence of one or more mutations in a biological sample from the patient, wherein the one or more mutations is at an amino acid position selected from V573, F589, G595 and G667 of the TrkA polypeptide of SEQ ID NO:1; V619, F633, G639 and G709 of the TrkB polypeptide of SEQ ID NO:3; V603, F617, G623 and G696 of the TrkC polypeptide of SEQ ID NO:5; V1182, L1196, G1202 and 1269 of the ALK polypeptide of SEQ ID NO:7; and L2012, L2026, G2032 and 2101 of the ROS1 polypeptide of SEQ ID NO:9, wherein the presence of one or more of the mutations in the biological sample is indicative of an increased unresponsiveness in the patient to the treatment regimen.

[0107] In another aspect, some embodiments disclosed herein relate to methods for treating a patient having a cancer tumor, comprising (a) determining the presence of a nucleic acid encoding a mutated Trk protein in a tumor sample from the patient, wherein the mutated Trk protein comprises at least one mutation at an amino acid position selected from V573, F589, G595 and G667 of the TrkA polypeptide of SEQ ID NO:1; V619, F633, G639 and G709 of the TrkB polypeptide of SEQ ID NO:3; V603, F617, G623 and G696 of the TrkC polypeptide of SEQ ID NO:5; V1182, L1196, G1202 and 1269 of the ALK polypeptide of SEQ ID NO:7; and L2012, L2026, G2032 and 2101 of the ROS1 polypeptide of SEQ ID NO:9; (b) selecting a Trk inhibitor appropriate for the treatment of said tumor; and (c) administering said Trk inhibitor to the patient.

[0108] In one aspect, some embodiments disclosed herein relate to methods for treating a patient having a cancer tumor, wherein the cancer tumor contains a mutated Trk gene, and wherein the mutated Trk gene within the cancer tumor shows resistance or acquired resistance to treatment with Trk inhibitors. The methods, in some embodiments, include administering a therapeutically effective amount of a Trk inhibitor that is active against a polypeptide encoded by the mutated Trk gene to a patient in need thereof,

optionally in combination with radiotherapy, radio-immunotherapy and/or tumor resection by surgery.

[0109] In one aspect, some embodiments disclosed herein relate to methods for treating cancer in a patient comprising the steps of (a) selecting a patient with cancer having a Trk mutation; and (b) administering to said patient an inhibitor that is active against one or more of said Trk mutations.

[0110] In one aspect, some embodiments disclosed herein relate to methods for treating a patient having a cancer tumor, comprising (a) determining the presence of a mutated Trk protein in a tumor sample from said patient, said mutated Trk protein comprises at least one mutation at an amino acid position selected from V573, F589, G595 and G667 of the TrkA polypeptide of SEQ ID NO:1; V619, F633, G639 and G709 of the TrkB polypeptide of SEQ ID NO:3; V603, F617, G623 and G696 of the TrkC polypeptide of SEQ ID NO:5; V1182, L1196, G1202 and 1269 of the ALK polypeptide of SEQ ID NO:7; and L2012, L2026, G2032 and 2101 of the ROS1 polypeptide of SEQ ID NO:9; (b) selecting a Trk inhibitor appropriate for the treatment of the tumor; and (c) administering the Trk inhibitor to the patient.

[0111] In one aspect, some embodiments disclosed herein relate to methods for treating a cancer in a patient bearing a Trk mutation, wherein said subject has become resistant to at least one Trk inhibitor, comprising administering to said patient an effective amount of one or more inhibitors effective against multiple receptor tyrosine kinases.

[0112] In one aspect, some embodiments disclosed herein relate to methods for identifying a compound suitable for treatment of cancer in a patient who has become resistant to an inhibitor of a receptor tyrosine kinase resulting from one or more mutations in the receptor tyrosine kinase, comprising (a) acquiring knowledge of the presence of one or more mutations in a biological sample from said patient, wherein the one or more mutations is at an amino acid position selected from V573, F589, G595 and G667 of the TrkA polypeptide of SEQ ID NO:1; V619, F633, G639 and G709 of the TrkB polypeptide of SEQ ID NO:3; V603, F617, G623 and G696 of the TrkC polypeptide of SEQ ID NO:5; V1182, L1196, G1202 and 1269 of the ALK polypeptide of SEQ ID NO:7; and L2012, L2026, G2032 and 2101 of the ROS1 polypeptide of SEQ ID NO:9; (b) determining the ability of the compound to inhibit the receptor tyrosine kinase having one or more of the mutations;

and (c) identifying a compound as suitable for treatment of the patient if the compound inhibits the receptor tyrosine kinase having one or more of the mutations.

[0113] Implementations of the methods according to one or more of the above aspects and other aspects of the disclosure can include one or more of the following features. In some embodiments, the one or more mutations described herein includes one or more amino acid substitutions in the kinase catalytic domain of the receptor tyrosine kinase polypeptide. In some embodiments, the one or more one amino acid substitutions is at a position corresponding to an amino acid residue selected from the group consisting of the amino acid residues identified in FIG. 1 and/or TABLE 1 as conserved residues, and combinations of any thereof. In some embodiments, the one or more amino acid substitutions is at a position corresponding to an amino acid residue selected from V573, F589, G595 and G667 of the TrkA polypeptide of SEQ ID NO:1; V619, F633, G639 and G709 of the TrkB polypeptide of SEQ ID NO:3; V603, F617, G623 and G696 of the TrkC polypeptide of SEQ ID NO:5; V1182, L1196, G1202 and 1269 of the ALK polypeptide of SEQ ID NO:7; and L2012, L2026, G2032 and 2101 of the ROS1 polypeptide of SEQ ID NO:9.

[0114] In some embodiments, the one or more amino acid substitutions is at a position corresponding to amino acid residue V573 of the TrkA polypeptide of SEQ ID NO: 1. In some embodiments, the one or more amino acid substitutions is a Val-to-Met substitution at a position corresponding to amino acid residue V573 of the TrkA polypeptide (V573M). In some embodiments, the one or more amino acid substitutions is at a position corresponding to amino acid residue F589 of the TrkA polypeptide of SEQ ID NO: 1. In some embodiments, the one or more amino acid substitutions is a Phe-to-Leu substitution at a position corresponding to amino acid residue F589 of the TrkA polypeptide (F589L). In some embodiments, the one or more amino acid substitutions is at a position corresponding to amino acid residue G595 of the TrkA polypeptide of SEQ ID NO: 1. In some embodiments, the one or more amino acid substitutions is a Gly-to-Arg substitution at a position corresponding to amino acid residue G595 of the TrkA polypeptide (G595R). In some embodiments, the one or more amino acid substitutions is at a position corresponding to amino acid residue G667 of the TrkA polypeptide of SEQ ID NO: 1. In some embodiments, the one or more amino acid substitutions is a Gly-to-Cys substitution at a position corresponding to amino acid residue G667 of the TrkA polypeptide (G667C). In

some embodiments, the one or more amino acid substitutions is a Gly-to-Ala substitution at a position corresponding to amino acid residue G667 of the TrkA polypeptide (G667A). In some embodiments, the one or more amino acid substitutions is a Gly-to-Ser substitution at a position corresponding to amino acid residue G667 of the TrkA polypeptide (G667S).

[0115] In some embodiments, the one or more amino acid substitutions is at a position corresponding to amino acid residue V619 of the TrkB polypeptide of SEQ ID NO: 3. In some embodiments, the one or more amino acid substitutions is a Val-to-Met substitution at a position corresponding to amino acid residue V619 of the TrkB polypeptide (V619M). In some embodiments, the one or more amino acid substitutions is at a position corresponding to amino acid residue F633 of the TrkB polypeptide of SEQ ID NO: 3. In some embodiments, the one or more amino acid substitutions is a Phe-to-Leu substitution at a position corresponding to amino acid residue F633 of the TrkB polypeptide (F633L). In some embodiments, the one or more amino acid substitutions is at a position corresponding to amino acid residue G639 of the TrkB polypeptide of SEQ ID NO: 3. In some embodiments, the one or more amino acid substitutions is a Gly-to-Arg substitution at a position corresponding to amino acid residue G639 of the TrkB polypeptide (G639R). In some embodiments, the one or more amino acid substitutions is at a position corresponding to amino acid residue G709 of the TrkB polypeptide of SEQ ID NO: 3. In some embodiments, the one or more amino acid substitutions is a Gly-to-Cys substitution at a position corresponding to amino acid residue G709 of the TrkB polypeptide (G709C). In some embodiments, the one or more amino acid substitutions is a Gly-to-Ala substitution at a position corresponding to amino acid residue G709 of the TrkB polypeptide (G709A). In some embodiments, the one or more amino acid substitutions is a Gly-to-Ser substitution at a position corresponding to amino acid residue G709 of the TrkB polypeptide (G709S).

[0116] In some embodiments, the one or more amino acid substitutions is at a position corresponding to amino acid residue V603 of the TrkC polypeptide of SEQ ID NO: 5. In some embodiments, the one or more amino acid substitutions is a Val-to-Met substitution at a position corresponding to amino acid residue V603 of the TrkC polypeptide (V603M). In some embodiments, the one or more amino acid substitutions is at a position corresponding to amino acid residue F617 of the TrkC polypeptide of SEQ ID NO: 5. In some embodiments, the one or more amino acid substitutions is at a position corresponding

to amino acid residue G623 of the TrkC polypeptide of SEQ ID NO: 5. In some embodiments, the one or more amino acid substitutions is a Phe-to-Leu substitution at a position corresponding to amino acid residue F623 of the TrkC polypeptide (F623L). In some embodiments, the one or more amino acid substitutions is a Gly-to-Arg substitution at a position corresponding to amino acid residue G623 of the TrkC polypeptide (G623R). In some embodiments, the one or more amino acid substitutions is at a position corresponding to amino acid residue G696 of the TrkC polypeptide of SEQ ID NO: 5. In some embodiments, the one or more amino acid substitutions is a Gly-to-Cys substitution at a position corresponding to amino acid residue G696 of the TrkC polypeptide (G696C). In some embodiments, the one or more amino acid substitutions is a Gly-to-Ala substitution at a position corresponding to amino acid residue G696 of the TrkC polypeptide (G696A). In some embodiments, the one or more amino acid substitutions is a Gly-to-Ser substitution at a position corresponding to amino acid residue G696 of the TrkC polypeptide (G696S).

[0117] In some embodiments, the methods disclosed herein relate to treat, reduce the symptoms of, ameliorate the symptoms of, delay the onset of, or otherwise pharmaceutically address a cancer condition in a patient that has been previously treated with one or more receptor tyrosine kinase inhibitors and has developed at least partial resistance to one or more such inhibitors.

[0118] In some embodiments, the methods disclosed herein relate to treat, reduce the symptoms of, ameliorate the symptoms of, delay the onset of, or otherwise pharmaceutically address a cancer condition in a patient that has been previously treated with one or more receptor tyrosine kinase inhibitors and has developed at least partial resistance to one or more such inhibitors. Non-limiting examples of such receptor tyrosine kinase inhibitors include AZ-23, BMS-754807, bosutinib, cabozantinib, ceritinib, crizotinib, entrectinib, foretinib, GNF 5837, GW441756, imatinib mesylate, K252a, LOXO-101, MGCD516, nilotinib hydrochloride monohydrate, NVP-TAE684, PF-06463922, rebastinib, staurosporine, sorafenib tosylate, sunitinib malate, TSR-011, and any combinations thereof (TABLE 2). In some embodiments, the methods disclosed herein relate to treat, reduce the symptoms of, ameliorate the symptoms of, delay the onset of, or otherwise pharmaceutically address a cancer condition in a patient that has been previously treated with entrectinib.

TABLE 2. Non-limiting examples of chemotherapeutic agents

Compound Name	CAS Registry No.	Chemical Name	Reference
crizotinib	877399-52-5	(R)-3-[1-(2,6-Dichloro-3-fluorophenyl)ethoxy]-5-[1-(piperidin-4-yl)-1H-pyrazol-4-yl]pyridin-2-amine	United States Patent No. 7,230,098
entrectinib	1108743-60-7	N-[5-(3,5-difluorobenzyl)-1H-indazol-3-yl]-4-(4-methyl-piperazin-1-yl)-2-(tetrahydro-pyran-4-ylamino)-benzamide	United States Patent No. 8,299,057
NVP-TAE684	761439-42-3	5-chloro-N2-[2-methoxy-4-[4-(4-methyl-1-piperazinyl)-1-piperidinyl]phenyl]-N4-[2-[(1-methylethyl)sulfonyl]phenyl]- 2,4-Pyrimidinediamine	United States Patent No. 7,964,592
foretinib	937176-80-2	1-N'-[3-fluoro-4-[6-methoxy-7-(3-morpholin-4-ylpropoxy)quinolin-4-yl]oxyphenyl]-1-N-(4-fluorophenyl)cyclopropane-1,1-dicarboxamide	United States Patent No. 8,497,284
BMS-754807	1001350-96-4	(2S)-1-[4-[(5-cyclopropyl-1H-pyrazol-3-yl)amino]pyrrolo[2,1-f][1,2,4]triazin-2-yl]-N-(6-fluoropyridin-3-yl)-2-methylpyrrolidine-2-carboxamide	United States Patent No. 7,534,792
GNF 5837	1033769-28-6	1-[2-fluoro-5-(trifluoromethyl)phenyl]-3-[4-methyl-3-[[(3Z)-2-oxo-3-(1H-pyrrol-2-ylmethylidene)-1H-indol-6-yl]amino]phenyl]urea	WO 2008073480
rebastinib	1020172-07-9	4-[4-[(5-tert-butyl-2-quinolin-6-ylpyrazol-3-yl)carbamoylamino]-3-fluorophenoxy]-N-methylpyridine-2-carboxamide	United States Patent No. 7,790,756
GW441756	504433-23-2	3-[(1-methylindol-3-yl)methylidene]-1H-pyrrolo[3,2-b]pyridin-2-one	United States Patent No. 7,015,231
cabozantinib	849217-68-1	1-N-[4-(6,7-dimethoxyquinolin-4-yl)oxyphenyl]-1-N'-(4-fluorophenyl)cyclopropane-1,1-dicarboxamide	United States Patent No. 7,579,473
bosutinib	380843-75-4	4-(2,4-dichloro-5-methoxyanilino)-6-methoxy-7-[3-(4-methylpiperazin-1-yl)propoxy]quinoline-3-carbonitrile	WO 2004075898
Compound 2	1034974-86-1	N-[5-(3,5-difluoro-benzenesulfonyl)-1H-indazol-3-yl]-2-((R)-2-methoxy-1-methyl-ethylamino)-4-(4-methyl-piperazin-1-yl) benzamide	United States Patent No. 8,114,865
TSR-011	1388225-79-3	N-[1,3-dihydro-6-[[4-(1-hydroxy-1-methylethyl)-1-piperidinyl]methyl]-1-[cis-4-[[(1-methylethyl)amino]carbonyl]cyclohexyl]-2H-benzimidazol-2-ylidene]-3,5-difluoro-, [N(E)]- benzamide,	*Journal of Medicinal Chemistry*, Volume 55, Issue 14, pp. 6523-6540, 2012

MGCD516	1123837-84-2	N-[3-fluoro-4-[[2-[5-[[(2-methoxyethyl)amino]methyl]-2-pyridinyl]thieno[3,2-b]pyridin-7-yl]oxy]phenyl]-N'-(4-fluorophenyl)- 1,1-cyclopropanedicarboxamide	United States Patent No. 8,404,846
ceritinib	1032900-25-6	5-chloro-2-N-(5-methyl-4-piperidin-4-yl-2-propan-2-yloxyphenyl)-4-N-(2-propan-2-ylsulfonylphenyl)pyrimidine-2,4-diamine	United States Patent No. 8,372,858
LOXO-101	1223403-58-4	(3S)-N-[5-[(2R)-2-(2,5-difluorophenyl)-1-pyrrolidinyl]pyrazolo[1,5-a]pyrimidin-3-yl]-3-hydroxy-1-pyrrolidinecarboxamide	United States Patent No. 8,513,263
PF-06463922	1454846-35-5	(10R)- 7-amino-12-fluoro-10,15,16,17-tetrahydro-2,10,16-trimethyl-15-oxo-2H-4,8-Methenopyrazolo[4,3-h][2,5,11]benzoxadiazacyclotetradecine-3-carbonitrile	United States Patent No. 8,680,111
AZ-23	915720-21-7	5-chloro-2-N-[(1S)-1-(5-fluoropyridin-2-yl)ethyl]-4-N-(3-propan-2-yloxy-1H-pyrazol-5-yl)pyrimidine-2,4-diamine	United States Patent No. 8,1149,89
K252a	99533-80-9	9,12-Epoxy-1H-diindolo[1,2,3-fg:3',2',1'-kl]pyrrolo[3,4-i][1,6]benzodiazocine-10-carboxylic acid, 2,3,9,10,11,12-hexahydro-10-hydroxy-9-methyl-1-oxo-, methyl ester, (9S,10R,12R)-	United States Patent No. 4,555,402
Staurosporine	62996-74-1	9,13-Epoxy-1H,9H-diindolo[1,2,3-gh:3',2',1'-lm]pyrrolo[3,4-j][1,7]benzodiazonin-1-one, 2,3,10,11,12,13-hexahydro-10-methoxy-9-methyl-11-(methylamino)-, (9S,10R,11R,13R)-	Commercially available; *Journal of Antibiotics* Volume 30, Issue 4, pp.75-82, 1977

[0119] In some embodiments of the methods disclosed herein, the one or more mutations in a receptor tyrosine kinase polypeptide confers resistance or acquired resistance to treatment with entrectinib, rebastinib, or a pharmaceutically acceptable salt thereof.

[0120] Some embodiments of the methods disclosed herein comprise selecting a chemotherapeutic agent appropriate for the treatment of the cancer, and administering a therapeutically effective amount of the selected chemotherapeutic agent to the patient. Non-limiting examples of such chemotherapeutic agents include those listed in TABLE 2, or any pharmaceutically acceptable salt thereof. In some embodiments, the selected chemotherapeutic agent is selected from the group consisting of entrectinib, NVP-TAE684, rebastinib, Compound 2, and any pharmaceutically acceptable salt thereof.

[0121] The methods and compounds according to the present disclosure can be deployed for selecting and/or treating a patient having any cancer. Non-limiting examples of

suitable cancers to be treated include anaplastic large-cell lymphoma (ALCL), colorectal cancer (CRC), cholangiocarcinoma, gastric, glioblastomas (GBM), leiomyosarcoma, melanoma, non-small cell lung cancer (NSCLC), squamous cell lung cancer, neuroblastoma (NB), ovarian cancer, pancreatic cancer, prostate cancer, medullary thyroid cancer, breast cancer, papillary thyroid cancer, or any combination thereof.

[0122] Some embodiments of the methods disclosed herein relate to treat, reduce the symptoms of, ameliorate the symptoms of, delay the onset of, or otherwise pharmaceutically address a cancer condition selected from anaplastic large-cell lymphoma (ALCL), colorectal cancer (CRC), cholangiocarcinoma, gastric, glioblastomas (GBM), leiomyosarcoma, melanoma, non-small cell lung cancer (NSCLC), squamous cell lung cancer, neuroblastoma (NB), ovarian cancer, pancreatic cancer, prostate cancer, medullary thyroid cancer, breast cancer, papillary thyroid cancer, in which one or more mutations in a receptor tyrosine kinase polypeptide selected from TrkA, TrkB, TrkC, ALK and ROS1 might play a role by selecting chemotherapeutic agent appropriate for the treatment of the cancer condition, and administering a therapeutically effective amount of the selected chemotherapeutic agent to the patient.

[0123] The types of biological samples suitable for use in the methods described herein are not particularly limited. In some embodiments, the biological sample comprises sputum, bronchoalveolar lavage, pleural effusion, tissue, whole blood, serum, plasma, buccal scrape, saliva, cerebrospinal fluid, urine, stool, circulating tumor cells, circulating nucleic acids, bone marrow, or any combination thereof. In yet some embodiments, the biological sample includes whole blood and blood components. In some embodiments, the blood component comprises plasma. In yet other embodiments, the biological sample includes cells or tissue. In some embodiments, the tissue is a tumor or cancer tissue.

[0124] In some embodiments of the methods disclosed herein, the acquiring knowledge of one or more molecular alterations from an analytical assay performed on a biological sample obtained from a patient. The analytical assay can generally be any analytical assay known to those having ordinary skill in the art, and can be for example an antibody-based assay, a nucleotide-based assay, or an enzymatic activity assay. Non-limited examples of suitable analytical assays include nucleic acid sequencing, polypeptide sequencing, restriction digestion, capillary electrophoresis, nucleic acid amplification-based

assays, nucleic acid hybridization assay, comparative genomic hybridization, real-time PCR, quantitative reverse transcription PCR (qRT-PCR), PCR-RFLP assay, HPLC, mass-spectrometric genotyping, fluorescent in-situ hybridization (FISH), next generation sequencing (NGS), and a kinase activity assay. Other examples of suitable analytical assays include ELISA, immunohistochemistry, western blotting, mass spectrometry, flow cytometry, protein-microarray, immunofluorescence, a multiplex detection assay, or any combination thereof.

[0125] In some embodiments, an electrophoretic mobility assay is used to acquire the knowledge of the one or more molecular alterations in the biological sample obtained from a patient. For example, a nucleic acid sequence encoding the mutation is detected by amplifying the nucleic acid region corresponding to the one or more alterations in a receptor tyrosine kinase gene and comparing the electrophoretic mobility of the amplified nucleic acid to the electrophoretic mobility of the corresponding region in a wild-type receptor tyrosine kinase gene.

[0126] In some embodiments, the analytical assay used to acquire the knowledge of the one or more molecular alterations in the biological sample involves polymerase chain reactions (PCR) or nucleic acid amplification-based assays. A number of PCR-based analytical assays known in the art are suitable for the methods disclosed herein, comprising but not limited to real-time PCR, quantitative reverse transcription PCR (qRT-PCR), and PCR-RFLP assay.

[0127] In some embodiments, the analytical assay used to acquire the knowledge of the one or more molecular alterations in the biological sample involves determining a nucleic acid sequence and/or an amino acid sequence comprising the one or more molecular alterations. In some embodiments, the nucleic acid sequence comprising the one or more molecular alterations from a cancer patient is sequenced. In some embodiments, the sequence is determined by a next generation sequencing procedure. As used herein "next-generation sequencing" refers to oligonucleotide sequencing technologies that have the capacity to sequence oligonucleotides at speeds above those possible with conventional sequencing methods (*e.g.* Sanger sequencing), due to performing and reading out thousands to millions of sequencing reactions in parallel. Non-limiting examples of next-generation sequencing methods/platforms include Massively Parallel Signature Sequencing (Lynx Therapeutics);

solid-phase, reversible dye-terminator sequencing (Solexa/Illumina); DNA nanoball sequencing (Complete Genomics); SOLiD technology (Applied Biosystems); 454 pyro-sequencing (454 Life Sciences/Roche Diagnostics); ion semiconductor sequencing (ION Torrent); and technologies available from Pacific Biosciences, Intelligen Bio-systems, Oxford Nanopore Technologies, and Helicos Biosciences.

[0128] Accordingly, in some embodiments, the NGS procedure used in the methods disclosed herein can comprise pyrosequencing, sequencing by synthesis, sequencing by ligation, or a combination of any thereof. In some embodiments, the NGS procedure is performed by an NGS platform selected from Illumina, Ion Torrent, Qiagen, Invitrogen, Applied Biosystem, Helicos, Oxford Nanopore, Pacific Biosciences, and Complete Genomics.

[0129] In some embodiments, FISH analysis can be used to identify the chromosomal mutations resulting in the one or more molecular alterations such as the mutated genes or mutated gene products (*i.e.* polypeptides) as described herein. For example, to perform FISH, at least a first probe tagged with a first detectable label can be designed to target a mutated gene of a mutated polypeptide, and at least a second probe tagged with a second detectable label can be designed to target the corresponding wild-type gene or wild-type polypeptide such that one of ordinary skill in the art observing the probes can determine that a relevant gene or gene product is present in the sample. Generally, FISH assays are performed using formalin-fixed, paraffin-embedded tissue sections that are placed on slides. For example, the DNA from the biological samples is denatured to single-stranded form and subsequently allowed to hybridize with the appropriate DNA probes that can be designed and prepared using methods and techniques known to those having ordinary skill in the art. Following hybridization, any unbound probe may be removed by a series of washes and the nuclei of the cells are counter-stained with DAPI (4',6 diamidino-2-phenylindole), a DNA-specific stain that fluoresces blue. Hybridization of the probe or probes are viewed using a fluorescence microscope equipped with appropriate excitation and emission filters, allowing visualization of the fluorescent signals. Other variations of the FISH method known in the art are also suitable for evaluating a patient selected in accordance with the methods disclosed herein.

[0130] In some embodiments, the analytical assay used to acquire the knowledge of the one or more molecular alterations in the biological sample involves a nucleic acid hybridization assay. The term "hybridization", as used herein, refers generally to the ability of nucleic acid molecules to join via complementary base strand pairing. Such hybridization may occur when nucleic acid molecules are contacted under appropriate conditions and/or circumstances. As used herein, two nucleic acid molecules are said to be capable of specifically hybridizing to one another if the two molecules are capable of forming an anti-parallel, double-stranded nucleic acid structure. A nucleic acid molecule is said to be the "complement" of another nucleic acid molecule if they exhibit complete complementarity. As used herein, nucleic acid molecules are said to exhibit "complete complementarity" when every nucleotide of one of the molecules is complementary to its base pairing partner nucleotide of the other. Two molecules are said to be "minimally complementary" if they can hybridize to one another with sufficient stability to permit them to remain annealed to one another under at least conventional "low-stringency" conditions. In some instances, the molecules are said to be "complementary" if they can hybridize to one another with sufficient stability to permit them to remain annealed to one another under conventional "high-stringency" conditions. Nucleic acid molecules that hybridize to other nucleic acid molecules, *e.g.*, at least under low stringency conditions are said to be "hybridizable cognates" of the other nucleic acid molecules. Conventional stringency conditions are described by Sambrook *et al.*, *Molecular Cloning, A Laboratory Handbook,* Cold Spring Harbor Laboratory Press, 1989), and by Haymes *et al.* In: *Nucleic Acid Hybridization, A Practical Approach*, IRL Press, Washington, D.C. (1985). Departures from complete complementarity are therefore permissible, as long as such departures do not completely preclude the capacity of the molecules to form a double-stranded structure. Thus, in order for a nucleic acid molecule or fragment thereof to serve as a primer or probe it needs only be sufficiently complementary in sequence to be able to form a stable double-stranded structure under the particular solvent and salt concentrations employed.

[0131] Appropriate stringency conditions which promote DNA hybridization include, for example, 6.0× sodium chloride/sodium citrate (SSC) at about 45°C, followed by a wash of 2.0×SSC at about 50°C. In addition, the temperature in the wash step can be increased from low stringency conditions at room temperature, about 22°C, to high

stringency conditions at about 65°C. Both temperature and salt may be varied, or either the temperature or the salt concentration may be held constant while the other variable is changed. These conditions are known to those skilled in the art, or can be found in *Current Protocols in Molecular Biology*, John Wiley & Sons, N.Y. (1989), 6.3.1- 6.3.6. For example, low stringency conditions may be used to select nucleic acid sequences with lower sequence identities to a target nucleic acid sequence. One may wish to employ conditions such as about 0.15 M to about 0.9 M sodium chloride, at temperatures ranging from about 20°C to about 55°C. High stringency conditions may be used to select for nucleic acid sequences with higher degrees of identity to the disclosed nucleic acid sequences (Sambrook *et al.*, 1989, supra). In one embodiment, high stringency conditions involve nucleic acid hybridization in about 2×SSC to about 10×SSC (diluted from a 20×SSC stock solution containing 3 M sodium chloride and 0.3 M sodium citrate, pH 7.0 in distilled water), about 2.5× to about 5× Denhardt's solution (diluted from a 50× stock solution containing 1% (w/v) bovine serum albumin, 1% (w/v) ficoll, and 1% (w/v) polyvinylpyrrolidone in distilled water), about 10 mg/mL to about 100 mg/mL fish sperm DNA, and about 0.02% (w/v) to about 0.1% (w/v) SDS, with an incubation at about 50°C to about 70°C for several hours to overnight. High stringency conditions are preferably provided by 6×SSC, 5× Denhardt's solution, 100 mg/mL sheared and denatured salmon sperm DNA, and 0.1% (w/v) SDS, with incubation at 55×C for several hours. Hybridization is generally followed by several wash steps. The wash compositions generally comprise 0.5×SSC to about 10×SSC, and 0.01% (w/v) to about 0.5% (w/v) SDS with a 15-min incubation at about 20°C to about 70°C. Preferably, the nucleic acid segments remain hybridized after washing at least one time in 0.1×SSC at 65°C. In some instances, very high stringency conditions may be used to select for nucleic acid sequences with much higher degrees of identity to the disclosed nucleic acid sequences. Very high stringency conditions are defined as prehybridization and hybridization at 42°C in 5×SSPE, 0.3% SDS, 200 μg/mL sheared and denatured salmon sperm DNA, and 50% formamide and washing three times each for 15 minutes using 2×SSC, 0.2% SDS at 70°C.

[0132] In some embodiments, the analytical assay used to acquire the knowledge of the one or more molecular alterations in the biological sample involves a nucleic acid hybridization assay that includes contacting nucleic acids derived from the biological sample with a nucleic acid probe comprising (1) a nucleic acid sequence complementary to a nucleic

acid sequence encoding the one or more mutations and further comprising (2) a detectable label.

[0133] In some embodiments are provided such methods, wherein the knowledge of the presence of the one or more molecular alterations is obtained from an assay performed simultaneously on a plurality of biological samples. In some embodiments, the plurality of biological samples may be assayed in a multitest platform.

[0134] As used herein, the term "multitest platform" is intended to encompass any suitable means to contain one or more reaction mixtures, suspensions, or detection reactions. As such, the outcomes of a number of screening events can be assembled onto one surface, resulting in a "multitest platform" having, or consisting of multiple elements or parts to do more than one experiment simultaneously. It is intended that the term "multitest platform" encompasses protein chips, microtiter plates, multi-well plates, microcards, test tubes, petri plates, trays, slides, and the like. In some embodiments, multiplexing can further include simultaneously conducting a plurality of screening events in each of a plurality of separate biological samples. For example, the number of biological samples analyzed can be based on the number of spots on a slide and the number of tests conducted in each spot. In another example, the number of biological samples analyzed can be based on the number of wells in a multi-well plate and the number of tests conducted in each well. For example, 6-well, 12-well, 24-well, 48-well, 96-well, 384-well, 1536-well or 3456-well microtiter plates can be useful in the presently disclosed methods, although it will be appreciated by those in the art, not each microtiter well need contain a patient biological sample. Depending on the size of the microtiter plate and the number of the individual biological samples in each well, very high numbers of tests can be run simultaneously. In some embodiments, the plurality of biological samples includes at least 6, 12, 24, 48, 96, 200, 384, 400, 500, 1000, 1250, 1500, or 3000 sample.

[0135] In some embodiments, knowledge is acquired from an antibody-based assay, comprising but not limited to ELISA, immunohistochemistry, western blotting, mass spectrometry, flow cytometry, protein-microarray, immunofluorescence, a multiplex detection assay, or any combination thereof. In some embodiments, the antibody-based assay includes the use of one or more antibodies that selectively bind to one or more of TrkA, TrkB, TrkC, ALK, and ROS1 polypeptides.

[0136] In some embodiments of the methods disclosed herein, the chemotherapeutic agents, or a pharmaceutically acceptable salt thereof, are selected for administration or are administered to an individual or patient having cancer, optionally in combination with at least one additional chemotherapeutic agent. In some embodiments, entrectinib, rebastinib, NVP-TAE684, staurosporine, Compound 2, or a pharmaceutically acceptable salt thereof is used in the methods disclosed herein as the chemotherapeutic agent that is appropriate for treating cancer.

[0137] In some embodiments, the chemotherapeutic agents described herein, or a pharmaceutically acceptable salt thereof, is administered at a therapeutically effective amount to the patient. As used herein, the term "therapeutically effective amount" means that amount of the compound or compounds being administered which will relieve to some extent one or more of the symptoms of the disorder being treated. In reference to the treatment of a cancer, a therapeutically effective amount refers to that amount which has the effect of (1) reducing the size of a cancer tumor, (2) inhibiting (that is, slowing to some extent, preferably stopping) cancer tumor metastasis, (3) inhibiting to some extent (that is, slowing to some extent, preferably stopping) cancer tumor growth, and/or, (4) relieving to some extent (or, preferably, eliminating) one or more symptoms associated with the cancer.

[0138] This amount will vary depending upon a variety of factors, comprising but not limited to the characteristics of the bioactive compositions and formulations disclosed herein (comprising activity, pharmacokinetics, pharmacodynamics, and bioavailability thereof), the physiological condition of the subject treated (comprising age, sex, disease type and stage, general physical condition, responsiveness to a given dosage, and type of medication) or cells, the nature of the pharmaceutically acceptable carrier or carriers in the formulation, and the route of administration. Further, an effective or therapeutically effective amount may vary depending on whether the one or more bioactive compositions and formulations disclosed herein is administered alone or in combination with other drug(s), other therapy/therapies or other therapeutic method(s) or modality/modalities. One skilled in the clinical and pharmacological arts will be able to determine an effective amount or therapeutically effective amount through routine experimentation, namely by monitoring a cell's or subject's response to administration of the one or more bioactive compositions and formulations disclosed herein and adjusting the dosage accordingly. Additional guidance

with regard to this aspect can be found in, for example, Remington: *The Science and Practice of Pharmacy*, 21st Edition, Univ. of Sciences in Philadelphia (USIP), Lippincott Williams & Wilkins, Philadelphia, PA, 2005.

[0139] In some embodiments of the methods disclosed herein, the selected chemotherapeutic agents, or a pharmaceutically acceptable salt thereof, is administered as a single therapeutic agent or in combination with one or more additional therapeutic agents.

[0140] In some embodiments of the methods disclosed herein, the selected chemotherapeutic agent, or a pharmaceutically acceptable salt thereof, are administered to a patient having or suffering from cancer in an amount ranging from about 200 mg/m^{2}to about 1600 mg/m^2, or from about 200 mg/m^{2}to about 1200 mg/m^2, or from about 200 mg/m^{2}to about 1000 mg/m^2, or from about 400 mg/m^{2}to about 1200 mg/m^2, or from about 400 mg/m^{2}to about 1000 mg/m^2, or from about 800 mg/m^{2}to about 1000 mg/m^2, or from about 800 mg/m^{2}to about 1200 mg/m^2, or from about 800 mg/m^{2}to about 1200 mg/m^2, or from about 800 mg/m^{2}to about 1600 mg/m^2. In some embodiments, the chemotherapeutic agents described above are administered to the patient in an amount of about 200 mg/m^2, about 300 mg/m^2, about 400 mg/m^2, about 500 mg/m^2, about 600 mg/m^2, about 700 mg/m^2, about 800 mg/m^2, about 900 mg/m^2, about 1000 mg/m^2, about 1100 mg/m^2, about 1200 mg/m^2, about 1300 mg/m^2, about 1400 mg/m^2, about 1500 mg/m^2, about 1600 mg/m^2, about 1700 mg/m^2, about 1800 mg/m^2, about 1900 mg/m^2, or about 2000 mg/m^2. In some embodiments, the selected chemotherapeutic agent, or a pharmaceutically accepted salt thereof, is administered to a patient or individual having or suffering from cancer in multiple dosages for a treatment period of 2 to 50 days. In some embodiments, the selected chemotherapeutic agent, or a pharmaceutically accepted salt thereof, is administered to a patient or individual having or suffering from cancer in multiple dosages of about 50 to about 200 mg/kg per dose over a treatment period of 5 to 42 days. In some embodiments, the selected chemotherapeutic agent, or a pharmaceutically accepted salt thereof, is administered to a patient having or suffering from cancer with an oral dosage of about 60 mg/kg twice a day (BID), seven times per week. In some embodiments, the selected chemotherapeutic agent, or a pharmaceutically accepted salt thereof, is administered to a patient having or suffering from cancer with an oral dosage of about 60 mg/kg twice a day (BID), seven times per week for six weeks, on alternate weekly basis (*i.e.* one week on one week off).

[0141] Some embodiments include any of the methods described herein, the selected chemotherapeutic agent, or a pharmaceutically acceptable salt thereof, are administered to a patient having or suffering from cancer in an amount ranging from about 0.01 mg /kg to about 100 mg/kg, or from about 0.02 mg/kg to about 50 mg/kg, or from about 0.05 mg/kg to about 25 mg/kg, or from about 0.1 mg/kg to about 20 mg/kg, or from about 0.2 mg/kg to about 10 mg/kg, or from about 0.5 mg/kg to about 5 mg/kg, or from about 1 mg/kg to about 2 mg/kg.

[0142] In some embodiments of the methods disclosed herein, the chemotherapeutic agents described herein may be administered to a cancer patient in need thereof by administration to the patient of a pharmaceutical composition comprising one or more such agents. In particular, such pharmaceutical compositions may comprise one or more of the chemotherapeutic agents described herein, or a pharmaceutically acceptable salt thereof, and at least one pharmaceutically acceptable excipient.

[0143] In some embodiments, such pharmaceutical compositions can comprise a physical admixture of the various ingredients in solid, liquid, or gelcap form. Other embodiments can comprise at least two separated ingredients in a single dosage unit or dosage form, such as, for example, a two- or three-layer tablet in which at least two active ingredients are located in separate layers or regions of the tablet, optionally separated by a third material, such as, for example, a sugar layer or other inert barrier to prevent contact between the first two ingredients. In other embodiments, two or more active ingredients are separately formulated into individual dosage units, which are then packaged together for ease of administration. One embodiment comprises a package containing a plurality of individual dosage units. This embodiment may, for example, comprise a blister package. In one embodiment of a blister package, multiple blister-packed dosage units are present on a single sheet, and those units that are to be administered together are packaged in the same or adjacent blisters of the blister pack. Alternatively, any other packaging can be used in which two active ingredients are packaged together for concurrent or sequential use.

[0144] Some embodiments relate to the use of any of the chemotherapeutic agents as described herein, or a pharmaceutically acceptable salt thereof, in the manufacture of a medicament for the treatment of abnormal cell growth in a mammal. The present disclosure further relates to the use of any of the chemotherapeutic agents as described herein, or a

pharmaceutically acceptable salt thereof, in the manufacture of a medicament for the treatment of abnormal cell growth in a mammal wherein the abnormal cell growth is cancerous or non-cancerous. In some embodiments, the abnormal cell growth is cancerous. In another embodiment, the abnormal cell growth is non-cancerous.

[0145] Some embodiments relate to pharmaceutical compositions comprising a chemotherapeutic agent described herein, or a pharmaceutically acceptable salt thereof, a pharmaceutically acceptable carrier and, optionally, at least one additional medicinal or pharmaceutical agent. In some embodiments, the at least one additional medicinal or pharmaceutical agent is an anti-cancer agent as described below.

[0146] The pharmaceutically acceptable carrier may comprise a conventional pharmaceutical carrier or excipient. Suitable pharmaceutical carriers include inert diluents or fillers, water and various organic solvents (such as hydrates and solvates). The pharmaceutical compositions may, if desired, contain additional ingredients such as flavorings, binders, excipients and the like. Thus for oral administration, tablets containing various excipients, such as citric acid may be employed together with various disintegrants such as starch, alginic acid and certain complex silicates and with binding agents such as sucrose, gelatin and acacia. Additionally, lubricating agents such as magnesium stearate, sodium lauryl sulfate and talc are often useful for tableting purposes. Solid compositions of a similar type may also be employed in soft and hard filled gelatin capsules. Non-limiting examples of materials, therefore, include lactose or milk sugar and high molecular weight polyethylene glycols. When aqueous suspensions or elixirs are desired for oral administration the active compound therein may be combined with various sweetening or flavoring agents, coloring matters or dyes and, if desired, emulsifying agents or suspending agents, together with diluents such as water, ethanol, propylene glycol, glycerin, or combinations thereof.

[0147] The pharmaceutical composition may, for example, be in a form suitable for oral administration as a tablet, capsule, pill, powder, sustained release formulations, solution suspension, for parenteral injection as a sterile solution, suspension or emulsion, for topical administration as an ointment or cream or for rectal administration as a suppository.

[0148] Exemplary parenteral administration forms include solutions or suspensions of active compounds in sterile aqueous solutions, for example, aqueous

propylene glycol or dextrose solutions. Such dosage forms may be suitably buffered, if desired.

[0149] The pharmaceutical composition may be in unit dosage forms suitable for single administration of precise dosages.

[0150] In some embodiments, the composition comprises a therapeutically effective amount of a compound as disclosed herein and a pharmaceutically acceptable carrier.

[0151] The compounds described herein may be formulated into pharmaceutical compositions as described below in any pharmaceutical form recognizable to the skilled artisan as being suitable. Pharmaceutical compositions of the disclosure comprise a therapeutically effective amount of at least one compound disclosed herein and an inert, pharmaceutically acceptable carrier or diluent.

[0152] To treat or prevent diseases or conditions mediated by one or more of the mutated receptor tyrosine kinase disclosed herein, a pharmaceutical composition is administered in a suitable formulation prepared by combining a therapeutically effective amount of at least one compound (as an active ingredient) with one or more pharmaceutically suitable carriers, which may be selected, for example, from diluents, excipients and auxiliaries that facilitate processing of the active compounds into the final pharmaceutical preparations.

[0153] The pharmaceutical carriers employed may be either solid or liquid. Exemplary solid carriers are lactose, sucrose, talc, gelatin, agar, pectin, acacia, magnesium stearate, stearic acid and the like. Exemplary liquid carriers are syrup, peanut oil, olive oil, water and the like. Similarly, the inventive compositions may include time-delay or time-release material known in the art, such as glyceryl monostearate or glyceryl distearate alone or with a wax, ethylcellulose, hydroxypropylmethylcellulose, methylmethacrylate or the like. Further additives or excipients may be added to achieve the desired formulation properties. For example, a bioavailability enhancer, such as Labrasol, Gelucire or the like, or formulator, such as CMC (carboxy-methylcellulose), PG (propyleneglycol), or PEG (polyethyleneglycol), may be added. Gelucire®, a semi-solid vehicle that protects active ingredients from light, moisture and oxidation, may be added, *e.g.*, when preparing a capsule formulation.

[0154] If a solid carrier is used, the preparation can be tableted, placed in a hard gelatin capsule in powder or pellet form, or formed into a troche or lozenge. The amount of solid carrier may vary, but generally will be from about 25 mg to about 1 g. If a liquid carrier is used, the preparation may be in the form of syrup, emulsion, soft gelatin capsule, sterile injectable solution or suspension in an ampoule or vial or non-aqueous liquid suspension. If a semi-solid carrier is used, the preparation may be in the form of hard and soft gelatin capsule formulations. The inventive compositions are prepared in unit-dosage form appropriate for the mode of administration, *e.g.* parenteral or oral administration.

[0155] To obtain a stable water-soluble dose form, a salt of a compound may be dissolved in an aqueous solution of an organic or inorganic acid, such as a 0.3 M solution of succinic acid or citric acid. If a soluble salt form is not available, the agent may be dissolved in a suitable co-solvent or combinations of co-solvents. Examples of suitable co-solvents include alcohol, propylene glycol, polyethylene glycol 300, polysorbate 80, glycerin and the like in concentrations ranging from 0 to 60% of the total volume. In an exemplary embodiment, a compound is dissolved in DMSO and diluted with water. The composition may also be in the form of a solution of a salt form of the active ingredient in an appropriate aqueous vehicle such as water or isotonic saline or dextrose solution.

[0156] Proper formulation is dependent upon the route of administration selected. For injection, the agents of the compounds may be formulated into aqueous solutions, preferably in physiologically compatible buffers such as Hanks solution, Ringer's solution, or physiological saline buffer. For transmucosal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

[0157] For oral administration, the compounds can be formulated by combining the active compounds with pharmaceutically acceptable carriers known in the art. Such carriers enable the compounds of the disclosure to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions and the like, for oral ingestion by a subject to be treated. Pharmaceutical preparations for oral use can be obtained using a solid excipient in admixture with the active ingredient (agent), optionally grinding the resulting mixture, and processing the mixture of granules after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients include: fillers such as sugars,

comprising lactose, sucrose, mannitol, or sorbitol; and cellulose preparations, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, gum, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carboxymethylcellulose, or polyvinylpyrrolidone (PVP). If desired, disintegrating agents may be added, such as crosslinked polyvinyl pyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate.

[0158] Dragee cores are provided with suitable coatings. For this purpose, concentrated sugar solutions may be used, which may optionally contain gum arabic, polyvinyl pyrrolidone, Carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for identification or to characterize different combinations of active agents.

[0159] Pharmaceutical preparations that can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a plasticizer, such as glycerol or sorbitol. The push-fit capsules can contain the active ingredients in admixture with fillers such as lactose, binders such as starches, and/or lubricants such as talc or magnesium stearate, and, optionally, stabilizers. In soft capsules, the active agents may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols. In addition, stabilizers may be added. All formulations for oral administration should be in dosages suitable for such administration. For buccal administration, the compositions may take the form of tablets or lozenges formulated in conventional manner.

[0160] For administration intranasally or by inhalation, the compounds for use according to the present disclosure may be conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebuliser, with the use of a suitable propellant, *e.g.*, dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of gelatin for use in an inhaler or insufflator and the like may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

[0161] The compounds may be formulated for parenteral administration by injection, *e.g.*, by bolus injection or continuous infusion. Formulations for injection may be

presented in unit-dosage form, *e.g.*, in ampoules or in multi-dose containers, with an added preservative. The compositions may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents.

[0162] Pharmaceutical formulations for parenteral administration include aqueous solutions of the active compounds in water-soluble form. Additionally, suspensions of the active agents may be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes. Aqueous injection suspensions may contain substances that increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Optionally, the suspension may also contain suitable stabilizers or agents that increase the solubility of the compounds to allow for the preparation of highly concentrated solutions.

[0163] Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, *e.g.* sterile pyrogen-free water, before use.

[0164] In addition to the formulations described above, the compounds may also be formulated as a depot preparation. Such long-acting formulations may be administered by implantation (for example, subcutaneously or intramuscularly) or by intramuscular injection. Thus, for example, the compounds may be formulated with suitable polymeric or hydrophobic materials (for example, as an emulsion in an acceptable oil) or ion-exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt. A pharmaceutical carrier for hydrophobic compounds is a co-solvent system comprising benzyl alcohol, a non-polar surfactant, a water-miscible organic polymer, and an aqueous phase. The co-solvent system may be a VPD co-solvent system. VPD is a solution of 3% w/v benzyl alcohol, 8% w/v of the non-polar surfactant polysorbate 80, and 65% w/v polyethylene glycol 300, made up to volume in absolute ethanol. The VPD co-solvent system (VPD: 5 W) contains VPD diluted 1:1 with a 5% dextrose in water solution. This co-solvent system dissolves hydrophobic compounds well, and itself produces low toxicity upon systemic administration. The proportions of a co-solvent system may be suitably varied without destroying its solubility and toxicity characteristics. Furthermore, the identity of the co-solvent components may be varied: for example, other low-toxicity non-polar surfactants

may be used instead of polysorbate 80; the fraction size of polyethylene glycol may be varied; other biocompatible polymers may replace polyethylene glycol, *e.g.* polyvinyl pyrrolidone; and other sugars or polysaccharides may be substituted for dextrose.

[0165] Alternatively, other delivery systems for hydrophobic pharmaceutical compounds may be employed. Liposomes and emulsions are known examples of delivery vehicles or carriers for hydrophobic drugs. Certain organic solvents such as dimethylsulfoxide also may be employed, although usually at the cost of greater toxicity due to the toxic nature of DMSO. Additionally, the compounds may be delivered using a sustained-release system, such as semipermeable matrices of solid hydrophobic polymers containing the therapeutic agent. Various sustained-release materials have been established and are known by those skilled in the art. Sustained-release capsules may, depending on their chemical nature, release the compounds for a few weeks up to over 100 days. Depending on the chemical nature and the biological stability of the therapeutic reagent, additional strategies for protein stabilization may be employed.

[0166] The pharmaceutical compositions also may comprise suitable solid- or gel-phase carriers or excipients. These carriers and excipients may provide marked improvement in the bioavailability of poorly soluble drugs. Examples of such carriers or excipients include calcium carbonate, calcium phosphate, sugars, starches, cellulose derivatives, gelatin, and polymers such as polyethylene glycols. Furthermore, additives or excipients such as Gelucire®, Capryol®, Labrafil®, Labrasol®, Lauroglycol®, Plurol®, Peceol®, Transcutol® and the like may be used.

[0167] Further, the pharmaceutical composition may be incorporated into a skin patch for delivery of the drug directly onto the skin.

[0168] It will be appreciated that the actual dosages of the agents of this disclosure will vary according to the particular agent being used, the particular composition formulated, the mode of administration, and the particular site, host, and disease being treated. Those skilled in the art using conventional dosage-determination tests in view of the experimental data for a given compound may ascertain optimal dosages for a given set of conditions. For oral administration, an exemplary daily dose generally employed will be from about 0.001 to about 1000 mg/kg of body weight, with courses of treatment repeated at appropriate intervals.

[0169] Furthermore, the pharmaceutically acceptable formulations may contain a compound or compounds, or a salt or solvate thereof, in an amount of about 10 mg to about 2000 mg, or from about 10 mg to about 1500 mg, or from about 10 mg to about 1000 mg, or from about 10 mg to about 750 mg, or from about 10 mg to about 500 mg, or from about 25 mg to about 500 mg, or from about 50 to about 500 mg, or from about 100 mg to about 500 mg. Furthermore, the pharmaceutically acceptable formulations may contain a compound, or a salt or solvate thereof, in an amount of about 50 mg, about 100 mg, about 150 mg, about 200 mg, about 250 mg, about 300 mg, about 350 mg, about 400 mg, about 450 mg, or about 500 mg.

[0170] Additionally, the pharmaceutically acceptable formulations may contain a compound, or a salt or solvate thereof, in an amount from about 0.5 w/w % to about 95 w/w %, or from about 1 w/w % to about 95 w/w %, or from about 1 w/w % to about 75 w/w %, or from about 5 w/w % to about 75 w/w %, or from about 10 w/w % to about 75 w/w %, or from about 10 w/w % to about 50 w/w %.

[0171] The compounds disclosed herein, or salts or solvates thereof, may be administered to a mammal suffering from abnormal cell growth, such as a human, either alone or as part of a pharmaceutically acceptable formulation, once a week, once a day, twice a day, three times a day, or four times a day, or even more frequently.

[0172] Those of ordinary skill in the art will understand that with respect to the compounds, the particular pharmaceutical formulation, the dosage, and the number of doses given per day to a mammal requiring such treatment, are all choices within the knowledge of one of ordinary skill in the art and can be determined without undue experimentation.

[0173] Administration of the compounds disclosed herein may be effected by any method that enables delivery of the compounds to the site of action. These methods include oral routes, intraduodenal routes, parenteral injection (comprising intravenous, subcutaneous, intramuscular, intravascular or infusion), topical, and rectal administration. Bolus doses can be used, or infusions over a period of 1, 2, 3, 4, 5, 10, 15, 20, 30, 60, 90, 120 or more minutes, or any intermediate time period can also be used, as can infusions lasting 3, 4, 5, 6, 7, 8, 9, 10. 12, 14 16, 20, 24 or more hours or lasting for 1-7 days or more. Infusions can be administered by drip, continuous infusion, infusion pump, metering pump, depot formulation, or any other suitable means.

[0174] Dosage regimens may be adjusted to provide the optimum desired response. For example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form, as used herein, refers to physically discrete units suited as unitary dosages for the mammalian subjects to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms are dictated by and directly dependent on (a) the unique characteristics of the chemotherapeutic agent and the particular therapeutic or prophylactic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active compound for the treatment of sensitivity in patients.

[0175] Thus, the skilled artisan would appreciate, based upon the disclosure provided herein, that the dose and dosing regimen is adjusted in accordance with methods well-known in the therapeutic arts. That is, the maximum tolerable dose can be readily established, and the effective amount providing a detectable therapeutic benefit to a patient may also be determined, as can the temporal requirements for administering each agent to provide a detectable therapeutic benefit to the patient. Accordingly, while certain dose and administration regimens are exemplified herein, these examples in no way limit the dose and administration regimen that may be provided to a patient in practicing the present disclosure.

[0176] It is to be noted that dosage values may vary with the type and severity of the condition to be alleviated, and may include single or multiple doses. It is to be further understood that for any particular subject, specific dosage regimens should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions, and that dosage ranges set forth herein are exemplary only and are not intended to limit the scope or practice of the claimed composition. For example, doses may be adjusted based on pharmacokinetic or pharmacodynamic parameters, which may include clinical effects such as toxic effects and/or laboratory values. Thus, the present disclosure encompasses intra-patient dose-escalation as determined by the skilled artisan. Determining appropriate dosages and regimens for

administration of the chemotherapeutic agent are well-known in the relevant art and would be understood to be encompassed by the skilled artisan once provided the teachings disclosed herein.

[0177] The discussion of the general methods given herein is intended for illustrative purposes only. Other alternative methods and alternatives will be apparent to those of skill in the art upon review of this disclosure, and are to be included within the spirit and purview of this application.

EXAMPLES

[0178] Additional alternatives are disclosed in further detail in the following examples, which are not in any way intended to limit the scope of this disclosure or the claims.

EXAMPLE 1

Generation of KM12 and Ba/F3-Tel/TrkA cell lines resistant to entrectinib

[0179] This Example describes the generation of entrectinib-resistant KM12 cell lines and entrectinib-resistant BA/F3-TEL/TRKA cell lines.

[0180] Schematic illustrations for selection and characterization of entrectinib-resistant KM12 cells are shown in FIG. 3 and FIG. 4. Cells of human colorectal cell line KM12 which harbors a TrkA fusion gene TPM3-TrkA were treated with 0, 1, 3, 10 nM of entrectinib initially in two independent sets of flasks (labeled A and B) in the complete culture media (RPMI medium (GIBCO®) + 10% FBS (fetal bovine serum) + Penicillin and Streptomycin). Culture media containing 0.1% DMSO (*i.e.* untreated control) or entrectinib were changed every 3-4 days and the cultured cells were split about once a week. KM12 cells treated with 10 nM of entrectinib were subsequently cultured in the presence of 30 nM of entrectinib for approximately two weeks after initial treatment of 10 nM of entrectinib. Approximately four weeks after treatment, the cells were sequentially treated with 100 nM of entrectinib and, after another about 4-week period, were treated with 300 nM of entrectinib. At the end of each stage of treatment, cell aliquots were analyzed for growth inhibition by entrectinib for 3-day treatment using CellTiter Glo® (Promega). RNA/DNA was extracted

from each of the cell samples. RT-PCR and sequencing analysis were performed by BioSettia (San Diego, CA).

[0181] Schematic illustrations for selection and characterization of entrectinib-resistant Ba/F3-Tel/TrkA cells are shown in FIG. 3 and FIG. 8. Input Ba/F3-Tel/TrkA cell line was an engineered cell line harboring a recombinant TrkA fusion gene ETV6-TrkA. Ba/F3-Tel/TrkA cells were treated with 0 and 3 nM of entrectinib initially in two independent sets of flasks (labeled A and B) in the complete culture media (RPMI + 10% FBS + Penicillin and Streptomycin). Seven days after the initial treatment, Ba/F3-Tel/TrkA cells treated with 3 nM of entrectinib were subsequently cultured in 10 and 30 nM of entrectinib in two independent sets (A and B) of flasks for approximately two weeks. Cell viability was evaluated by trypan blue and counted every two days (FIG. 9). At Day 24, the cells cultured at 3nM of entrectinib were set up in triplicates (that is, 3 replicates from set A and 3 replicates from set B) and incubated with 6, 12, or 24 nM of entrectinib. The survived cell pools, named Ba/F3-Tel/TrkA-10nMA, Ba/F3-Tel/TrkA-6nMA1, Ba/F3-Tel/TrkA-6nMA2, Ba/F3-Tel/TrkA-6nMA3, Ba/F3-Tel/TrkA-6nMB1, Ba/F3-Tel/TrkA-6nMB2, Ba/F3-Tel/TrkA-6nMB3, Ba/F3-Tel/TrkA-12nMA1, Ba/F3-Tel/TrkA-12nMA2, Ba/F3-Tel/TrkA-12nMA3, Ba/F3-Tel/TrkA-12nMB2 and Ba/F3-Tel/TrkA-12nMB3, were expended and further characterized. Cells of the parental line and the entrectinib-resistant cells were analyzed for growth inhibition by entrectinib for 3-day treatment using CellTiter Glo (Promega). RNA/DNA was extracted from each of the cell samples. RT-PCR and sequencing analysis were performed by BioSettia (San Diego, CA).

EXAMPLE 2

Cellular IC50 of RTK inhibitors as determined by growth inhibition studies in KM12 cells and BA/F3-TEL/TRKA cells

[0182] This Example describes a general procedure developed to evaluate the anti-proliferative activity of RTK inhibitors, *e.g.* entrectinib, in parental KM12 cells and entrectinib-resistant KM12 cells. Cells of a parental KM12 line and an entrectinib-resistant KM12 line were trypsinized and seeded at 5,000 cells per well in 96-well assay white plates (Costar #3610), followed by an overnight incubation in the complete media without entrectinib. The next day, different concentrations of each of the RTK inhibitors, *e.g.*

entrectinib (0 to 1 μM), were added to the wells. Each treatment condition was performed in duplicate. Similarly, Ba/F3-Tel/TrkA cells were seeded at 5,000 cells per well in 96-well assay white plates (Costar #3610) in the complete media without entrectinib and, on the next day, were treated with different concentrations of each of the RTK inhibitors, *e.g.* entrectinib (0 to 1 μM) in duplicates. Three days after incubation, cell viabilities were measured by luciferase-based ATP level detection using CellTiter-Glo® reagents (Promega) and IC50s were determined by 4-parameter curve fit with variable slope.

EXAMPLE 3

Generation of Ba/F3-TPM3 and Ba/F3-TPM3-TrkA-G959R Cell Lines

[0183] This Example describes studies performed to generate transgenic Ba/F3 cells expressing either a wild-type protein TPM3-TrkA or a TPM3-TrkA-G595R fusion protein. A cDNA encoding TPM3-TrkA fusion was cloned from a KM12 parental cell line and entrectinib-resistant cells by a PCR-based technique and subsequently inserted into a lentiviral vector pVL-EF1a-MCS-IRES-Puro (BioSettia, San Diego, CA). After confirmation of the cDNA inserts by direct sequencing, vesicular stomatitis virus GP (VSVG)-pseudo-typed lentiviruses containing either the TPM3-TrkA cDNA or the TPM3-TrkA-G595R cDNA were transduced into the murine IL-3 dependent pro-B cell Ba/F3 at different multiplicity of infections (MOIs) with 8 μg/mL of polybrene (EMD Millipore). The transduced Ba/F3 cells were selected in the murine IL-3 containing RPMI media supplemented with 10% FBS and 1 μg/mL of puromycin for two weeks. The stable cell pools were further selected in RPMI media (GIBCO®) supplemented with 10% FBS (fetal bovine serum) and without murine IL-3 for 4 weeks.

EXAMPLE 4

Isolation and characterization of entrectinib-resistant KM12 cells

[0184] Six samples (duplicated samples at each treatment) of parental KM12 cells were treated with 0.01% DMSO (v/v), 1 nM entrectinib, 3 nM entrectinib, or 10 nM entrectinib for about two weeks. No apparent change in morphology and doubling time for the cells was observed. At the end of the two-week treatment, the duplicated samples of the KM12 cells treated with 10 nM entrectinib were cultured in growth media containing 30 nM

of entrectinib. A slightly slower growth rate for these KM12-10nM treated cells was observed. As shown in at FIG. 5, in a 3-day growth inhibition study, KM12 cells (Set A) cultured in DMSO (vehicle) and 1-10 nM entrectinib displayed overlapping growth inhibition curves and similar IC50 values (TABLE 3 and FIG. 5). However, KM12-30 nM-A treated cells displayed an upshifted growth curve and a ~ 2-fold increase of IC50 value (TABLE 3), indicating a reduced sensitivity to entrectinib.

TABLE 3. IC50 values of the kinase inhibitor entrectinib in parental KM12 cells and entrectinib-resistant cell lines

Cell lines	KM12-DMSO-A	KM12-1nM A	KM12-3nM A	KM12-10nM A	KM12-30nM A
IC50 (nM)	1.26	1.57	1.64	1.71	3.41
R square	0.9795	0.9841	0.9747	0.9615	0.9626

[0185] When entrectinib concentration in the culture media was increased from 30 to 100 μM for about 4 weeks, KM12 cells of Set A became even less sensitive to entrectinib, as indicated by the upshift of the bottom plateau (FIG. 6) and increased IC50 values (TABLE 4).

TABLE 4. IC50 values of the RTK inhibitor entrectinib in parental KM12 cells and entrectinib-resistant cell lines

	KM12-DMSO	KM12-1nM A	KM12-3nM A	KM12-10nM A	KM12-30nM A	KM12-100nM A
IC50 (nM)	3.95	4.19	4.36	4.65	6.47	6.62
R square	0.9934	0.9952	0.9894	0.9343	0.9277	0.9055

[0186] KM12 cells of Set B were also tested for their sensitivity to entrectinib (FIG. 10 and Table 5). As shown in TABLE 5, drastic increases of IC50 values in cells of Set B were observed when these cells were cultured at 30 nM and higher concentrations of entrectinib. Additionally, the change in cells cultured in media containing 100 nM of entrectinib for 4 weeks was found to be genetically stable. This conclusion was drawn from the observation that following a 4-weeks culture period in the presence of 100 nM of entrectinib (KM12-100nM-B), the IC50 values were found stable even after entrectinib was

withdrawn from the cell culture media (KM12-100nM-B (no drug)), suggesting the change in these cells were at the genomic level.

TABLE 5. Sensitivity of KM12 cells of Set B to entrectinib as determined by IC50 values

entrectinib	KM12-DMSO-B	KM12-30nM-B	KM12-100nM-B	KM12-300nM-B	KM12-100nM-B (no drug)
IC50 (nM)	1.14	93.83	70.37	208.7	85.41
R square	0.9790	0.9656	0.9624	0.9282	0.9567

[0187] Following four weeks of treatment with 300 nM of entrectinib, RNA was isolated from each of the KM12 cell pools of both Set A and Set B, and subsequently subjected to RT-PCR and sequencing analysis. As shown at FIG. 7 and TABLE 6, no mutations in TrkA kinase domain were found in the cell pools of Set A, while the cells of Set B were found to possess two point mutations at position G595 and G667 in the TrkA kinase domain (TABLE 6). In particular, a Gly-to-Arg substitution was identified at residue G595 (i.e., G595R), and a Gly-to-Cys substitution was identified at residue G667 (i.e., G667C) (FIG. 7).

TABLE 6. Results from sequence analysis from entrectinib-treated cell pools of Set A and Set B

Sample ID	Mutations in TrkA Kinase Domain
KM12-DMSO-A	Wild-type sequences
KM12-30 nM-A	Wild-type sequences
KM12-100 nM-A	Wild-type sequences
KM12-100 nM-A (no drug)	Wild-type sequences
KM12-300 nM-A	Wild-type sequences
KM12-DMSO-B	Wild-type sequences
KM12-30 nM-B	G/T, Glycine/Cysteine, 667, exon 15
KM12-100 nM-B	G/T, Glycine/Cysteine, 667, exon 15
KM12-100 nM-B (no drug)	G/T, Glycine/Cysteine, 667, exon 15
KM12-300 nM-B	G/A, Glycine/Arginine, 595, exon 14

[0188] Without being bound by any particular theory, two mechanisms of resistance are believed possible. In Set A, the resistance of KM12 could be a bypass mechanism, in which other signal transduction pathways have been affected. This possibility is supported by the observation that there is no mutation in the TPM3-trkA gene. In Set B,

the change of nucleotide G to T (see, TABLE 6 and FIG. 7) resulted in a missense mutation (G667C) in exon 15 in KM12 cells cultured in entrectinib between 30-100 nM. However, when the cells were cultured in entrectinib concentrations ranging from 100 nM to 300 nM for another 4 weeks, a G to A change (FIG. 7) resulted in G595R in exon 14, but no G667C mutation was observed in these cells.

[0189] KM12 cells (cultured in 100 nM entrectinib) bearing the G667C mutation were found to be genetically stable because withdrawing the 100 nM of entrectinib for 4 weeks did not reverse the G667C mutation (TABLE 6). In the sequence alignment of FIG. 1, the amino acid numbering of TrkA is with reference to the full-length sequence of TrkA having GenBank accession number NP_002520.2. The corresponding amino acid numberings of TrkB and TrkC are shown in TABLES 1 and 7.

TABLE 7. Concordance positions of conserved amino acid residues in the kinase domains of human TrkA, TrkB, and TrkC polypeptides

Gene Name	Species	GeneBank ID	Length (aa)	residue	Residue
NTRK1, TrkA	Human	NP_002520.2 NM_002529.3	796	G595	G667
NTRK2, TrkB	Human	NP_006171.2 NM_006180.3	838	G639	G709
NTRK3, TrkC	Human	NP_001012338.1 NM_001012338.2	839	G623	G696

EXAMPLE 5

Isolation and characterization of entrectinib-resistant Ba/F3-tel/trkA cells

[0190] Parental Ba/F3-Tel/TrkA cells were treated with the RTK inhibitor entrectinib at treatment regimens as described in Example 4 above. Entrectinib-resistant Ba/F3-Tel/TrkA cells were isolated and subsequently characterized by using the procedures described in Example 4. Cell pools of 10 nM entrectinib-resistant Ba/F3- Tel/TrkA were established after 2-week selection (FIG. 9). Remarkably, as showed in FIGs. 11A and 11B, a 10nM entrectinib-resistant Ba/F3-Tel/TrkA-10nMA cell pool displayed an IC50 which was >100 fold higher than that of the control parental line, indicating a drastic reduction of these cells' sensitivity to entrectinib. As showed at FIG. 15, these entrectinib-resistant Baf3-trkA (A) cells were found to harbor the same G667C and G595R mutations as discussed above in

Example 4 with regard to the entrectinib-resistant KM12 cell lines. Additionally, as shown in FIG. 19, a different treatment of Ba/F3-Tel/TrkA at 12 nM Entrectinib resulted in a G667C change in multiple clones, which was also the same change identified in Entrectinib-KM12 resistant cells as discussed above in Example 4.

EXAMPLE 6

Identification of compounds capable of inhibiting growth of entrectinib-resistant KM12 cells and entrectinib-resistant Ba/F3-tel/trkA cells

[0191] This example describes a study performed to screen a number of chemical compounds for their ability to inhibit the proliferation of mutant KM12 and Ba/F3-tel/trkA cells harboring either G595R or G667C mutation, using the experimental procedure described in Example 2 above. Such compounds, once identified, would be useful in the treatment of cancer patients that have developed resistance to an inhibitor of a receptor tyrosine kinase. In this experiment, each of the following cell lines: Ba/F3-tel/trkA, Ba/F3-tel/trkA-10nMA(G595R), KM12-DMSO, KM12-30nM101-B (G667C), KM12-100nM101-B (G667C), KM12-300nM101-B (G595R) was screened against a number of chemical compounds. Exemplifications of such compounds are listed in TABLEs 2 and 8-9.

[0192] As showed in FIGs. 27 and 30, and TABLES 8-9 below, entrectinib, rebastinib, staurosporine, NVP-TAE684, and Compound 2 each showed significant inhibitory activity against mutant cells harboring the TrkA-G595C mutation or the TrkA-G667C mutation.

TABLE 8. IC50 values of six candidate compounds tested against parental KM12 and Ba/F3-tel/trkA cell lines (WT) and respective mutant cell lines harboring the RKA-G595C mutation or the G595R mutation.

Inhibitor	BaF3-tel/trkA_WT	BaF3-tel/trkA_G595R	KM12-TPM3/trkA_WT	KM12-TPM3/trkA_G667C	KM12-TPM3/trkA_G595R
Entrectinib	2.8	>1 uM	3.4	367.4	>1 uM
Compound 2	2.3	846.2	6.1	465.7	424.5
ceritinib	>1 uM	>1 uM	268.7	>1 uM	>1 uM
LOXO-101	33.0	>1 uM	27.0	>1 uM	>1 uM
PF06463922	385.6	>1 uM	205.6	>1 uM	>1 uM

crizotinib (PF1066) Xalkori	179.2	432.8	76.6	>1 uM	>1 uM

TABLE 9. IC50 values of four candidate compounds tested against parental Ba/F3-tel/trkA, Ba/F3-tel/trkB, Ba/F3-tel/trkC cell lines (WT) and mutant BaF3-tel/trkA cell line harboring the G595R mutation.

	IC50 (nM)			
Name by company	**BaF3-tel/trkA**	**BaF3-tel/trkA-10nMA(G595R)**	**BaF3-tel/trkB**	**BaF3-tel/trkC**
entrectinib	2.5	1099.0	8.7	5.5
AZ-23	1.7	91.4	3.3	2.2
K252a	10.9	133.3	45.3	14.3
Starausporine	2.0	6.5	4.2	3.3

EXAMPLE 7

Activities of Entrectinib and LOXO-101 in the inhibition of growth of Ba/F3 Cell Lines Expressing NTRK1 Wild-Type and Various Mutant NTRK1

[0193] This example describes a study performed to study the ability of the entrectinib and LOXO-101 compounds to inhibit the proliferation of wild-type and mutant Ba/F3-tel/trkA_cells harboring various mutations, using the experimental procedure described in Example 2 above . In this experiment, each of entrectinib and LOXO-101 was screened against the mutants in TABLE 10.

TABLE 10. IC50 values of entrectinib and LOXO-101 tested against Ba/F3 Cell Lines Expressing NTRK1 Wild-Type and Various Mutant NTRK1

Cell lines	**NTRK1 Mutation**	**Entrectinib (nM)**	**LOXO-101 (nM)**
Ba/F3-LNMA-NTRK1		2.3	11.9
Ba/F3-LNMA-NTRK1-V573M	V573M	24.2	621.9
Ba/F3-LNMA-NTRK1-G667A	G667A	5.1	74.4
Ba/F3-LNMA-NTRK1-G667S	G667S	14.6	197.7

Ba/F3-LMNA-NTRK1 F589L	F589L	9.7	>1000
Ba/F3-LNMA-NTRK1-G595R	G595R	>1000	>1000
Ba/F3-TPM3-NTRK1		5.0	15.1
Ba/F3-TPM3-NTRK1-G667C	G667C	69.0	>1000
Ba/F3-TPM3-NTRK1-G595R	G595R	>1000	>1000

EXAMPLE 8

Activities of Entrectinib, LOXO-101, and Staurosporine in the inhibition of growth of Ba/F3 Cell Lines Expressing NTRK1 Wild-Type and Various Mutant NTRK1

[0194] This example describes a study performed to study the ability of the entrectinib, LOXO-101, and staurosporine compounds to inhibit the proliferation of wild-type and mutant Ba/F3-tel/trkA cells harboring various mutations, using the experimental procedure described in Example 2 above. In this experiment, each of entrectinib, LOXO-101, and staurosporine was screened against the mutants in TABLE 11.

TABLE 11. IC50 values of entrectinib, LOXO-101 and staurosporine tested against Ba/F3 Cell Lines Expressing NTRK1 Wild-Type and Various Mutant NTRK1

Cell Lines	Entrectinib (nM)	LOXO-101 (nM)	Staurosporine (nM)
BaF3-LMNA-NTRK1	2.4	15.4	2.2
BaF3-LMNA-NTRK1-F589L	2.9	>1000	1.6
BaF3-LMNA-NTRK1-G667A	3.8	61.4	0.6
BaF3-LMNA-NTRK1-G595R	>1000	>1000	3.9

[0195] All of the references disclosed herein, including but not limited to journal articles, textbooks, patents and patent applications, are hereby incorporated by reference for

the subject matter discussed herein and in their entireties. However, no admission is made that any reference cited herein constitutes prior art. Throughout this disclosure, various information sources are referred to and incorporated by reference. The information sources include, for example, scientific journal articles, patent documents, textbooks, and World Wide Web browser-inactive page addresses. The reference to such information sources is solely for the purpose of providing an indication of the general state of the art at the time of filing. While the contents and teachings of each and every one of the information sources can be relied on and used by one of skill in the art to make and use the embodiments disclosed herein, any discussion and comment in a specific information source should no way be considered as an admission that such comment was widely accepted as the general opinion in the field.

[0196] The discussion of the general methods given herein is intended for illustrative purposes only. It is not intended to be exhaustive or to limit the disclosure. Individual aspects or features of a particular embodiment are generally not limited to that particular embodiment, but, where applicable, are interchangeable and can be used in a selected embodiment, even if not specifically shown or described. It is expressly contemplated that any aspect or feature of the present disclosure can be combined with any other aspect, features, or combination of aspects and features disclosed herein. Other alternative methods and embodiments will be apparent to those of skill in the art upon review of this disclosure, and are to be included within the spirit and purview of this application.

[0197] The term "comprise" and variants of the term such as "comprises" or "comprising" are used herein to denote the inclusion of a stated integer or stated integers but not to exclude any other integer or any other integers, unless in the context or usage an exclusive interpretation of the term is required.

CLAIMS:

1. A method for treating a patient having a cancer tumor, comprising

a) determining the presence of a nucleic acid encoding a mutated Trk protein in a tumor sample from said patient, wherein said mutated Trk protein comprises at least one mutation at an amino acid position selected from:

i. V573, F589, G595 and G667 of the TrkA polypeptide set forth in SEQ ID NO: 1;

ii. V619, F633, G639 and G709 of the TrkB polypeptide set forth in SEQ ID NO: 3; and

iii. V603, F617, G623 and G696 of the TrkC polypeptide set forth in SEQ ID NO: 5; and

b) administering to said patient a Trk-inhibiting compound.

2. The method of claim 1, wherein said one or more amino acid mutations is at a position corresponding to amino acid residue V573 of the TrkA polypeptide.

3. The method of claim 2, wherein said one or more amino acid mutations is a Val-to-Met substitution (V573M).

4. The method of claim 1, wherein said one or more amino acid mutations is at a position corresponding to amino acid residue F589 of the TrkA polypeptide.

5. The method of claim 4, wherein said one or more amino acid mutations is a Phe-to-Leu substitution (F598L).

6. The method of claim 1, wherein said one or more mutations is at a position corresponding to amino acid residue G595 of the TrkA polypeptide.

7. The method of claim 6, wherein said one or more mutations is a Gly-to-Arg substitution (G595R).

8. The method of claim 1, wherein said one or more mutations is at a position corresponding to amino acid residue G667 of the TrkA polypeptide.

9. The method of claim 8, wherein said one or more mutations is a Gly-to-Cys substitution (G667C).

10. The method of claim 8, wherein said one or more amino acid mutations is a Gly-to-Ala substitution (G667A).

11. The method of claim 8, wherein said one or more amino acid mutations is a Gly-to-Ser substitution (G667S).

12. The method of claim 1, wherein said one or more amino acid mutations is at a position corresponding to amino acid residue V619 of the TrkB polypeptide.

13. The method of claim 12, wherein said one or more amino acid mutations is a Val-to-Met substitution (V619M).

14. The method of claim 1, wherein said one or more amino acid mutations is at a position corresponding to amino acid residue F633 of the TrkB polypeptide.

15. The method of claim 14, wherein said one or more amino acid mutations is a Phe-to-Leu substitution (F633L).

16. The method of claim 1, said one or more mutations is at a position corresponding to amino acid residue G639 of the TrkB polypeptide.

17. The method of claim 16, wherein said one or more mutations is a Gly-to-Arg substitution (G639R).

18. The method of claim 1, wherein said one or more mutations is at a position corresponding to amino acid residue G709 of the TrkB polypeptide.

19. The method of claim 18, wherein said one or more mutations is a Gly-to-Cys substitution (G709C).

20. The method of claim 18, wherein said one or more amino acid mutations is a Gly-to-Ala substitution (G709A).

21. The method of claim 18, wherein said one or more amino acid mutations is a Gly-to-Ser substitution (G709S).

22. The method of claim 1, wherein said one or more amino acid mutations is at a position corresponding to amino acid residue V603 of the TrkC polypeptide.

23. The method of claim 22, wherein said one or more amino acid mutations is a Val-to-Met substitution (V603M).

24. The method of claim 1, wherein said one or more amino acid mutations is at a position corresponding to amino acid residue F617 of the TrkC polypeptide.

25. The method of claim 24, wherein said one or more amino acid mutations is a Phe-to-Leu substitution (F617L).

26. The method of claim 1, wherein said one or more mutations is at a position corresponding to amino acid residue G623 of the TrkC polypeptide.

27. The method of claim 26, wherein said one or more mutations is a Gly-to-Arg substitution (G623R).

28. The method of claim 1, wherein said one or more mutations is at a position corresponding to amino acid residue G696 of the TrkC polypeptide.

29. The method of claim 29, wherein said one or more mutations is a Gly-to-Cys substitution (G696C).

30. The method of claim 28, wherein said one or more amino acid mutations is a Gly-to-Ala substitution (G696A).

31. The method of claim 28, wherein said one or more amino acid mutations is a Gly-to-Ser substitution (G696S).

32. The method of any one of claims 1 to 31, wherein said cancer is selected from anaplastic large-cell lymphoma (ALCL), colorectal cancer (CRC), cholangiocarcinoma, gastric, glioblastomas (GBM), leiomyosarcoma, melanoma, non-small cell lung cancer (NSCLC), squamous cell lung cancer, neuroblastoma (NB), ovarian cancer, pancreatic cancer, prostate cancer, medullary thyroid cancer, breast cancer, papillary thyroid cancer, or any combination thereof.

33. Use of a Trk-inhibiting compound in the manufacture of a medicament for the treatment of a patient having a cancer tumor, wherein said tumor is determined to contain a nucleic acid encoding a mutated Trk protein, and wherein said mutated Trk protein comprises at least one mutation at an amino acid position selected from:

(i) V573, F589, G595 and G667 of the TrkA polypeptide set forth in SEQ ID NO: 1;

(ii) V619, F633, G639 and G709 of the TrkB polypeptide set forth in SEQ ID NO: 3; and

(iii) V603, F617, G623 and G696 of the TrkC polypeptide set forth in SEQ ID NO: 5.

34. The use of claim 33, wherein said one or more amino acid mutations is at a position corresponding to amino acid residue V573 of the TrkA polypeptide.

35. The use of claim 34, wherein said one or more amino acid mutations is a Val-to-Met substitution (V573M).

36. The use of claim 33, wherein said one or more amino acid mutations is at a position corresponding to amino acid residue F589 of the TrkA polypeptide.

37. The use of claim 36, wherein said one or more amino acid mutations is a Phe-to-Leu substitution (F598L).

38. The use of claim 33, wherein said one or more mutations is at a position corresponding to amino acid residue G595 of the TrkA polypeptide.

39. The use of claim 38, wherein said one or more mutations is a Gly-to-Arg substitution (G595R).

40. The use of claim 33, wherein said one or more mutations is at a position corresponding to amino acid residue G667 of the TrkA polypeptide.

41. The use of claim 40, wherein said one or more mutations is a Gly-to-Cys substitution (G667C).

42. The use of claim 40, wherein said one or more amino acid mutations is a Gly-to-Ala substitution (G667A).

43. The use of claim 40, wherein said one or more amino acid mutations is a Gly-to-Ser substitution (G667S).

44. The use of claim 33, wherein said one or more amino acid mutations is at a position corresponding to amino acid residue V619 of the TrkB polypeptide.

45. The use of claim 44, wherein said one or more amino acid mutations is a Val-to-Met substitution (V619M).

46. The use of claim 33, wherein said one or more amino acid mutations is at a position corresponding to amino acid residue F633 of the TrkB polypeptide.

47. The use of claim 46, wherein said one or more amino acid mutations is a Phe-to-Leu substitution (F633L).

48. The use of claim 33, said one or more mutations is at a position corresponding to amino acid residue G639 of the TrkB polypeptide.

49. The use of claim 48, wherein said one or more mutations is a Gly-to-Arg substitution (G639R).

50. The use of claim 33, wherein said one or more mutations is at a position corresponding to amino acid residue G709 of the TrkB polypeptide.

51. The use of claim 50, wherein said one or more mutations is a Gly-to-Cys substitution (G709C).

52. The use of claim 50, wherein said one or more amino acid mutations is a Gly-to-Ala substitution (G709A).

53. The use of claim 50, wherein said one or more amino acid mutations is a Gly-to-Ser substitution (G709S).

54. The use of claim 33, wherein said one or more amino acid mutations is at a position corresponding to amino acid residue V603 of the TrkC polypeptide.

55. The use of claim 54, wherein said one or more amino acid mutations is a Val-to-Met substitution (V603M).

56. The use of claim 44, wherein said one or more amino acid mutations is at a position corresponding to amino acid residue F617 of the TrkC polypeptide.

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57. The use of claim 56, wherein said one or more amino acid mutations is a Phe-to-Leu substitution (F617L).

58. The use of claim 33, wherein said one or more mutations is at a position corresponding to amino acid residue G623 of the TrkC polypeptide.

59. The use of claim 58, wherein said one or more mutations is a Gly-to-Arg substitution (G623R).

60. The use of claim 33, wherein said one or more mutations is at a position corresponding to amino acid residue G696 of the TrkC polypeptide.

61. The use of claim 60, wherein said one or more mutations is a Gly-to-Cys substitution (G696C).

62. The use of claim 60, wherein said one or more amino acid mutations is a Gly-to-Ala substitution (G696A).

63. The use of claim 60, wherein said one or more amino acid mutations is a Gly-to-Ser substitution (G696S).

64. The use of any one of claims 33 to 63, wherein said cancer is selected from anaplastic large-cell lymphoma (ALCL), colorectal cancer (CRC), cholangiocarcinoma, gastric, glioblastomas (GBM), leiomyosarcoma, melanoma, non-small cell lung cancer (NSCLC), squamous cell lung cancer, neuroblastoma (NB), ovarian cancer, pancreatic cancer, prostate cancer, medullary thyroid cancer, breast cancer, papillary thyroid cancer, or any combination thereof.

Dated: 24 November 2017

```
SEQ ID NO:3          -----EGGPDAVIIGMTKIPVIENPQYFGITNSQLKPDTFVQHIKRHNIVLKRELGEGAF 565
SEQ ID NO:5          -----DAGPDTVVIGMTRIPVIENPQYFRQGHNCHKPDTYVQHIKRRDIVLKRELGEGAF 549
SEQ ID NO:1          -----EGKGSGLQG-----HIIENPQYF--------SDACVHHIKRRDIVLKWELGEGAF 521
SEQ ID NO:9          TVLINEDKELAELRGLAAGVGLANACYAIHTLPTQEEIENLPAFPREKLTLRLLLGSGAF 1956
SEQ ID NO:7          AMQMELQSPEYKLSKLRTSTIMTDYNPNYCFAGKTSSISDLKEVPRKNITLIRGLGHGAF 1127
                                     : :               : . *.:.*   ** ***

SEQ ID NO:3          GKVFLAECYNLCPE-QDKILVAVKTLKDASDN-ARKDFHREAELLTNLQHEHIVKFYGVC 623
SEQ ID NO:5          GKVFLAECYNLSPT-KDKMLVAVKALKDPTLA-ARKDFQREAELLTNLQHEHIVKFYGVC 607
SEQ ID NO:1          GKVFLAECHNLLPE-QDKMLVAVKALKEASES-ARQDFQREAELLTMLQHQHIVRFFGVC 579
SEQ ID NO:9          GEVYEGTAVDILGVGSGEIKVAVKTLKKGSTDQEKIEFLKEAHLMSKFNHPNILKQLGVC 2016
SEQ ID NO:7          GEVYEGQVSGMPND-PSPLQVAVKTLPEVCSEQDELDFLMEALIISKFNHQNIVRCIGVS 1186
                     *:*: .   .:     . : ****:* .      . :*  ** ::: ::* :*::  **.

SEQ ID NO:3          VEGDPLIMVFEYMKHGDLNKFLRAHGPDAVLMAEGNP---PTELTQSQMLHIAQQIAAGM 680
SEQ ID NO:5          GDGDPLIMVFEYMKHGDLNKFLRAHGPDAMILVDGQPRQAKGELGLSQMLHIASQIASGM 667
SEQ ID NO:1          TEGRPLLMVFEYMRHGDLNRFLRSHGPDAKLLAGGED-VAPGPLGLGQLLAVASQVAAGM 638
SEQ ID NO:9          LLNEPQYILLELMEGGDLLTYLRKARMATFYGP---------LLTLVDLVDLCVDISKGC 2067
SEQ ID NO:7          LQSLPRFILLELMAGGDLKSFLRETRPRPSQPS---------SLAMLDLLHVARDIACGC 1237
                       . *  ::.:* *  ***  :**     .              *   ::: :. ::: *

SEQ ID NO:3          VYLASQHFVHRDLATRNCLVGEN-----LLVKIGDFGMSRDVYSTDYYR----------- 724
SEQ ID NO:5          VYLASQHFVHRDLATRNCLVGAN-----LLVKIGDFGMSRDVYSTDYYRLFNPSGNDFCI 722
SEQ ID NO:1          VYLAGLHFVHRDLATRNCLVGQG-----LVVKIGDFGMSRDIYSTDYYR----------- 682
SEQ ID NO:9          VYLERMHFIHRDLAARNCLVSVKDYTSPRIVKIGDFGLARDIYKNDYYR----------- 2116
SEQ ID NO:7          QYLEENHFIHRDIAARNCLLTCPG--PGRVAKIGDFGMARDIYRASYYR----------- 1284
                      **   **:***:*:****:         :.******::**:*  .***

SEQ ID NO:3          ---VGGHTMLPIRWMPPESIMYRKFTTESDVWSLGVVLWEIFTYGKQPWYQLSNNEVIEC 781
SEQ ID NO:5          WCEVGGHTMLPIRWMPPESIMYRKFTTESDVWSFGVILWEIFTYGKQPWFQLSNTEVIEC 782
SEQ ID NO:1          ---VGGRTMLPIRWMPPESILYRKFTTESDVWSFGVVLWEIFTYGKQPWYQLSNTEAIDC 739
SEQ ID NO:9          ---KRGEGLLPVRWMAPESLMDGIFTTQSDVWSFGILIWEILTLGHQPYPAHSNLDVLNY 2173
SEQ ID NO:7          ---KGGCAMLPVKWMPPEAFMEGIFTSKTDTWSFGVLLWEIFSLGYMPYPSKSNQEVLEF 1341
                          *  :**::**.**:::   **:::*.**:*:::***:: *  *:   ** :.::

SEQ ID NO:3          ITQGRVLQRPRTCPQEVYELMLGCWQREPHMRKNIKGIHTLLQNLA-------------- 827
SEQ ID NO:5          ITQGRVLERPRVCPKEVYDVMLGCWQREPQQRLNIKEIYKILHALG-------------- 828
SEQ ID NO:1          ITQGRELERPRACPPEVYAIMRGCWQREPQQRHSIKDVHARLQALA-------------- 785
SEQ ID NO:9          VQTGGRLEPPRNCPDDLWNLMTQCWAQEPDQRPTFHRIQDQLQLFRNFFLNSIYKSRDEA 2233
SEQ ID NO:7          VTSGGRMDPPKNCPGPVYRIMTQCWQHQPEDRPNFAIILERIEYCTQ--------DPDVI 1393
                     :  *  :: *: **  :: :*  ** ::*. * .:  :  :  :.
```

FIG. 1

KM12 cell line

- Colorectal cancer (CRC) cell line, patient derived, adherent
- TPM3-TrkA (Exon1-7 of TPM3 fused to Exon*10-17 of TrkA)

BaF3-tel-TrkA cell line

- Mouse Pro B cells, suspension, recombinant cell lines
- ETV6-TrkA (Exon1-6 of ETV6 fused to Exon* 12-17 of TrkA)

IC50 (nM)	Entrectinib	Entrectinib
Cell lines	Nerviaro	Ignyta
BaF3-tel/trkA	2.8	2.6-4.2
KM12	17	1.7-7

*:1st exon in the coding sequence as exon1

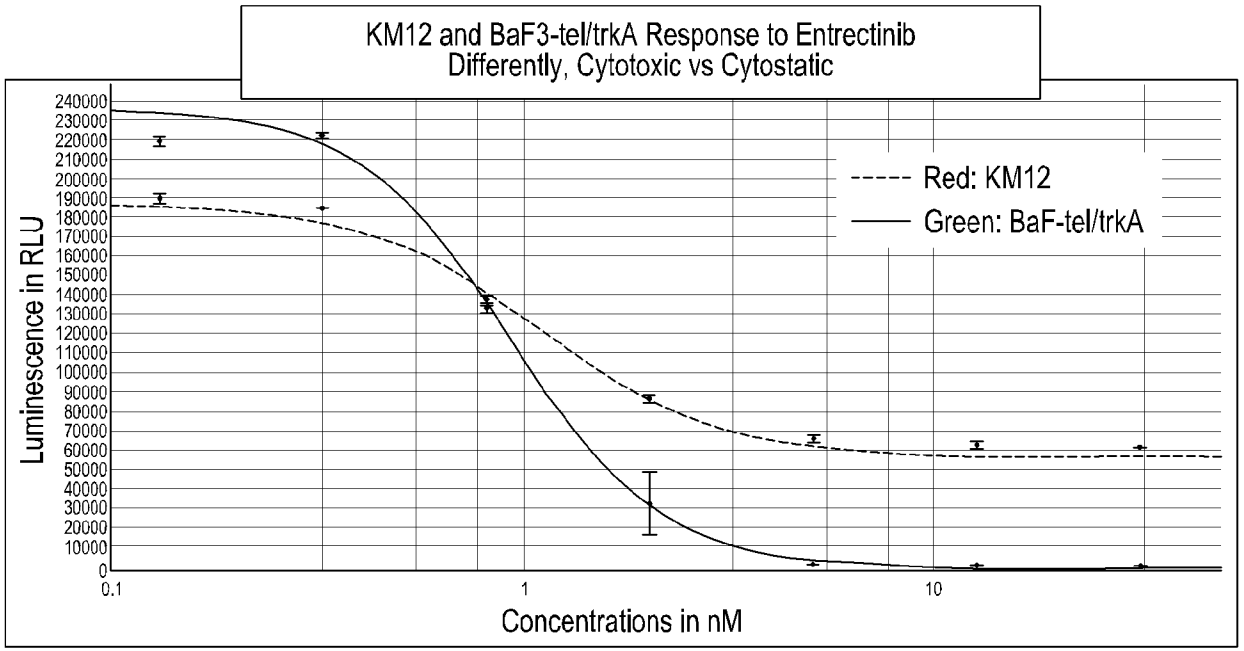


FIG. 2

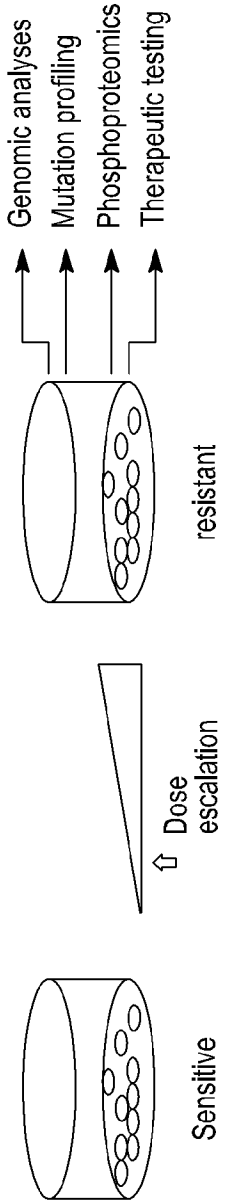

Genomic analyses
Mutation profiling
Phosphoproteomics
Therapeutic testing
resistant
Dose escalation
Sensitive

FIG. 3

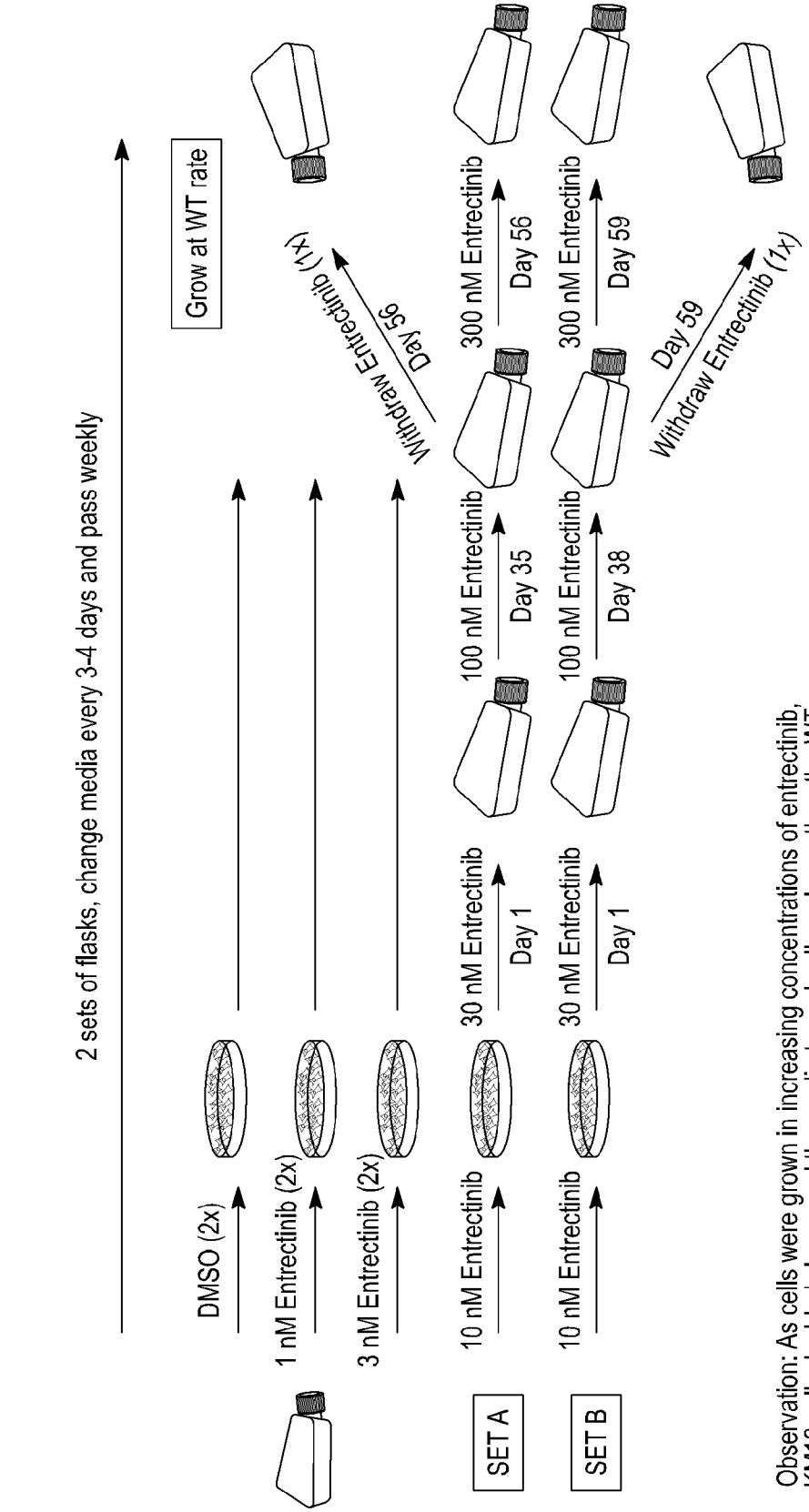

2 sets of flasks, change media every 3-4 days and pass weekly
DMSO (2x)
1 nM Entrectinib (2x)
3 nM Entrectinib (2x)
Grow at WT rate
Day 56
Withdraw Entrectinib (1x)
Day 59
Withdraw Entrectinib (1x)
SET A
SET B
10 nM Entrectinib
30 nM Entrectinib
Day 1
Day 35
100 nM Entrectinib
300 nM Entrectinib
Day 38
Day 59
Observation: As cells were grown in increasing concentrations of entrectinib, KM12 cells doubled slower and the media turned yellow slower than the WT initially, but in later time points KM12 cells and WT cells were growing at comparable rates.

FIG. 4

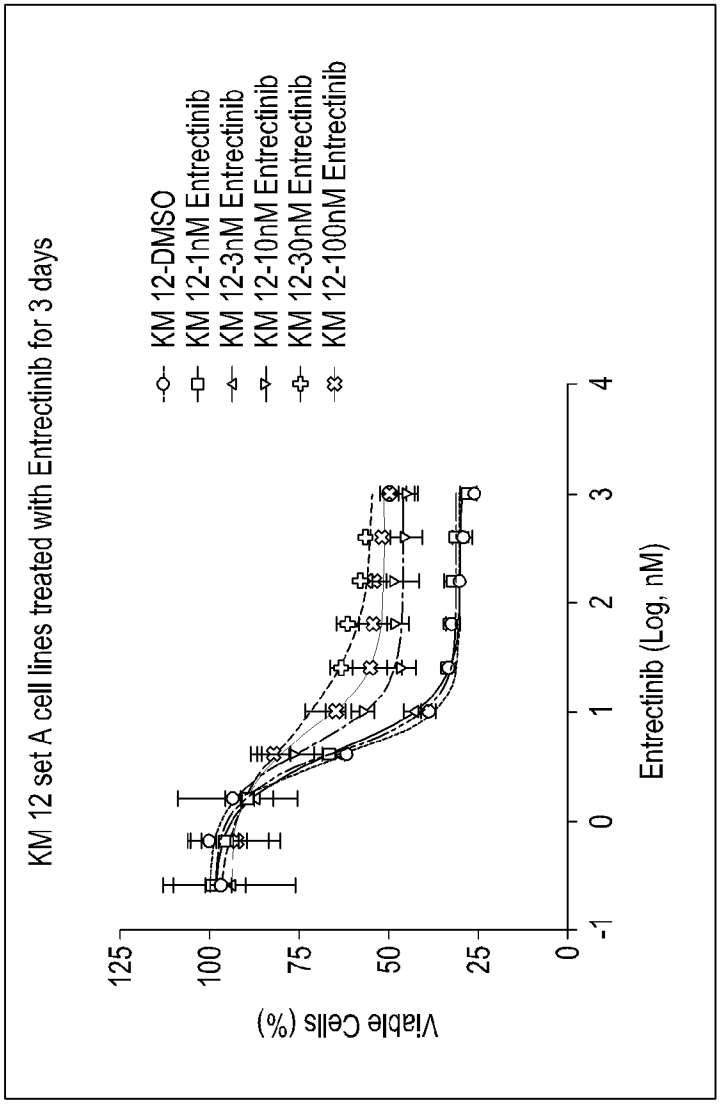

KM 12 set A cell lines treated with Entrectinib for 3 days
KM 12-DMSO
KM 12-1nM Entrectinib
KM 12-3nM Entrectinib
KM 12-10nM Entrectinib
KM 12-30nM Entrectinib
KM 12-100nM Entrectinib
Viable Cells (%)
125
100
75
50
25
0
-1
0
1
2
3
4
Entrectinib (Log, nM)

FIG. 5

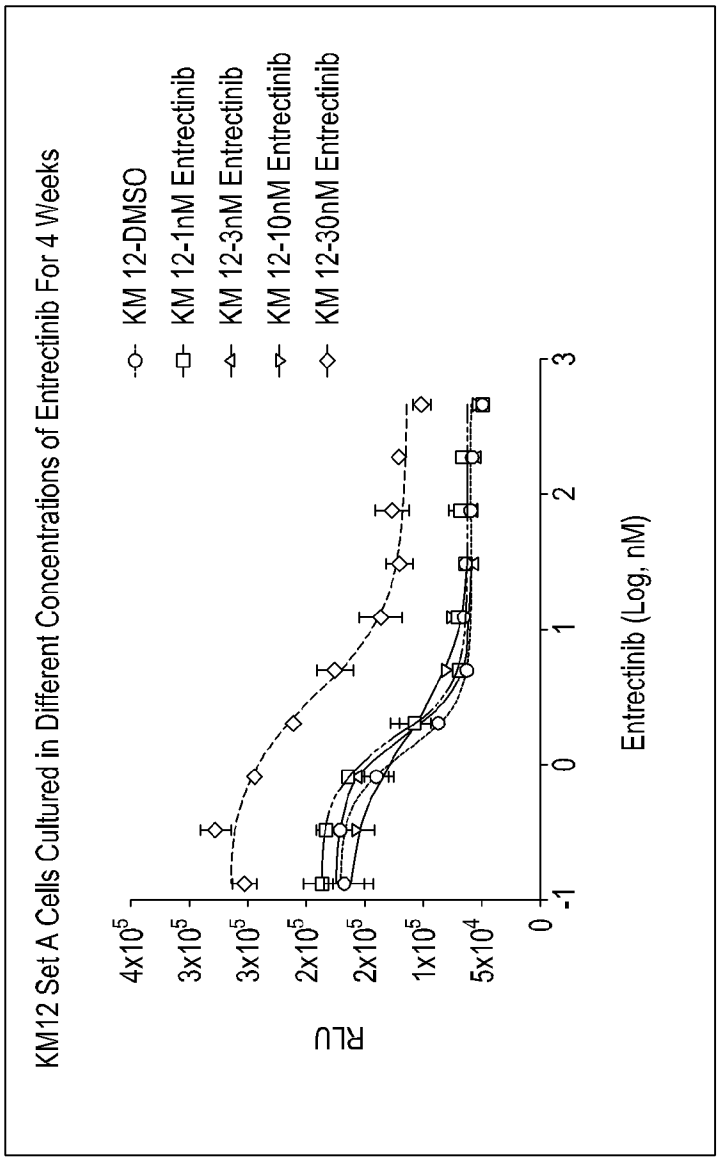

KM12 Set A Cells Cultured in Different Concentrations of Entrectinib For 4 Weeks
KM 12-DMSO
KM 12-1nM Entrectinib
KM 12-3nM Entrectinib
KM 12-10nM Entrectinib
KM 12-30nM Entrectinib
RLU
4x10^5
3x10^5
3x10^5
2x10^5
2x10^5
1x10^5
5x10^4
0
-1
0
1
2
3
Entrectinib (Log, nM)

FIG. 6

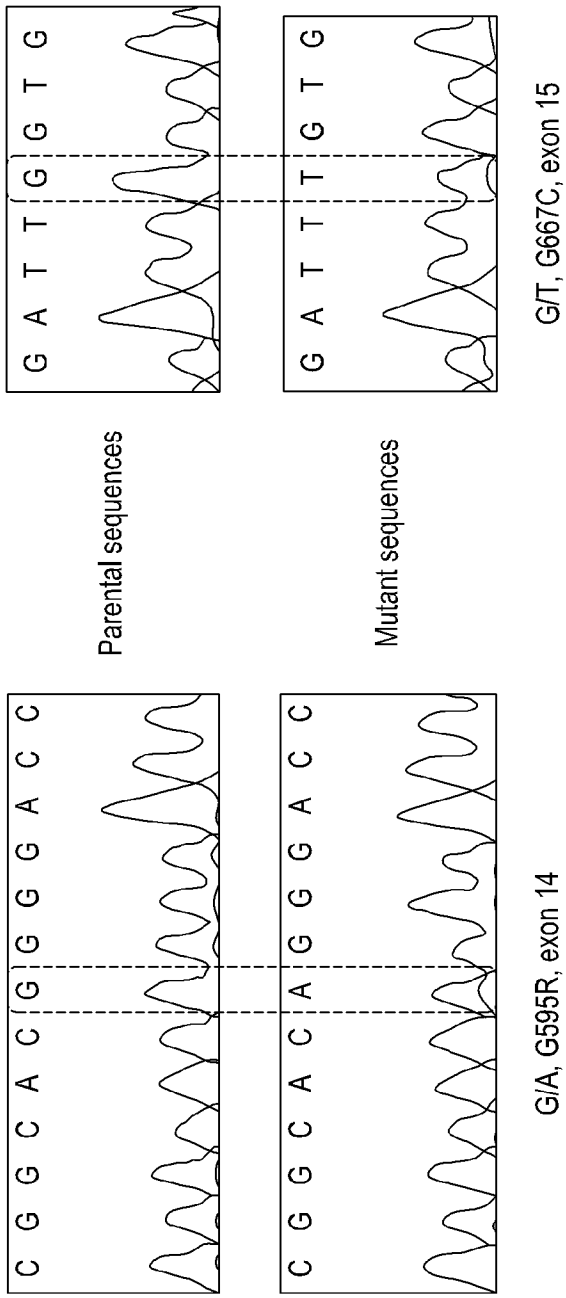

G A T T T G G T G
G A T T T G T G
G/T, G667C, exon 15
Parental sequences
Mutant sequences
C G G G C A C G G G G A C C
C G G G C A C A G G G A C C
G/A, G595R, exon 14

FIG. 7

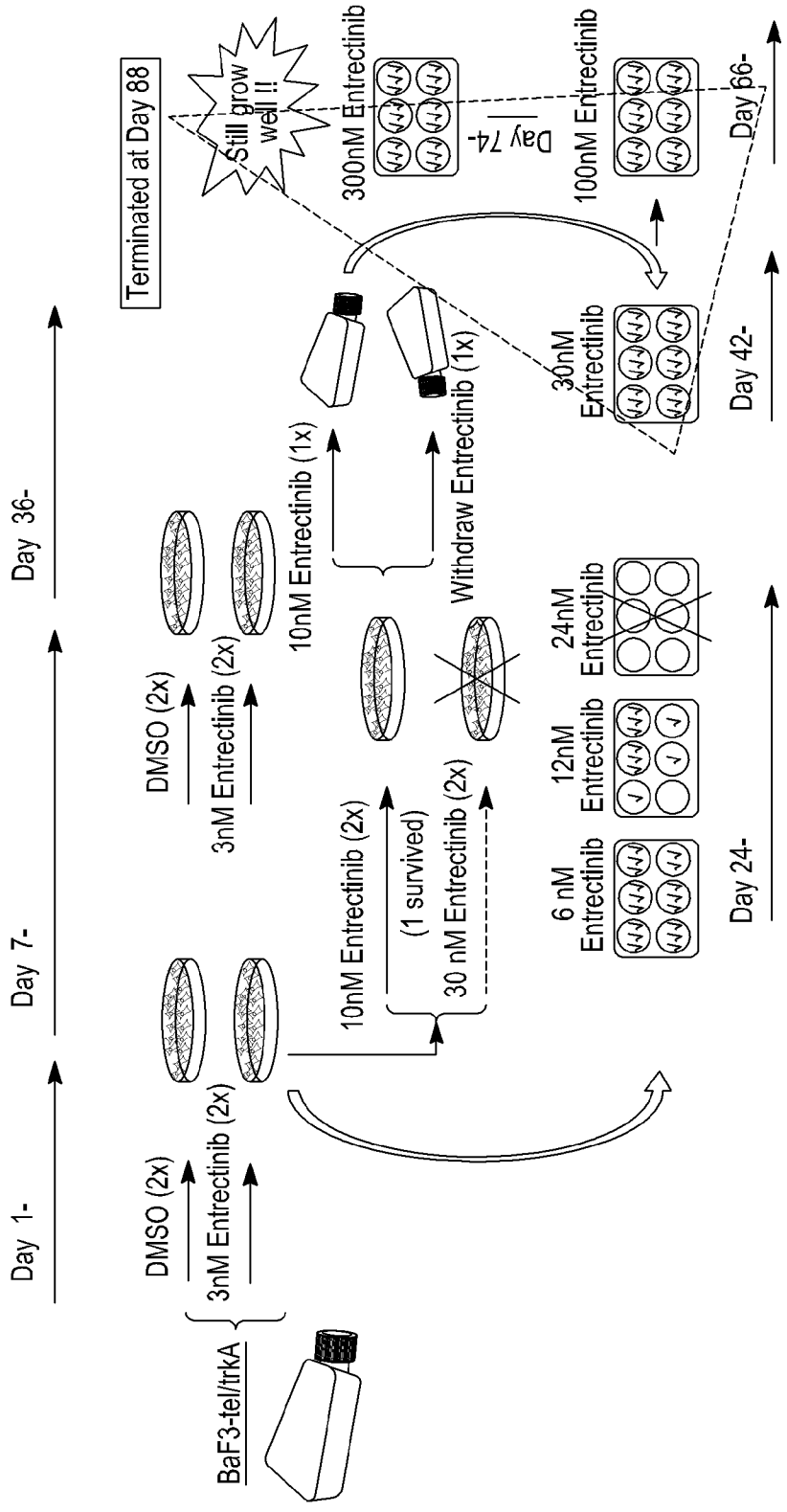
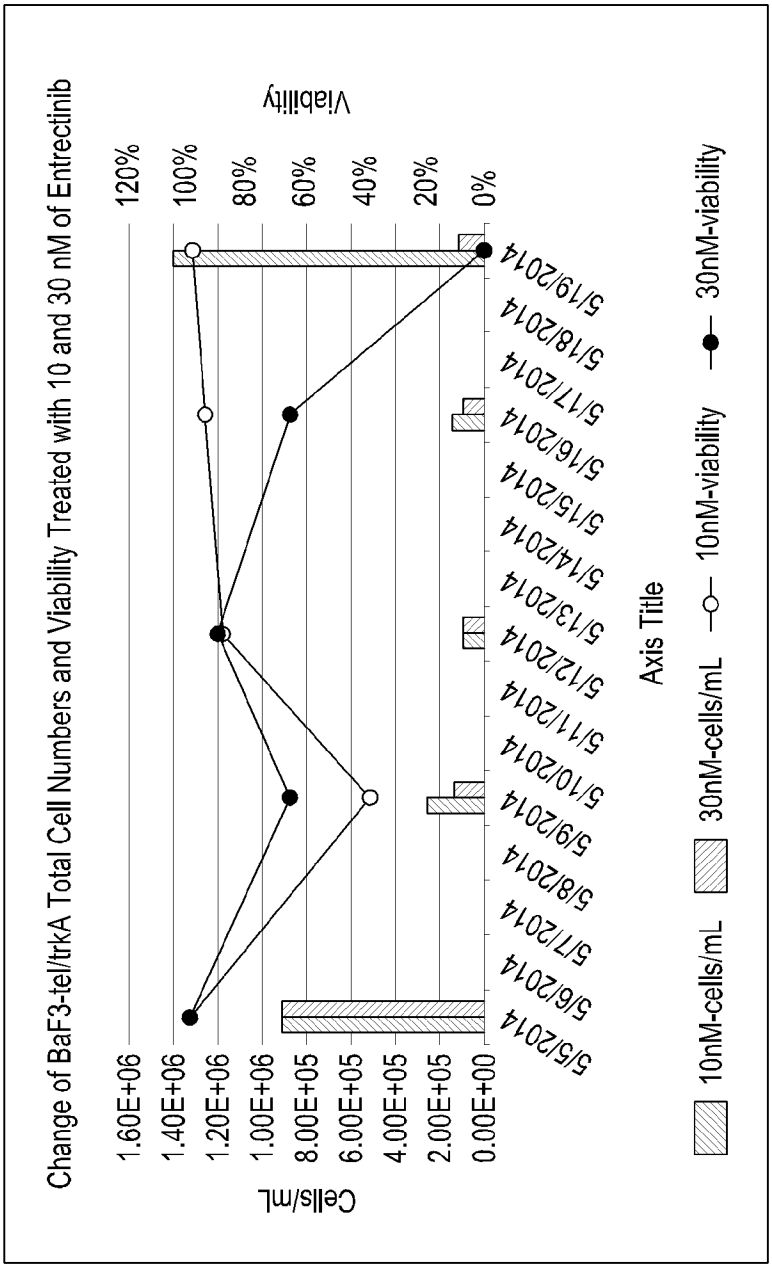

Day 1-
Day 7-
Day 36-
BaF3-tel/trkA
DMSO (2x)
3nM Entrectinib (2x)
10nM Entrectinib (2x)
(1 survived)
30 nM Entrectinib (2x)
DMSO (2x)
3nM Entrectinib (2x)
10nM Entrectinib (1x)
Withdraw Entrectinib (1x)
Terminated at Day 88
Still grow well !!
Day 24-
6 nM Entrectinib
12nM Entrectinib
24nM Entrectinib
Day 42-
30nM Entrectinib
Day 74-
300nM Entrectinib
100nM Entrectinib
Day 66-

FIG. 8



Note: BaF3-tel/trkA cells could not survive 30 nM Entrectinib treatment

FIG. 9

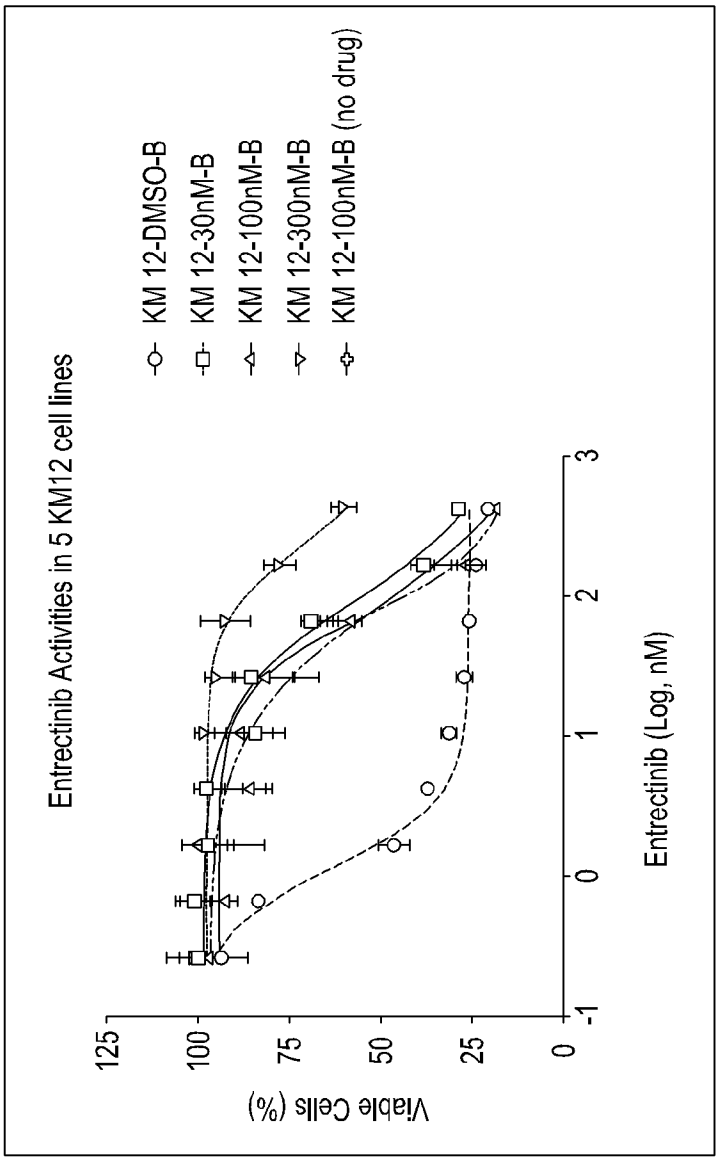

Entrectinib Activities in 5 KM12 cell lines
KM 12-DMSO-B
KM 12-30nM-B
KM 12-100nM-B
KM 12-300nM-B
KM 12-100nM-B (no drug)
Viable Cells (%)
125
100
75
50
25
0
-1
0
1
2
3
Entrectinib (Log, nM)

FIG. 10

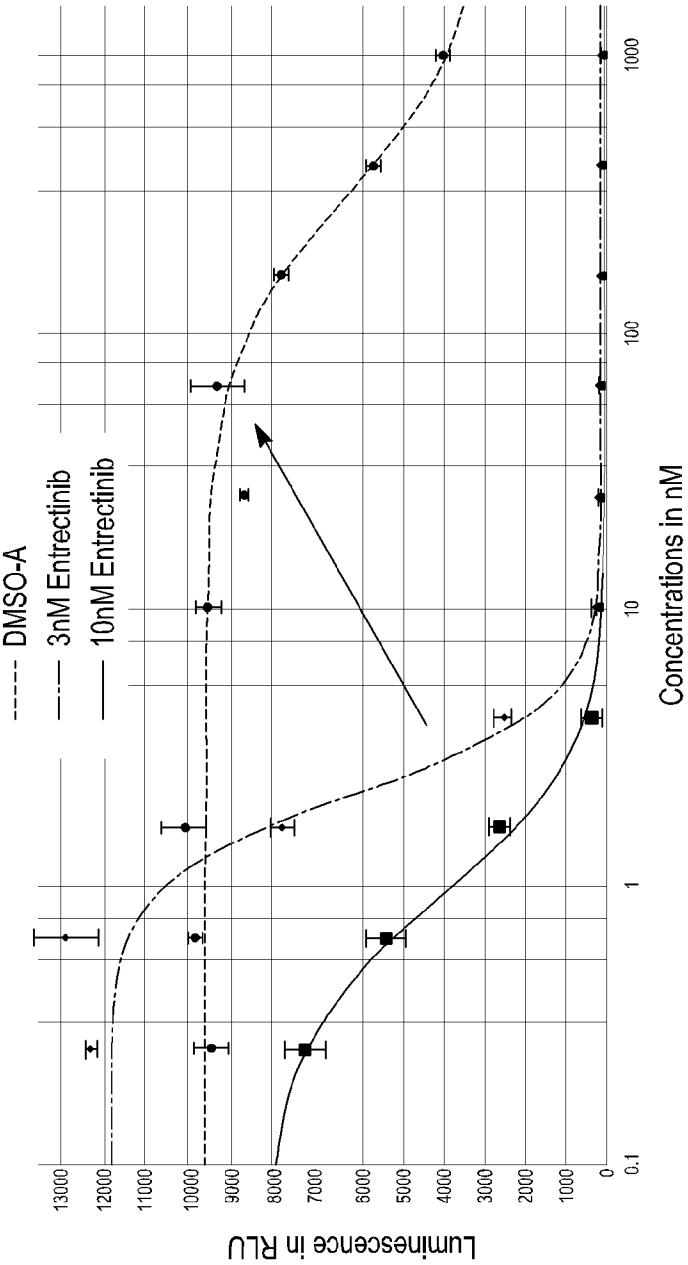
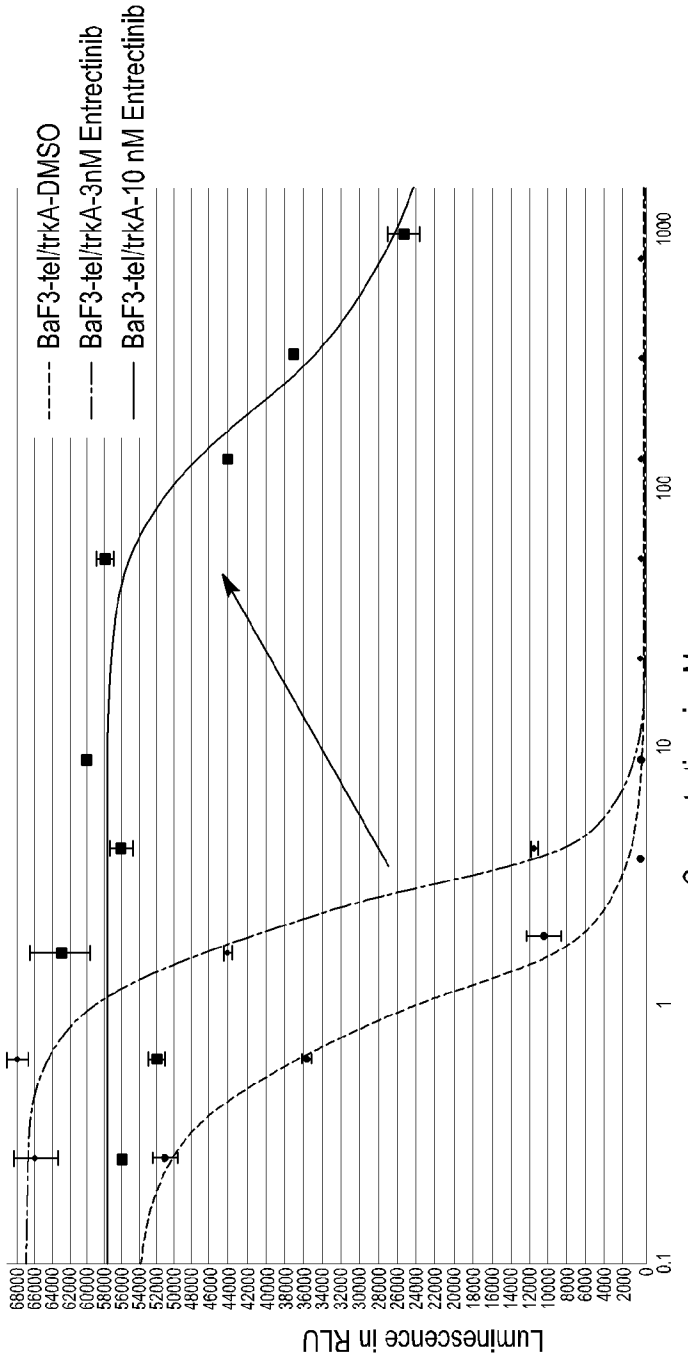

DMSO-A
3nM Entrectinib
10nM Entrectinib
Luminescence in RLU
13000
12000
11000
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
0.1
1
10
100
1000
Concentrations in nM

FIG. 11A



Treatment	DMSO-A	3nM Entrectinib-A	10nM Entrectinib-A
IC50 (nM)	0.94	2.20	316.39
r2	1.00	0.98	0.96
IC50 (nM)	0.79	2.07	273.48
r2	0.999	0.997	0.914

Note: Growth inhibition curve right-shifted and IC50 values changed dramatically

FIG. 11B

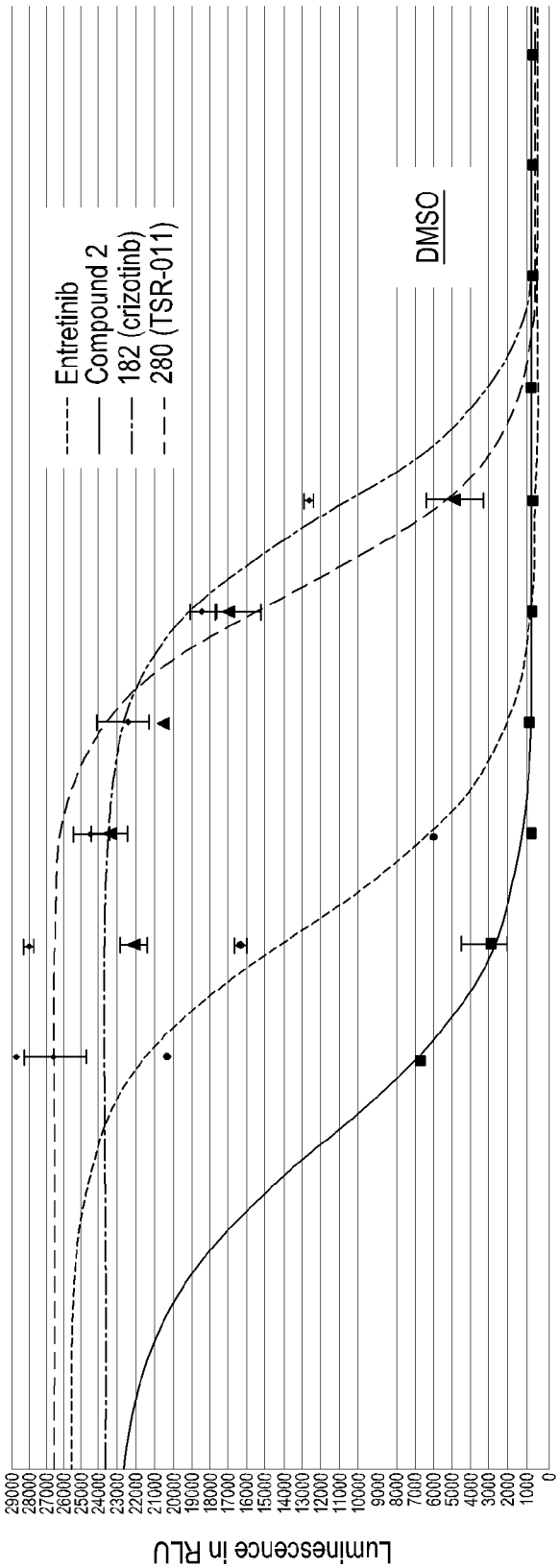

Luminescence in RLU
29000
28000
27000
26000
25000
24000
23000
22000
21000
20000
19000
18000
17000
16000
15000
14000
13000
12000
11000
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
Concentrations in nM
Entretinib
Compound 2
182 (crizotinb)
280 (TSR-011)
DMSO

FIG. 12A

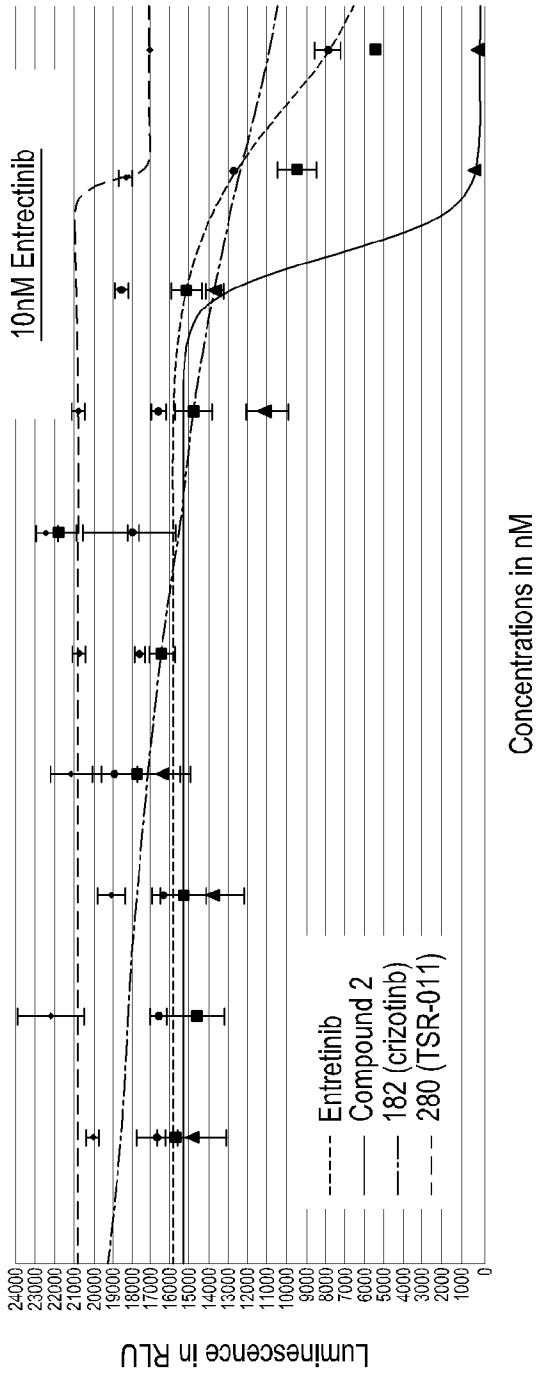

10nM Entrectinib
Luminescence in RLU
Concentrations in nM
Entretinib
Compound 2
182 (crizotinb)
280 (TSR-011)

FIG. 12B

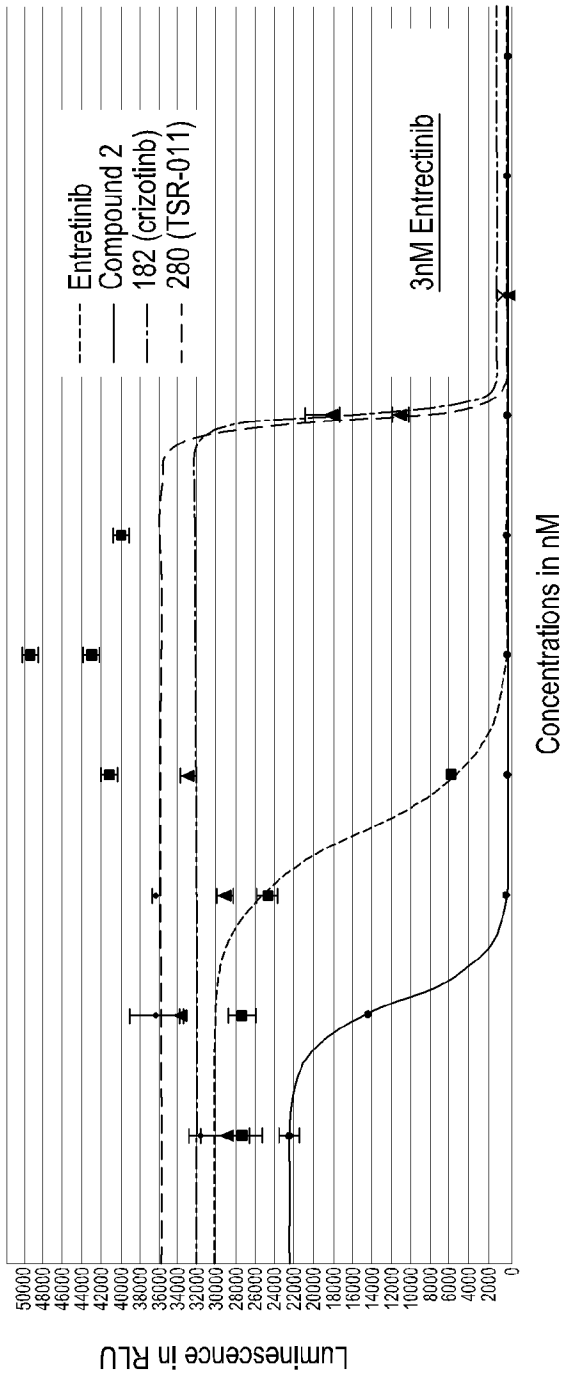

Entretinib
Compound 2
182 (crizotinb)
280 (TSR-011)
3nM Entrectinib
Concentrations in nM
Luminescence in RLU
50000
48000
46000
44000
42000
40000
38000
36000
34000
32000
30000
28000
26000
24000
22000
20000
18000
16000
14000
12000
10000
8000
6000
4000
2000
0

FIG. 12C

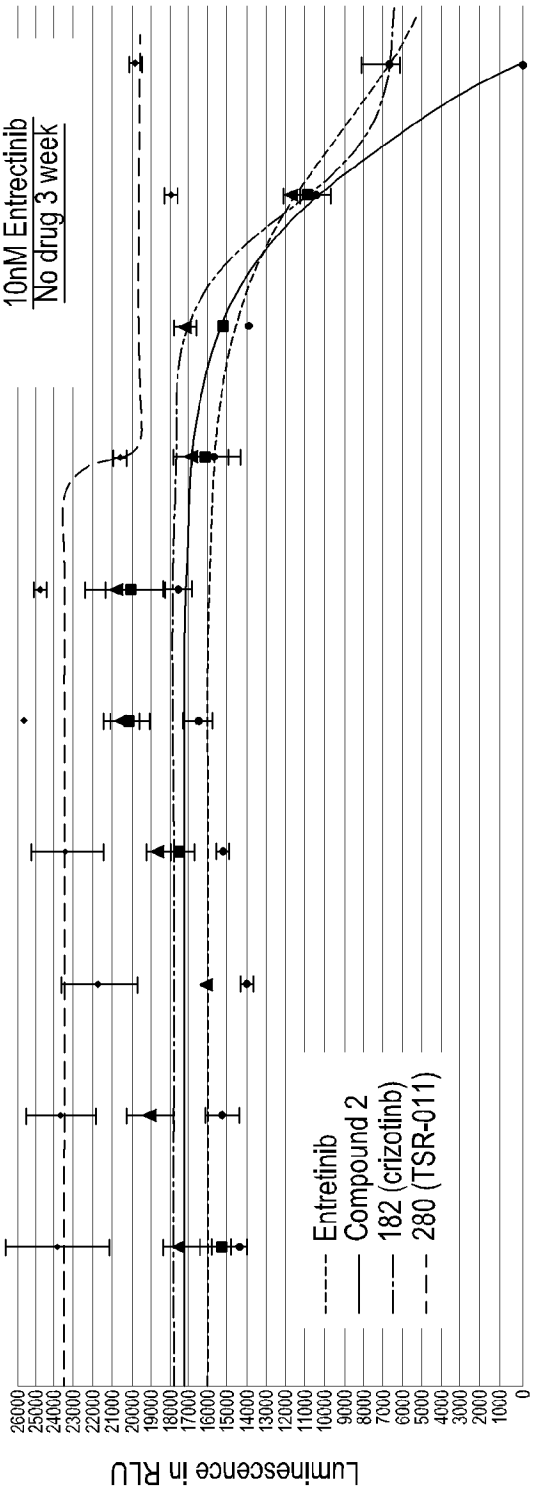

10nM Entrectinib
No drug 3 week
Entretinib
Compound 2
182 (crizotinb)
280 (TSR-011)
Luminescence in RLU
Concentrations in nM

FIG. 12D

Cell lines	Inhibitors	Entrectinib	Compound 2	Crizotinib	TSR-011
DMSO-A	EC50(nM)	0.81	0.14	23.54	12.67
	r^2	0.991	0.999	0.978	0.991
3nM Entrectinib-A	EC50(nM)	2.66	0.73	64.48	60.98
	r^2	0.953	1.000	0.901	0.905
10nM Entrectinib-A	EC50(nM)	682.83	222.14	1.52E+14	389.762
	r^2	0.776	0.894	0.272	0.623
10nM Entrectinib-A (no drug in culture)	EC50(nM)	642.20	818.11	355.82	62.091
	r^2	0.823	0.852	0.832	0.716

Note: The resistant cell pools were also less sensitive to crizotinib and TSR-011

FIG. 12E

Cell Line background:
KM12 Cell Line - TPM3-TrkA (Exon1-7 of TPM3 fused to Exon10-17 of TrkA)
BaF3-tel-TrkA Cell Line - ETV6-TrkA (Exon1-6 of ETV6 fused to Exon 12-17 of TrkA)

Cell line was provided in T-75 flasks
1. BaF3-tel-trkA (DMSO) (P39-19)
2. BaF3-tel-trkA (10nM Entrectinib) (P37-16)
3. KM12-A (DMSO) (P4-12)
4. KM12-A (100nM Entrectinib-resistant) (P5-11)

Results
1. RNA extraction and RT-PCR
2. Sequencing comparison between mutants (2,4) and control trkA (1,3)
3. If mutations are identified, fusion proteins are to be cloned in the lentiviral vector for activity confirmation in cells.

FIG. 13

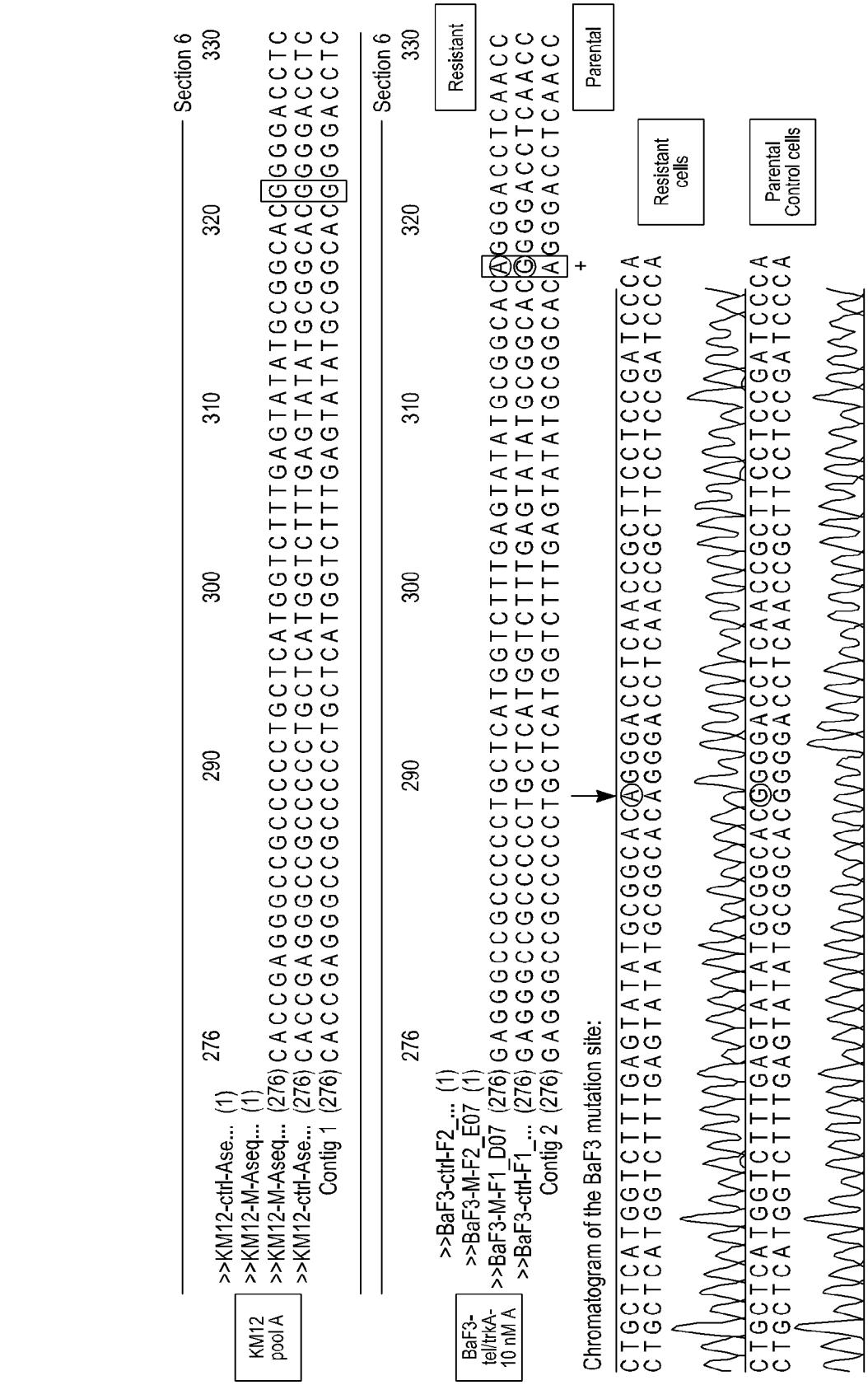

KM12 pool A
BaF3-tel/trkA-10 nM A
Section 6
276
290
300
310
320
330
>>KM12-ctrl-Ase... (1)
>>KM12-M-Aseq... (1)
>>KM12-M-Aseq... (276) CACCGAGGGCCGCCCCCTGCTCATGGTCTTTGAGTATATGCGGCACGGGACCTC
>>KM12-ctrl-Ase... (276) CACCGAGGGCCGCCCCCTGCTCATGGTCTTTGAGTATATGCGGCACGGGACCTC
Contig 1 (276) CACCGAGGGCCGCCCCCTGCTCATGGTCTTTGAGTATATGCGGCACGGGACCTC
>>BaF3-ctrl-F2_... (1)
>>BaF3-M-F2_E07 (1)
>>BaF3-M-F1_D07 (276) GAGGGCCGCCCCCTGCTCATGGTCTTTGAGTATATGCGGCACAGGGACCTCAACC
>>BaF3-ctrl-F1_... (276) GAGGGCCGCCCCCTGCTCATGGTCTTTGAGTATATGCGGCACGGGACCTCAACC
Contig 2 (276) GAGGGCCGCCCCCTGCTCATGGTCTTTGAGTATATGCGGCACAGGGACCTCAACC
Resistant
Parental
Chromatogram of the BaF3 mutation site:
CTGCTCATGGTCTTTGAGTATATGCGGCACAGGGACCTCAACCGCTTCCTCCGATCCCA
CTGCTCATGGTCTTTGAGTATATGCGGCACGGGACCTCAACCGCTTCCTCCGATCCCA
Resistant cells
Parental Control cells

FIG. 14

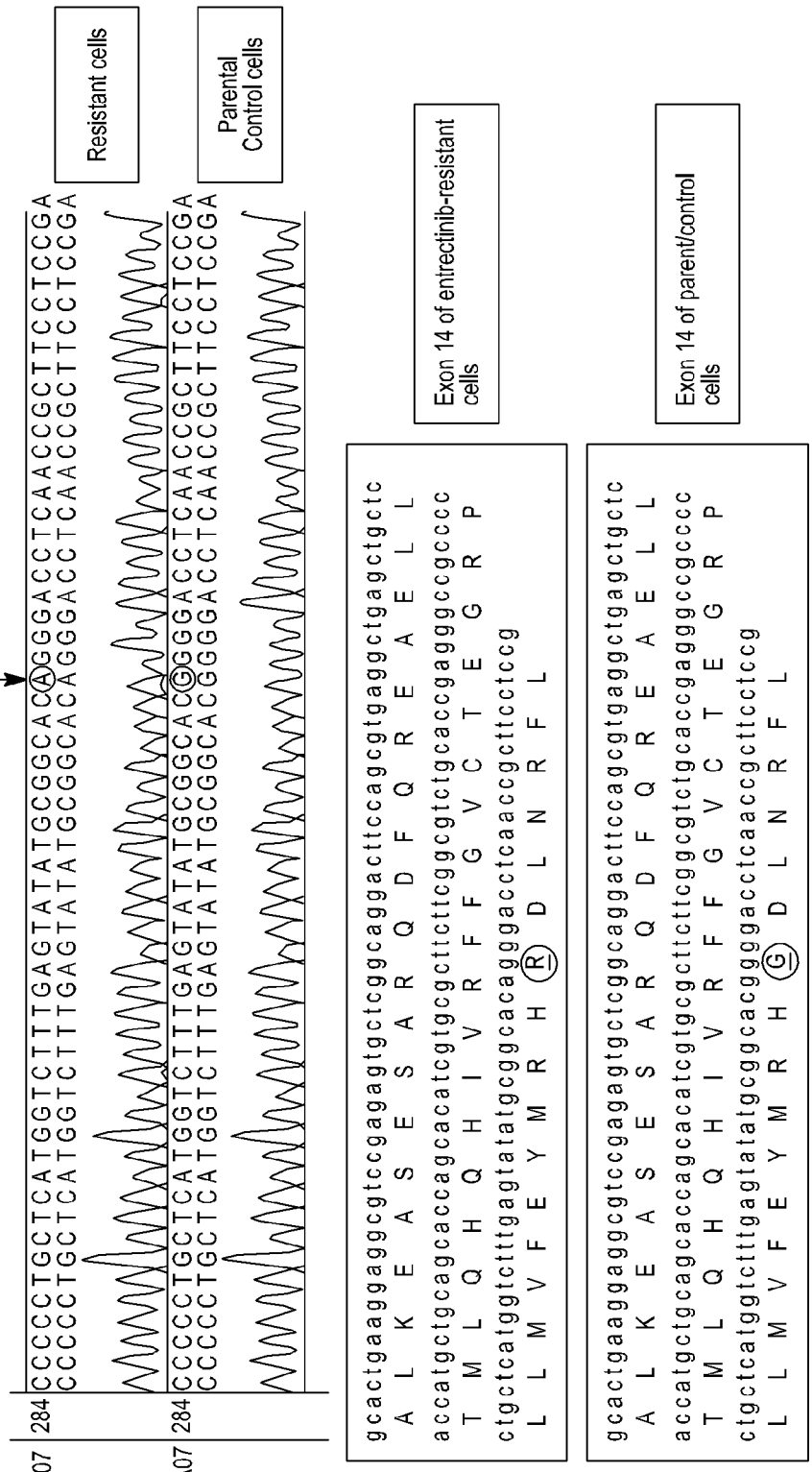

>>BaF3-M-F1_D07
284
>>BaF3-ctrl-F1_A07
284
Resistant cells
Parental Control cells
Exon 14 of entrectinib-resistant cells
Exon 14 of parent/control cells

FIG. 15

The binding mode of entrectinib with wild type TrkA was obtained using Glide docking implemented in Maestro[1]. Receptor coordinates were downloaded from PDB (PDB code: 4PMT) and properly prepared by adding protons, sampling water orientations and finally full energy minimization. Both G595R and G667C models were built with Maestro and subsequently subjected to energy minimization.

For the wild type, entrectinib makes extensive hydrogen bonding as well as hydrophobic interactions with the protein in the ATP pocket where both G595 and G667 are located (Fig. 16b). However, both G595R and G667C mutants create steric hindrance directly with Entrectinib, making it a much weaker binder with both mutants than the wild type (Fig. 16c and 16d).

1. Maestro Release 2015-1, Schrodinger, Inc, New York, NYC, 2015

FIG. 16

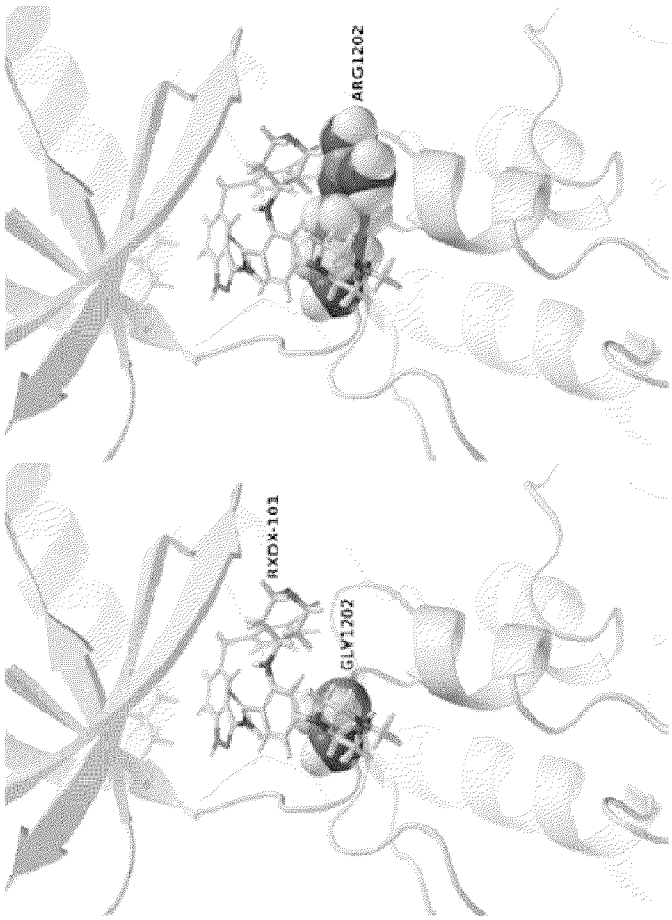


FIG. 17

Cell Line background:
KM12 - TPM3-TrkA (Exon1-7 of TPM3 fused to Exon10-17 of TrkA)
Ba/F3 - Tel/TrkA - ETV6-TrkA (Exon1-6 of ETV6 fused to Exon 12-17 of TrkA)
Extracted RNA provided
Results:
1. RNA extraction and RT-PCR
2. Sequencing comparison between mutants (2,4) and control trkA (1,3)
RNA Purified using Qiagen's DNA/RNA
extraction kit
-Quantified by Qubit 2.0

KM12

SampleID	Conc (mg/ml)
8/1, KM12-DMSO-A (P4-16)	1.91
8/1, KM12-A-30nM Entrectinib (P5-15)	1.26
8/1, KM12-A-100nM Entrectinib (no drug) (P5-9-6)	0.2
8/1, KM12-A-100nM Entrectinib (P5-15)	1.38
8/1, KM12-A-300nM (P5-9-6)	1.42
8/7 KM12 B-DMSO (P4-16)	2
8/7 KM12 B-100nM (no drug) (P5-10-6)	1.6
8/7 KM12 B-30nM (P5-16)	1.5
8/7 KM12 B-100nM (P5-16)	1.7
8/7 KM12 B-300nM (P5-10-16)	1.7

BaF3-tel/trkA

SampleID	Conc (mg/ml)
8/7 BaF3-tel/trkA-6nM A1(P37-4-20)	1.3
8/7 BaF3-tel/trkA-6nM A2(P37-4-20)	0.77
8/7 BaF3-tel/trkA-6nM A3(P37-4-20)	0.74
8/7 BaF3-tel/trkA-6nM B1(P37-4-16)	0.95
8/7 BaF3-tel/trkA-6nM B2(P37-4-19)	1.03
8/7 BaF3-tel/trkA-6nM B3(P37-4-20)	0.84
7/29 BaF3-tel/trkA-12nM Entrectinib-A1 (P37-4-15)	0.71
8/1 BaF3-tel/trkA-12nM Entrectinib-A2 (P37-4-15)	0.85
7/29 BaF3-tel/trkA-12nM Entrectinib-A3 (P37-4-17)	0.74
8/1, TrkA-B2-12nM (P37-4-8)	0.81
8/1 BaF3-tel/trkA-12nM Entrectinib-B3 (P37-4-14)	0.66

FIG. 18

KM12-B

treatment	Set A, mutation	Set B, mutation	Exon	Note
DMSO	None, exon 12-17	None, exon 12-17		Clean sequencing data,
30 nM Entrectinib	None, exon 12-17	G/T, Gly667Cys	15	Clean sequencing data,
100 nM Entrectinib	None, exon 12-17	G/T, Gly667Cys	15	Clean sequencing data,
100 nM Entrectinib (-drug)	None, exon 12-17	G/T, Gly667Cys	15	Clean sequencing data,
300 nM Entrectinib	None, exon 12-17	G/T, Gly595Arg	14	Clean sequencing data,

Baf3-tel-trkA

Resistant Entrectinib con	Pools	Set B, mutation	Exon	Note
6 nM	A1 (3 nM->6nM)	None, exon 12-17		
6 nM	A2 (3 nM->6nM)	None, exon 12-17		
6 nM	A3 (3 nM->6nM)	None, exon 12-17		
6 nM	B1 (3 nM->6nM)	None, exon 12-17		
6 nM	B2 (3 nM->6nM)	None, exon 12-17		
6 nM	B3 (3 nM->6nM)	None, exon 12-17		
10 nM	A (0 nM->10nM)	G/A, Gly595Arg	14	Clean sequencing data,
12 nM	A1(3 nM->12nM)	None, exon 12-17		
12 nM	A2(3 nM->12nM)	G/T, Gly667Cys, mixed	15	6 subclones are also mixed of WT and mutant (~50%)
12 nM	A3(3 nM->12nM)	G/T, Gly667Cys, mixed	15	
12 nM	B2(3 nM->12nM)	None, exon 12-17		
12 nM	B3(3 nM->12nM)	G/T, Gly667Cys, mixed	15	6 subclones are also mixed of WT and mutant (~50%)

FIG. 19

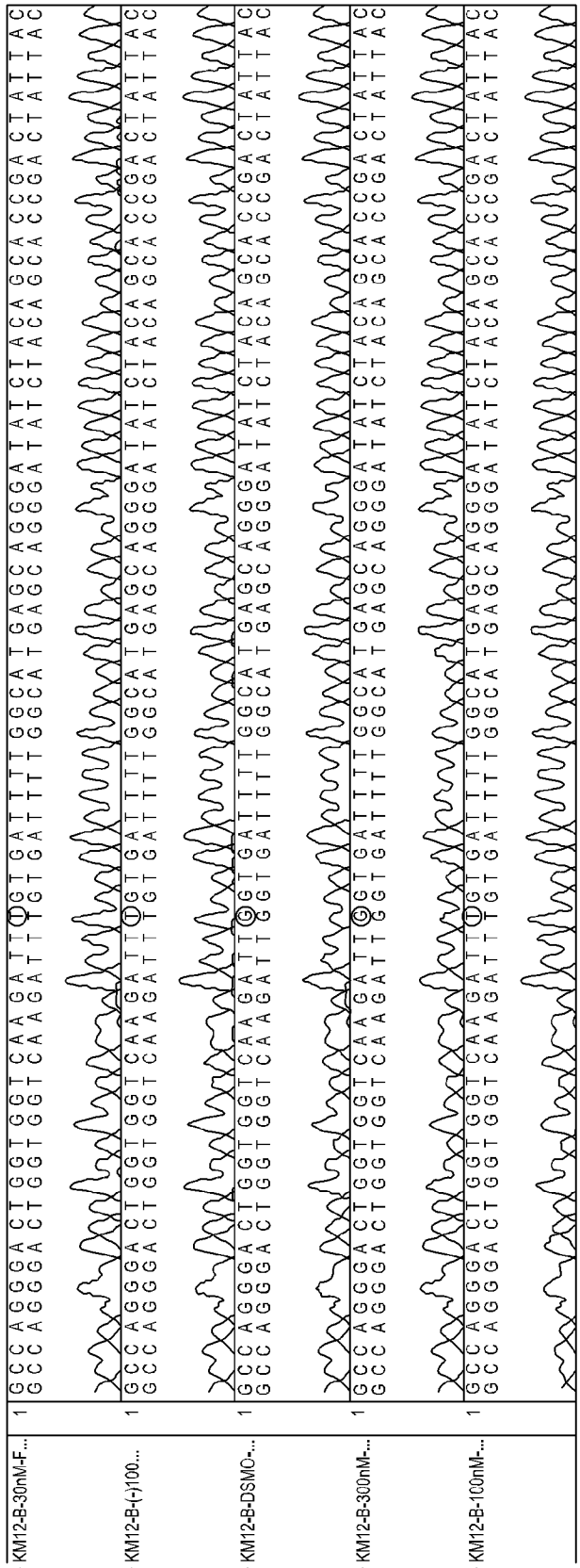

Chromatogram of mutation site for KM12-B corresponding to amino acid residue 545 of TrkA
>> KM12-B-30nM-F...
>> KM12-B-(-)100...
>> KM12-B-DSMO-...
>> KM12-B-300nM-...
>> KM12-B-100nM-...

FIG. 20

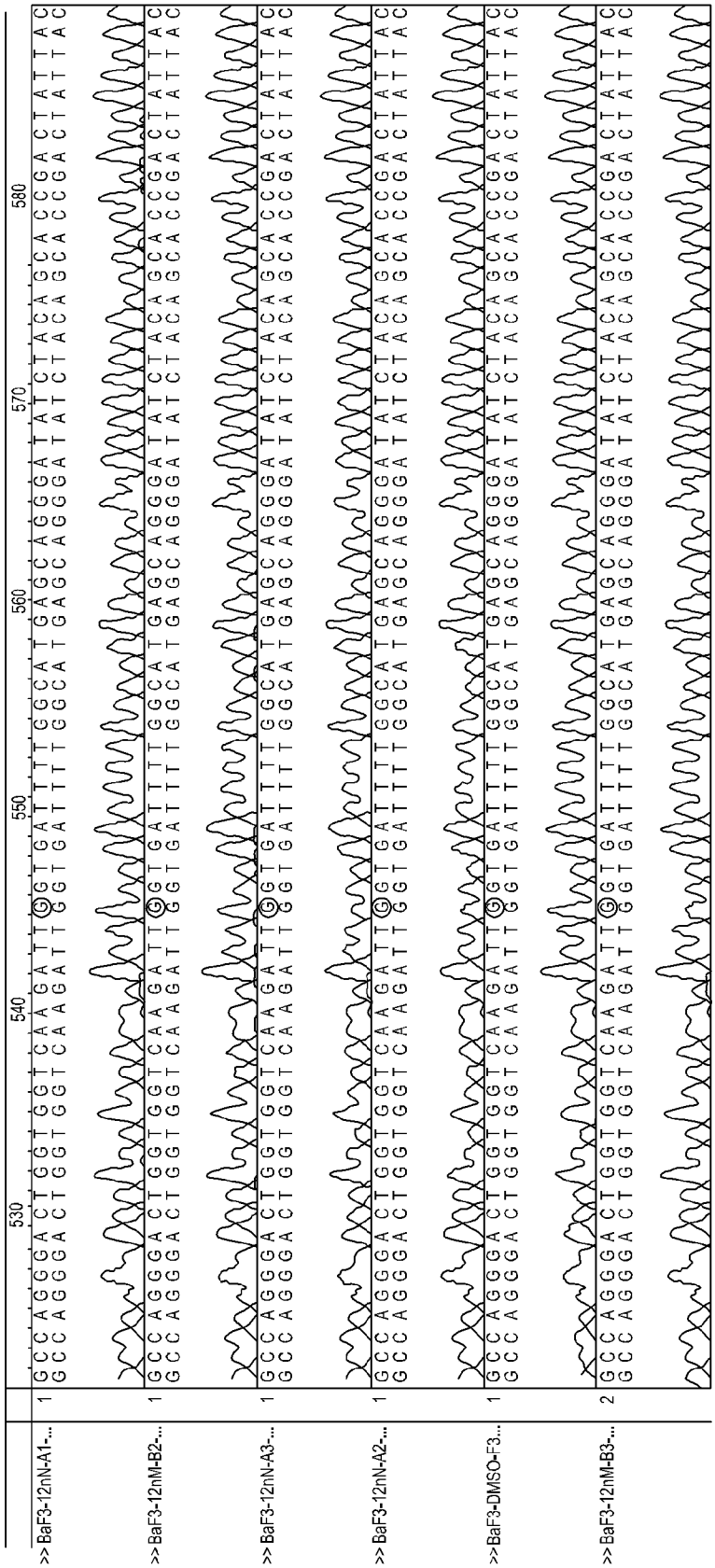

530
540
550
560
570
580
>>BaF3-12nN-A1-...
>>BaF3-12nM-B2-...
>>BaF3-12nN-A3-...
>>BaF3-12nN-A2-...
>>BaF3-DMSO-F3...
>>BaF3-12nM-B3-...

FIG. 21

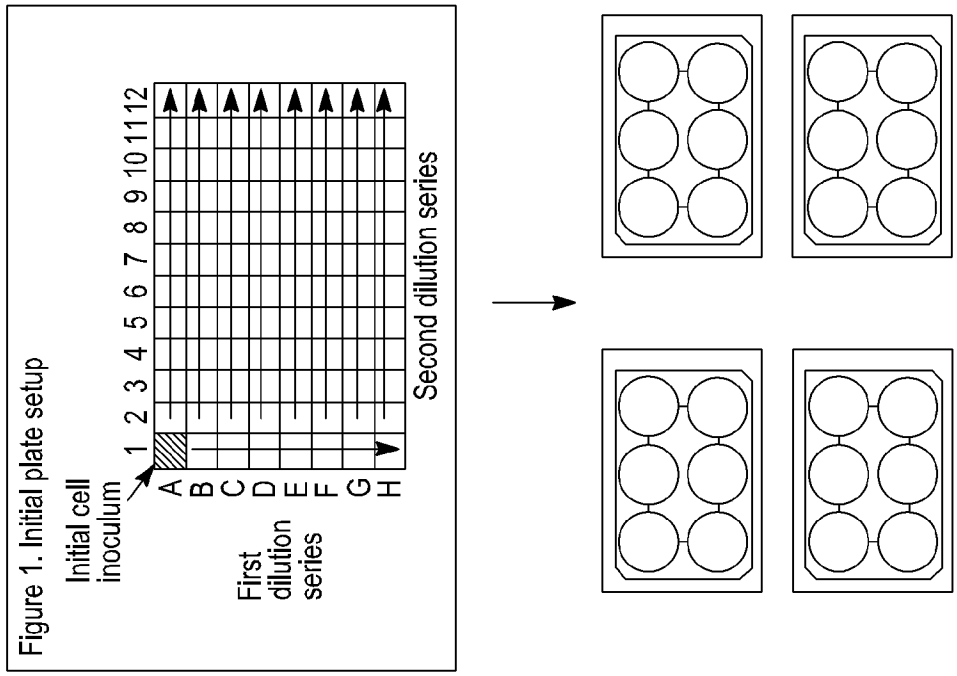


Figure 1. Initial plate setup
Initial cell inoculum
First dilution series
Second dilution series
1 2 3 4 5 6 7 8 9 10 11 12
A
B
C
D
E
F
G
H

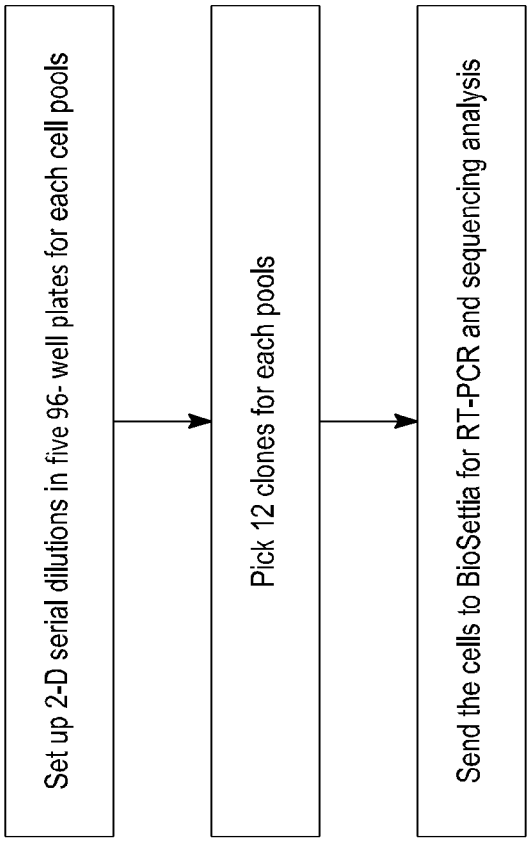

Set up 2-D serial dilutions in five 96- well plates for each cell pools
Pick 12 clones for each pools
Send the cells to BioSettia for RT-PCR and sequencing analysis

FIG. 22

Table Summary

Sample ID	Mutations
Baf3-tel/trkA-DMSO-A,P39-32	-
Baf3-tel/trkA-DMSO-B,P39-32	-
Baf3-tel/trkA-3nM-A,P37-33	no change
Baf3-tel/trkA-3nM-B,P37-33	no change
Baf3-tel/trkA-10nM-A,P37-18	G/A,Glycine/Arginine,exon 14
Baf3-tel/trkA-3nM-A-weekly,P37-18	no change

Sample ID	Mutations
BaF3-tel/trkA-12nMA2,#1-3E	Mixture,G & T,exon 15
BaF3-tel/trkA-12nMA2,#2-4F	Mixture,G & T,exon 15
BaF3-tel/trkA-12nMA2,#3-3F	Mixture,G & T,exon 15
BaF3-tel/trkA-12nMA2,#4-7B	Mixture,G & T,exon 15
BaF3-tel/trkA-12nMA2,#5-3G	Mixture,G & T,exon 15
BaF3-tel/trkA-12nMA2,#5-6B	Mixture,G & T,exon 15
BaF3-tel/trkA-12nMB3,#1-5C	Mixture,G & T,exon 15
BaF3-tel/trkA-12nMB3,#2-2H	Mixture,G & T,exon 15
BaF3-tel/trkA-12nMB3,#3-8H	Mixture,G & T,exon 15
BaF3-tel/trkA-12nMB3,#4-3E	Mixture,G & T,exon 15
BaF3-tel/trkA-12nMB3,#4-5C	Mixture,G & T,exon 15
BaF3-tel/trkA-12nMB3,#5-2H	Mixture,G & T,exon 15

Conclusion: All tested clones still have mixed G&T peaks in exon 15, G667C, suggesting multiple inserted DNA copies at the genomic level.

FIG. 23

Cell Lines	Cell type	Target
BaF3-tel/trkA	Engineering cell line, mouse pro B	TrkA fusion
BaF3-tel/trkB	Engineering cell line, mouse pro B	TrkB fusion
BaF3-tel/trkC	Engineering cell line, mouse pro B	TrkC fusion
Karpas 299	Lymphoma from human	ALK fusion
HCC-78	NSCLC carcinoma	Ros1 fusion
KM12	CRC	TrkA fusion

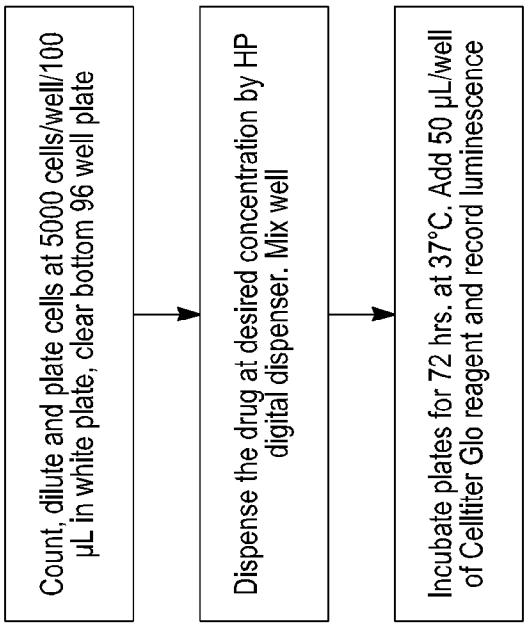


FIG. 24

IC_{50} (nM) using cell based (GI-CTG) assay

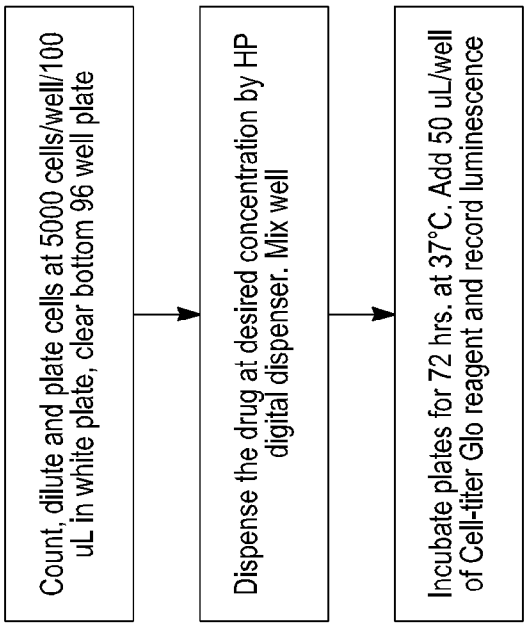
Compounds	BaF3-tel/trkA	BaF3-tel/trkA G595R	BaF3-tel/trkB	BaF3-tel/trkC	Karpas 299	HCC-78	KM12
Entrectinib	3.4	620.1	3.8	2.8	24.8	107.9	5.4
Crizotinib	78.0	>1000	73.6	57.0	18.2	139.0	76.1
NVP-TAE684	>1000	142.3	>1000	>1000	>1000	86.8	313.9
Foretinib	19.4	>1000	19.9	21.6	>1000	154.4	10.1
BMS-754807	109.3	>1000	37.8	112.1	>1000	232.9	121.4
GNF5837	8.9	>1000	4.7	46.7	>1000	>1000	17.7
Rebastinib	7.1	155.7	9.4	12.6	>1000	584.7	34.3
GW441756	199.4	>1000	405.9	>1000	>1000	382.3	297.5
Cabozantinib	86.3	>1000	48.4	49.4	>1000	14.9	111.9
Bosutinib	448.7	>1000	773.8	1562.9	>1000	>1000	74429.9
RXDX-102	0.8	>1000	**7.6**	**7.9**	78.0	413.0	1.7
TSR-011	34.7	>1000	**>1000**	**>1000**	114.2	114.0	44.4
MGCD516	**16.1**	>1000	**56.0**	**26.8**	12.3	81.5	**92.8**

Note:
1. All data are the average of at least two independent experiments.
2. The data presented in bold letters is from a single experiment.

FIG. 25

Compound	ALK(nM)	ROS1(nM)	TrkA(nM)	TrkB(nM)	TrkC(nM)	ACK1(nM)
Crizotinib	1.6	0.5	10	2.6	<1.0	188
Ceritinib/LDK378	1	1.5	7600	-	-	66
Alectinib-CH542802	1.1	3400	780	580	120	180
TSR-011	1.1	2500	9	8.7	1.9	990
P26113	0.9	1.7	2700	-	-	36
PLX7486	-	-	-	-	-	-
NVP-TAE684	0.4	.06	10.3	-	-	40
ASP-3026	6.8	10	10000	-	-	67
PF06463922	Pending	Pending	Pending	Pending	Pending	Pending
GSK1838705A	0.7	5.1	9100	-	-	2900
Dasatinib	10000	17000	2700	-	-	44
Foretinib	1500	50	5.3	7.7	2.4	330
Rebastinib/DCC-2036	1200	280	41	21	25	2200
LOXO-101	7142	53	1.1	<1	<1	215
Entrectinib	1.6	0.2	1.7	0.1	0.1	18
MGCD516	944	6.6	1.9	2.0	1.0	926
GW441756	2200	9400	32	-	-	1600
BMS-754807	123	<0.5	1	-	-	111
vemurafenib	>10000	>10000	6400	-	-	20
Bosutinib	61	4000	113	16	0.5	40
Cabozantinib	8000	59	39	36	30	650
AZD3463	2.3	1.3	200	-	-	77
dovitinib	640	>10000	8	21	2.4	2300
ENMD-2076	4100	197	14	35	3.1	600

FIG. 26



Cell Lines	Note	Target
BaF3-tel/trkA DMSO-A	WT	ETV6-trkA fusion
BaF3-tel/trkA 10nM-A	Selected by 10nM Entrectinib, G595R	ETV6-trkA fusion
KM12-DMSO-B	WT	TPM3-TrkA fusion
KM12-30nM-B KM12-100nM-B	Selected by 100nM Entrectinib, G667C	TPM3-TrkA fusion
KM12-300nM-B	Selected by 300nM Entrectinib, G595R	TPM3-TrkA fusion

FIG. 27

Name by company	Baf3-tel/trkA-WT	Baf3-tel/trkA10nMA-(G595R)	KM12 (trkA-WT)	KM12-100nMB (trkA-G667C)	KM12 300nMB (trkA-G595R)
Entrectinib	2.6	>1000	3.4	367.4	>1000
Compound 2	2.1	856.7	6.1	465.7	424.5
Ceritinib	>1000	>1000	268.7	>1000	>1000
LOXO-101	27.5	>1000	27.0	>1000	>1000
PF06463922	328.8	>1000	205.6	>1000	>1000
crizotinib	134.6	>1000	76.6	>1000	>1000

FIG. 28

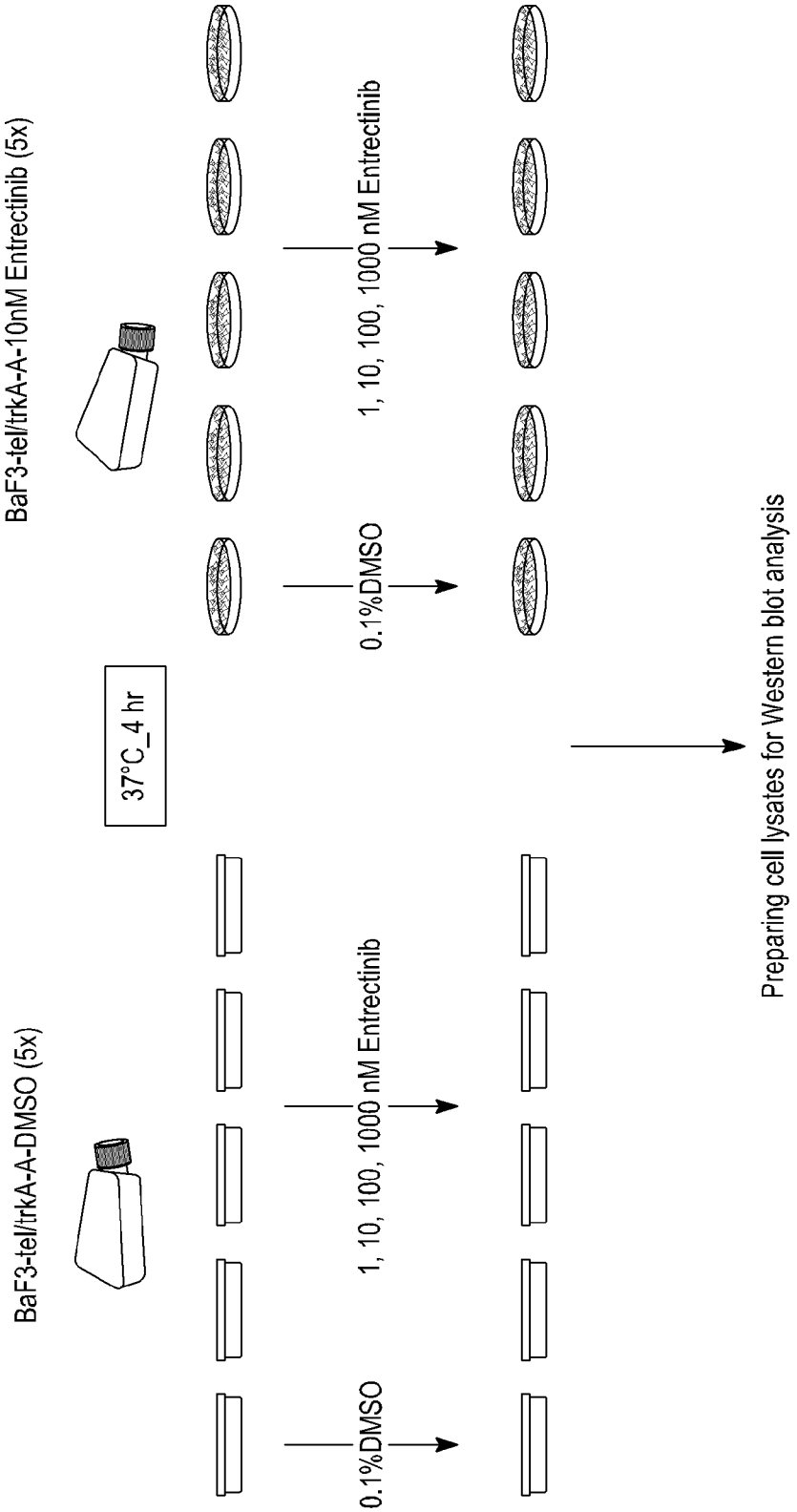

BaF3-tel/trkA-A-DMSO (5x)
BaF3-tel/trkA-A-10nM Entrectinib (5x)
37°C_4 hr
0.1%DMSO
1, 10, 100, 1000 nM Entrectinib
0.1%DMSO
1, 10, 100, 1000 nM Entrectinib
Preparing cell lysates for Western blot analysis

FIG. 29

Lane#	Sample (50 ug/sample)
1	Chemi Ladder
2	(BaF3-tel/trkA-10nMA Entrectinib +.1% DMSO
3	(BaF3-tel/trkA-10nMA Entrectinib)+1nM Entrectinib
4	(BaF3-tel/trkA-10nMA Entrectinib)+10nM Entrectinib
5	(BaF3-tel/trkA-10nMA Entrectinib)+100nM Entrectinib
6	(BaF3-tel/trkA-10nMA Entrectinib)+1000nM Entrectinib
7	(BaF3-tel/trkA-DMSO)+.1% DMSO
8	(BaF3-tel/trkA-DMSO)+1nM Entrectinib
9	(BaF3-tel/trkA-DMSO)+10nM Entrectinib
10	(BaF3-tel/trkA-DMSO)+100nM Entrectinib
11	(BaF3-tel/trkA-DMSO)+1000nM Entrectinib

Results:
1. Total trkA expression in both cell lines are similar.
2. pTrkA-Y490 in 10nM-Entrectinib-resistant BaF3-tel/trkA cells don't change in the presence of Entrectinib up to 1 uM.
3. Other down stream pathway proteins showed no correlation with Entrectinib treatment (data not shown)

FIG. 30

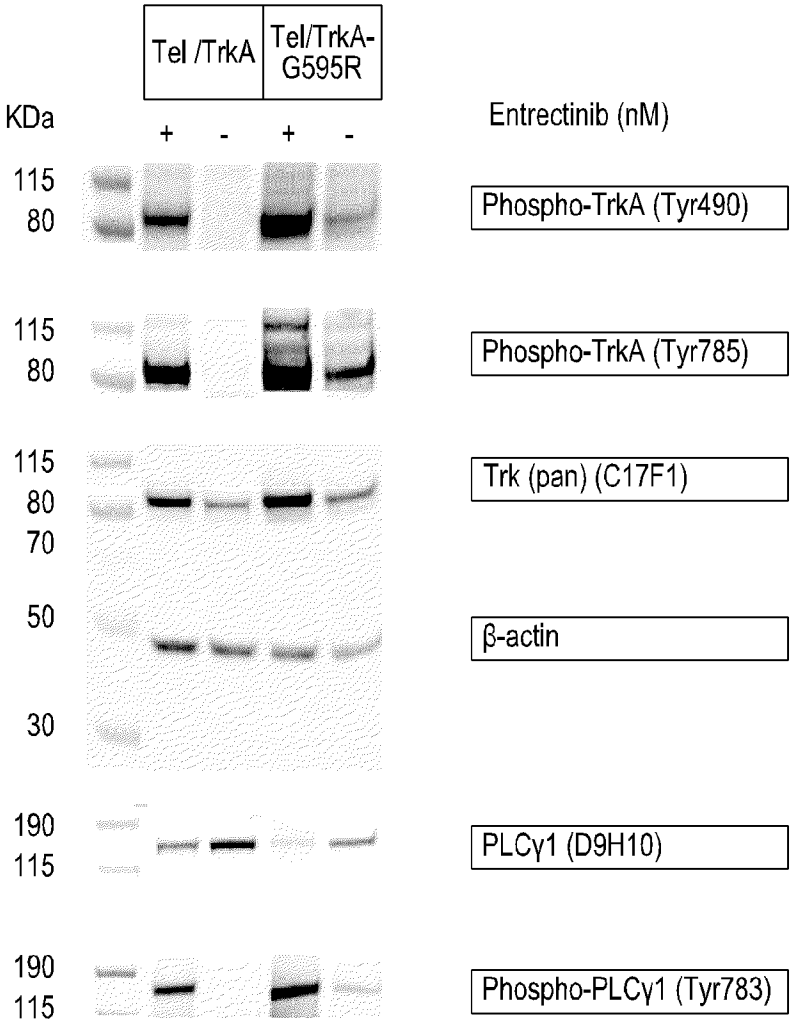

Tel /TrkA
Tel/TrkA-G595R
KDa
+ - + -
Entrectinib (nM)
115
80
Phospho-TrkA (Tyr490)
115
80
Phospho-TrkA (Tyr785)
115
80
70
Trk (pan) (C17F1)
50
β-actin
30
190
115
PLCγ1 (D9H10)
190
115
Phospho-PLCγ1 (Tyr783)

FIG. 31

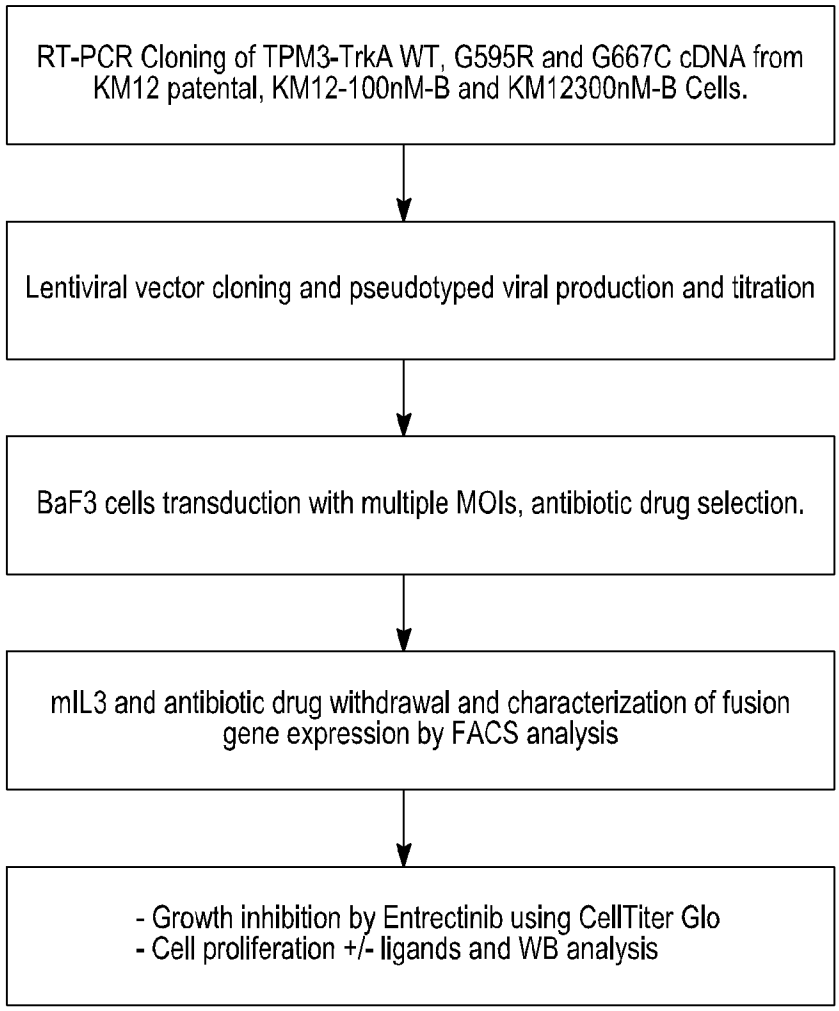

RT-PCR Cloning of TPM3-TrkA WT, G595R and G667C cDNA from KM12 patental, KM12-100nM-B and KM12300nM-B Cells.
Lentiviral vector cloning and pseudotyped viral production and titration
BaF3 cells transduction with multiple MOIs, antibiotic drug selection.
mIL3 and antibiotic drug withdrawal and characterization of fusion gene expression by FACS analysis
- Growth inhibition by Entrectinib using CellTiter Glo
- Cell proliferation +/- ligands and WB analysis

FIG. 32

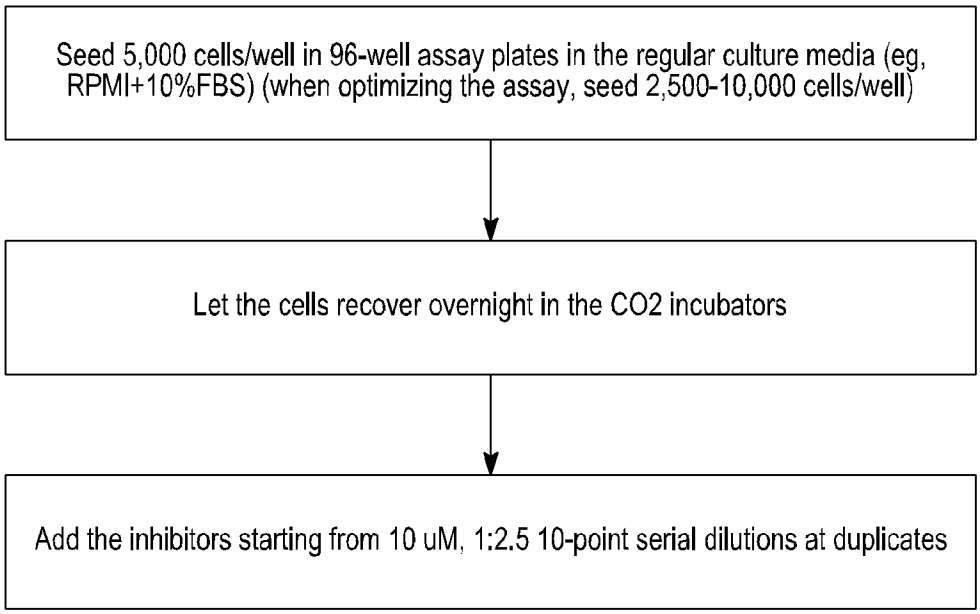
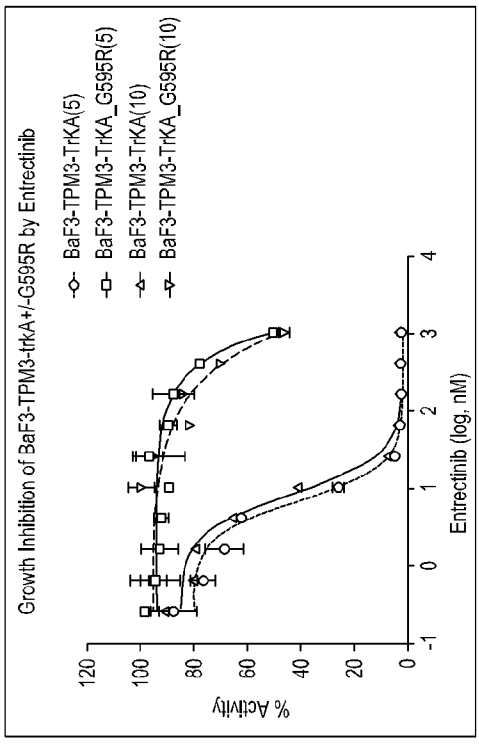
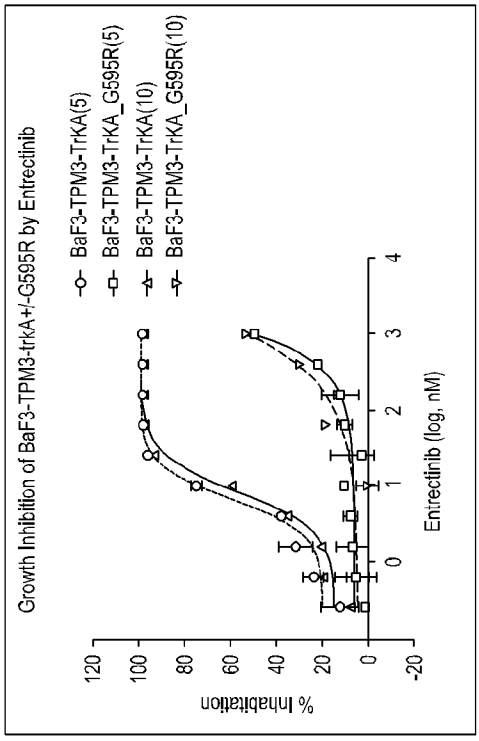

Seed 5,000 cells/well in 96-well assay plates in the regular culture media (eg, RPMI+10%FBS) (when optimizing the assay, seed 2,500-10,000 cells/well)
Let the cells recover overnight in the CO2 incubators
Add the inhibitors starting from 10 uM, 1:2.5 10-point serial dilutions at duplicates

FIG. 33





	BaF3-TPM3-TrKA(5)	BaF3-TPM3-TrKA_G595R(5)	BaF3-TPM3-TrKA(10)	BaF3-TPM3-TrKA_G595R(10)
IC50 (nM)	6.791	~ 121025	8.436	~ 9.108e+006
R square	0.9820	0.9046	0.9914	0.9253

FIG. 34

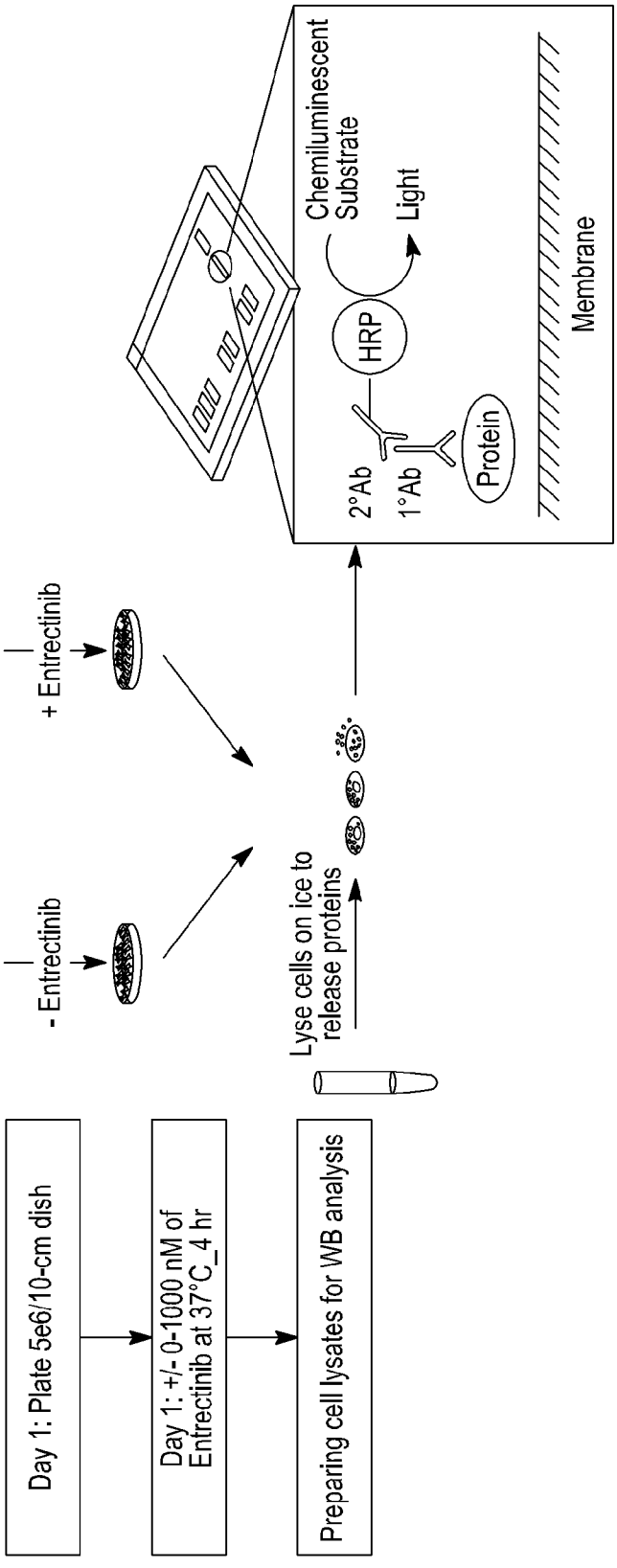

Day 1: Plate 5e6/10-cm dish
Day 1: +/- 0-1000 nM of Entrectinib at 37°C_4 hr
Preparing cell lysates for WB analysis
Lyse cells on ice to release proteins
- Entrectinib
+ Entrectinib
Chemiluminescent Substrate
Light
HRP
2°Ab
1°Ab
Protein
Membrane

FIG. 35

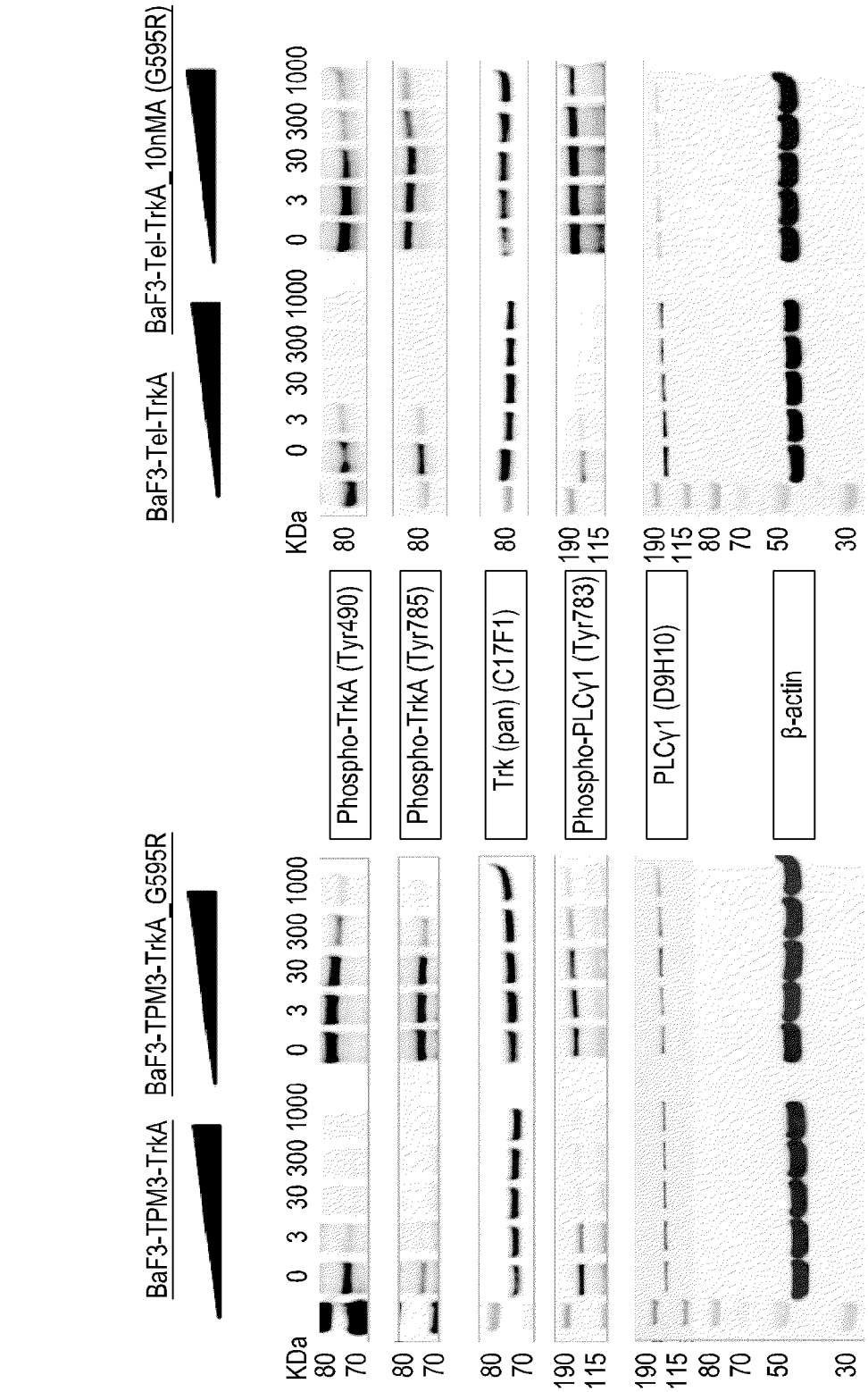

BaF3-TPM3-TrkA
BaF3-TPM3-TrkA_G595R
BaF3-Tel-TrkA
BaF3-Tel-TrkA_10nMA (G595R)
KDa
0 3 30 300 1000 0 3 30 300 1000
0 3 30 300 1000 0 3 30 300 1000
Phospho-TrkA (Tyr490)
Phospho-TrkA (Tyr785)
Trk (pan) (C17F1)
Phospho-PLCγ1 (Tyr783)
PLCγ1 (D9H10)
β-actin
80
70
190
115
50
30

FIG. 36

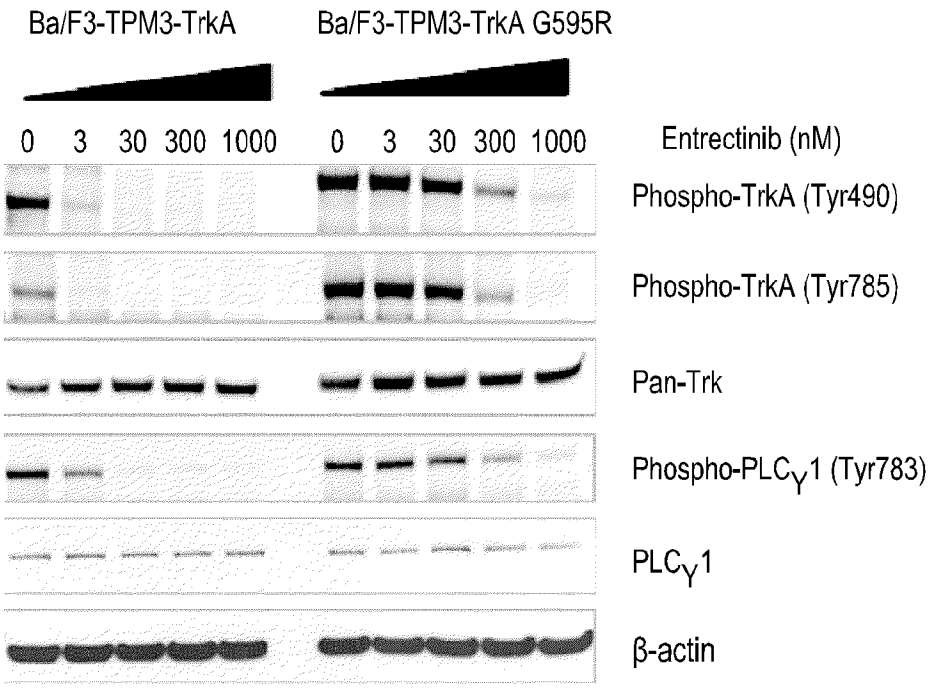

Ba/F3-TPM3-TrkA
Ba/F3-TPM3-TrkA G595R
0 3 30 300 1000
0 3 30 300 1000
Entrectinib (nM)
Phospho-TrkA (Tyr490)
Phospho-TrkA (Tyr785)
Pan-Trk
Phospho-PLCγ1 (Tyr783)
PLCγ1
β-actin

FIG. 37

SEQUENCE LISTING

<110> WEI, GE

<120> COMPOSITIONS AND METHODS FOR TREATING PATIENTS WITH RTK MUTANT CELLS

<130> IGNYT.051WO

<150> 62/168,237
<151> 2015-05-29

<150> 62/309,900
<151> 2016-03-17

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20 25 30

Ala Ala Pro Cys Pro Asp Ala Cys Cys Pro His Gly Ser Ser Gly Leu
35 40 45

Arg Cys Thr Arg Asp Gly Ala Leu Asp Ser Leu His His Leu Pro Gly
50 55 60

Ala Glu Asn Leu Thr Glu Leu Tyr Ile Glu Asn Gln Gln His Leu Gln
65 70 75 80

His Leu Glu Leu Arg Asp Leu Arg Gly Leu Gly Glu Leu Arg Asn Leu
85 90 95

Thr Ile Val Lys Ser Gly Leu Arg Phe Val Ala Pro Asp Ala Phe His
100 105 110

Phe Thr Pro Arg Leu Ser Arg Leu Asn Leu Ser Phe Asn Ala Leu Glu
115 120 125

Ser Leu Ser Trp Lys Thr Val Gln Gly Leu Ser Leu Gln Glu Leu Val
130 135 140

Leu Ser Gly Asn Pro Leu His Cys Ser Cys Ala Leu Arg Trp Leu Gln
145 150 155 160

Arg Trp Glu Glu Glu Gly Leu Gly Gly Val Pro Glu Gln Lys Leu Gln
165 170 175

Cys His Gly Gln Gly Pro Leu Ala His Met Pro Asn Ala Ser Cys Gly
180 185 190

Val Pro Thr Leu Lys Val Gln Val Pro Asn Ala Ser Val Asp Val Gly
195 200 205

Asp Asp Val Leu Leu Arg Cys Gln Val Glu Gly Arg Gly Leu Glu Gln
210 215 220

Ala Gly Trp Ile Leu Thr Glu Leu Glu Gln Ser Ala Thr Val Met Lys
225 230 235 240

Ser Gly Gly Leu Pro Ser Leu Gly Leu Thr Leu Ala Asn Val Thr Ser
245 250 255

Asp Leu Asn Arg Lys Asn Val Thr Cys Trp Ala Glu Asn Asp Val Gly
260 265 270

Arg Ala Glu Val Ser Val Gln Val Asn Val Ser Phe Pro Ala Ser Val
275 280 285

Gln Leu His Thr Ala Val Glu Met His His Trp Cys Ile Pro Phe Ser
290 295 300

Val Asp Gly Gln Pro Ala Pro Ser Leu Arg Trp Leu Phe Asn Gly Ser
305 310 315 320

Val Leu Asn Glu Thr Ser Phe Ile Phe Thr Glu Phe Leu Glu Pro Ala
325 330 335

Ala Asn Glu Thr Val Arg His Gly Cys Leu Arg Leu Asn Gln Pro Thr
340 345 350

His Val Asn Asn Gly Asn Tyr Thr Leu Leu Ala Ala Asn Pro Phe Gly
355 360 365

Gln Ala Ser Ala Ser Ile Met Ala Ala Phe Met Asp Asn Pro Phe Glu
370 375 380

Phe Asn Pro Glu Asp Pro Ile Pro Val Ser Phe Ser Pro Val Asp Thr
385 390 395 400

Asn Ser Thr Ser Gly Asp Pro Val Glu Lys Lys Asp Glu Thr Pro Phe

405 410 415

Gly Val Ser Val Ala Val Gly Leu Ala Val Phe Ala Cys Leu Phe Leu
420 425 430

Ser Thr Leu Leu Leu Val Leu Asn Lys Cys Gly Arg Arg Asn Lys Phe
435 440 445

Gly Ile Asn Arg Pro Ala Val Leu Ala Pro Glu Asp Gly Leu Ala Met
450 455 460

Ser Leu His Phe Met Thr Leu Gly Gly Ser Ser Leu Ser Pro Thr Glu
465 470 475 480

Gly Lys Gly Ser Gly Leu Gln Gly His Ile Ile Glu Asn Pro Gln Tyr
485 490 495

Phe Ser Asp Ala Cys Val His His Ile Lys Arg Arg Asp Ile Val Leu
500 505 510

Lys Trp Glu Leu Gly Glu Gly Ala Phe Gly Lys Val Phe Leu Ala Glu
515 520 525

Cys His Asn Leu Leu Pro Glu Gln Asp Lys Met Leu Val Ala Val Lys
530 535 540

Ala Leu Lys Glu Ala Ser Glu Ser Ala Arg Gln Asp Phe Gln Arg Glu
545 550 555 560

Ala Glu Leu Leu Thr Met Leu Gln His Gln His Ile Val Arg Phe Phe
565 570 575

Gly Val Cys Thr Glu Gly Arg Pro Leu Leu Met Val Phe Glu Tyr Met
580 585 590

Arg His Gly Asp Leu Asn Arg Phe Leu Arg Ser His Gly Pro Asp Ala
595 600 605

Lys Leu Leu Ala Gly Gly Glu Asp Val Ala Pro Gly Pro Leu Gly Leu
610 615 620

Gly Gln Leu Leu Ala Val Ala Ser Gln Val Ala Ala Gly Met Val Tyr
625 630 635 640

Leu Ala Gly Leu His Phe Val His Arg Asp Leu Ala Thr Arg Asn Cys
645 650 655

Leu Val Gly Gln Gly Leu Val Val Lys Ile Gly Asp Phe Gly Met Ser
660 665 670

Arg Asp Ile Tyr Ser Thr Asp Tyr Tyr Arg Val Gly Gly Arg Thr Met
675 680 685

Leu Pro Ile Arg Trp Met Pro Pro Glu Ser Ile Leu Tyr Arg Lys Phe
690 695 700

Thr Thr Glu Ser Asp Val Trp Ser Phe Gly Val Val Leu Trp Glu Ile
705 710 715 720

Phe Thr Tyr Gly Lys Gln Pro Trp Tyr Gln Leu Ser Asn Thr Glu Ala
725 730 735

Ile Asp Cys Ile Thr Gln Gly Arg Glu Leu Glu Arg Pro Arg Ala Cys
740 745 750

Pro Pro Glu Val Tyr Ala Ile Met Arg Gly Cys Trp Gln Arg Glu Pro
755 760 765

Gln Gln Arg His Ser Ile Lys Asp Val His Ala Arg Leu Gln Ala Leu
770 775 780

Ala Gln Ala Pro Pro Val Tyr Leu Asp Val Leu Gly
785 790 795

<210> 2
<211> 277
<212> PRT
<213> Homo sapiens

<220>
<223> kinase domain of tropomyosin receptor kinase A

<220>
<221> misc_feature
<223> fragment of the polypeptide sequence at SEQ ID NO: 1

<400> 2

His Ile Lys Arg Arg Asp Ile Val Leu Lys Trp Glu Leu Gly Glu Gly
1 5 10 15

Ala Phe Gly Lys Val Phe Leu Ala Glu Cys His Asn Leu Leu Pro Glu
20 25 30

Gln Asp Lys Met Leu Val Ala Val Lys Ala Leu Lys Glu Ala Ser Glu
35 40 45

Ser Ala Arg Gln Asp Phe Gln Arg Glu Ala Glu Leu Leu Thr Met Leu
50 55 60

Gln His Gln His Ile Val Arg Phe Phe Gly Val Cys Thr Glu Gly Arg
65 70 75 80

Pro Leu Leu Met Val Phe Glu Tyr Met Arg His Gly Asp Leu Asn Arg
85 90 95

Phe Leu Arg Ser His Gly Pro Asp Ala Lys Leu Leu Ala Gly Gly Glu
100 105 110

Asp Val Ala Pro Gly Pro Leu Gly Leu Gly Gln Leu Leu Ala Val Ala
115 120 125

Ser Gln Val Ala Ala Gly Met Val Tyr Leu Ala Gly Leu His Phe Val
130 135 140

His Arg Asp Leu Ala Thr Arg Asn Cys Leu Val Gly Gln Gly Leu Val
145 150 155 160

Val Lys Ile Gly Asp Phe Gly Met Ser Arg Asp Ile Tyr Ser Thr Asp
165 170 175

Tyr Tyr Arg Val Gly Gly Arg Thr Met Leu Pro Ile Arg Trp Met Pro
180 185 190

Pro Glu Ser Ile Leu Tyr Arg Lys Phe Thr Thr Glu Ser Asp Val Trp
195 200 205

Ser Phe Gly Val Val Leu Trp Glu Ile Phe Thr Tyr Gly Lys Gln Pro
210 215 220

Trp Tyr Gln Leu Ser Asn Thr Glu Ala Ile Asp Cys Ile Thr Gln Gly
225 230 235 240

Arg Glu Leu Glu Arg Pro Arg Ala Cys Pro Pro Glu Val Tyr Ala Ile
245 250 255

Met Arg Gly Cys Trp Gln Arg Glu Pro Gln Gln Arg His Ser Ile Lys
260 265 270

Asp Val His Ala Arg
275

<210> 3
<211> 838
<212> PRT
<213> Homo sapiens

<220>
<223> tropomyosin receptor kinase B, TrkB

<220>
<221> misc_feature
<223> NCBI Accession Number NP_006171.2

<400> 3

Met Ser Ser Trp Ile Arg Trp His Gly Pro Ala Met Ala Arg Leu Trp
1 5 10 15

Gly Phe Cys Trp Leu Val Val Gly Phe Trp Arg Ala Ala Phe Ala Cys
20 25 30

Pro Thr Ser Cys Lys Cys Ser Ala Ser Arg Ile Trp Cys Ser Asp Pro
35 40 45

Ser Pro Gly Ile Val Ala Phe Pro Arg Leu Glu Pro Asn Ser Val Asp
50 55 60

Pro Glu Asn Ile Thr Glu Ile Phe Ile Ala Asn Gln Lys Arg Leu Glu
65 70 75 80

Ile Ile Asn Glu Asp Asp Val Glu Ala Tyr Val Gly Leu Arg Asn Leu
85 90 95

Thr Ile Val Asp Ser Gly Leu Lys Phe Val Ala His Lys Ala Phe Leu

100 105 110

Lys Asn Ser Asn Leu Gln His Ile Asn Phe Thr Arg Asn Lys Leu Thr
115 120 125

Ser Leu Ser Arg Lys His Phe Arg His Leu Asp Leu Ser Glu Leu Ile
130 135 140

Leu Val Gly Asn Pro Phe Thr Cys Ser Cys Asp Ile Met Trp Ile Lys
145 150 155 160

Thr Leu Gln Glu Ala Lys Ser Ser Pro Asp Thr Gln Asp Leu Tyr Cys
165 170 175

Leu Asn Glu Ser Ser Lys Asn Ile Pro Leu Ala Asn Leu Gln Ile Pro
180 185 190

Asn Cys Gly Leu Pro Ser Ala Asn Leu Ala Ala Pro Asn Leu Thr Val
195 200 205

Glu Glu Gly Lys Ser Ile Thr Leu Ser Cys Ser Val Ala Gly Asp Pro
210 215 220

Val Pro Asn Met Tyr Trp Asp Val Gly Asn Leu Val Ser Lys His Met
225 230 235 240

Asn Glu Thr Ser His Thr Gln Gly Ser Leu Arg Ile Thr Asn Ile Ser
245 250 255

Ser Asp Asp Ser Gly Lys Gln Ile Ser Cys Val Ala Glu Asn Leu Val
260 265 270

Gly Glu Asp Gln Asp Ser Val Asn Leu Thr Val His Phe Ala Pro Thr
275 280 285

Ile Thr Phe Leu Glu Ser Pro Thr Ser Asp His His Trp Cys Ile Pro
290 295 300

Phe Thr Val Lys Gly Asn Pro Lys Pro Ala Leu Gln Trp Phe Tyr Asn
305 310 315 320

Gly Ala Ile Leu Asn Glu Ser Lys Tyr Ile Cys Thr Lys Ile His Val
325 330 335

Thr Asn His Thr Glu Tyr His Gly Cys Leu Gln Leu Asp Asn Pro Thr
340 345 350

His Met Asn Asn Gly Asp Tyr Thr Leu Ile Ala Lys Asn Glu Tyr Gly
355 360 365

Lys Asp Glu Lys Gln Ile Ser Ala His Phe Met Gly Trp Pro Gly Ile
370 375 380

Asp Asp Gly Ala Asn Pro Asn Tyr Pro Asp Val Ile Tyr Glu Asp Tyr
385 390 395 400

Gly Thr Ala Ala Asn Asp Ile Gly Asp Thr Thr Asn Arg Ser Asn Glu
405 410 415

Ile Pro Ser Thr Asp Val Thr Asp Lys Thr Gly Arg Glu His Leu Ser
420 425 430

Val Tyr Ala Val Val Val Ile Ala Ser Val Val Gly Phe Cys Leu Leu
435 440 445

Val Met Leu Phe Leu Leu Lys Leu Ala Arg His Ser Lys Phe Gly Met
450 455 460

Lys Asp Phe Ser Trp Phe Gly Phe Gly Lys Val Lys Ser Arg Gln Gly
465 470 475 480

Val Gly Pro Ala Ser Val Ile Ser Asn Asp Asp Asp Ser Ala Ser Pro
485 490 495

Leu His His Ile Ser Asn Gly Ser Asn Thr Pro Ser Ser Ser Glu Gly
500 505 510

Gly Pro Asp Ala Val Ile Ile Gly Met Thr Lys Ile Pro Val Ile Glu
515 520 525

Asn Pro Gln Tyr Phe Gly Ile Thr Asn Ser Gln Leu Lys Pro Asp Thr
530 535 540

Phe Val Gln His Ile Lys Arg His Asn Ile Val Leu Lys Arg Glu Leu
545 550 555 560

Gly Glu Gly Ala Phe Gly Lys Val Phe Leu Ala Glu Cys Tyr Asn Leu
565 570 575

Cys Pro Glu Gln Asp Lys Ile Leu Val Ala Val Lys Thr Leu Lys Asp
580 585 590

Ala Ser Asp Asn Ala Arg Lys Asp Phe His Arg Glu Ala Glu Leu Leu
595 600 605

Thr Asn Leu Gln His Glu His Ile Val Lys Phe Tyr Gly Val Cys Val
610 615 620

Glu Gly Asp Pro Leu Ile Met Val Phe Glu Tyr Met Lys His Gly Asp
625 630 635 640

Leu Asn Lys Phe Leu Arg Ala His Gly Pro Asp Ala Val Leu Met Ala
645 650 655

Glu Gly Asn Pro Pro Thr Glu Leu Thr Gln Ser Gln Met Leu His Ile
660 665 670

Ala Gln Gln Ile Ala Ala Gly Met Val Tyr Leu Ala Ser Gln His Phe
675 680 685

Val His Arg Asp Leu Ala Thr Arg Asn Cys Leu Val Gly Glu Asn Leu
690 695 700

Leu Val Lys Ile Gly Asp Phe Gly Met Ser Arg Asp Val Tyr Ser Thr
705 710 715 720

Asp Tyr Tyr Arg Val Gly Gly His Thr Met Leu Pro Ile Arg Trp Met
725 730 735

Pro Pro Glu Ser Ile Met Tyr Arg Lys Phe Thr Thr Glu Ser Asp Val
740 745 750

Trp Ser Leu Gly Val Val Leu Trp Glu Ile Phe Thr Tyr Gly Lys Gln
755 760 765

Pro Trp Tyr Gln Leu Ser Asn Asn Glu Val Ile Glu Cys Ile Thr Gln
770 775 780

Gly Arg Val Leu Gln Arg Pro Arg Thr Cys Pro Gln Glu Val Tyr Glu
785 790 795 800

Leu Met Leu Gly Cys Trp Gln Arg Glu Pro His Met Arg Lys Asn Ile
805 810 815

Lys Gly Ile His Thr Leu Leu Gln Asn Leu Ala Lys Ala Ser Pro Val

820 825 830

Tyr Leu Asp Ile Leu Gly
835

<210> 4
<211> 275
<212> PRT
<213> Homo sapiens

<220>
<223> kinase domain of tropomyosin receptor kinase B

<220>
<221> misc_feature
<223> fragment of the polypeptide sequence at SEQ ID NO: 3

<400> 4

His Ile Lys Arg His Asn Ile Val Leu Lys Arg Glu Leu Gly Glu Gly
1 5 10 15

Ala Phe Gly Lys Val Phe Leu Ala Glu Cys Tyr Asn Leu Cys Pro Glu
20 25 30

Gln Asp Lys Ile Leu Val Ala Val Lys Thr Leu Lys Asp Ala Ser Asp
35 40 45

Asn Ala Arg Lys Asp Phe His Arg Glu Ala Glu Leu Leu Thr Asn Leu
50 55 60

Gln His Glu His Ile Val Lys Phe Tyr Gly Val Cys Val Glu Gly Asp
65 70 75 80

Pro Leu Ile Met Val Phe Glu Tyr Met Lys His Gly Asp Leu Asn Lys
85 90 95

Phe Leu Arg Ala His Gly Pro Asp Ala Val Leu Met Ala Glu Gly Asn
100 105 110

Pro Pro Thr Glu Leu Thr Gln Ser Gln Met Leu His Ile Ala Gln Gln
115 120 125

Ile Ala Ala Gly Met Val Tyr Leu Ala Ser Gln His Phe Val His Arg
130 135 140

Asp Leu Ala Thr Arg Asn Cys Leu Val Gly Glu Asn Leu Leu Val Lys
145 150 155 160

Ile Gly Asp Phe Gly Met Ser Arg Asp Val Tyr Ser Thr Asp Tyr Tyr
165 170 175

Arg Val Gly Gly His Thr Met Leu Pro Ile Arg Trp Met Pro Pro Glu
180 185 190

Ser Ile Met Tyr Arg Lys Phe Thr Thr Glu Ser Asp Val Trp Ser Leu
195 200 205

Gly Val Val Leu Trp Glu Ile Phe Thr Tyr Gly Lys Gln Pro Trp Tyr
210 215 220

Gln Leu Ser Asn Asn Glu Val Ile Glu Cys Ile Thr Gln Gly Arg Val
225 230 235 240

Leu Gln Arg Pro Arg Thr Cys Pro Gln Glu Val Tyr Glu Leu Met Leu
245 250 255

Gly Cys Trp Gln Arg Glu Pro His Met Arg Lys Asn Ile Lys Gly Ile
260 265 270

His Thr Leu

275

<210> 5
<211> 839
<212> PRT
<213> Homo sapiens

<220>
<223> tropomyosin receptor kinase C, TrkC

<220>
<221> misc_feature
<223> NCBI Accession Number NP_001012338.1

<400> 5

Met Asp Val Ser Leu Cys Pro Ala Lys Cys Ser Phe Trp Arg Ile Phe
1 5 10 15

Leu Leu Gly Ser Val Trp Leu Asp Tyr Val Gly Ser Val Leu Ala Cys
20 25 30

Pro Ala Asn Cys Val Cys Ser Lys Thr Glu Ile Asn Cys Arg Arg Pro
35 40 45

Asp Asp Gly Asn Leu Phe Pro Leu Leu Glu Gly Gln Asp Ser Gly Asn
50 55 60

Ser Asn Gly Asn Ala Ser Ile Asn Ile Thr Asp Ile Ser Arg Asn Ile
65 70 75 80

Thr Ser Ile His Ile Glu Asn Trp Arg Ser Leu His Thr Leu Asn Ala
85 90 95

Val Asp Met Glu Leu Tyr Thr Gly Leu Gln Lys Leu Thr Ile Lys Asn
100 105 110

Ser Gly Leu Arg Ser Ile Gln Pro Arg Ala Phe Ala Lys Asn Pro His
115 120 125

Leu Arg Tyr Ile Asn Leu Ser Ser Asn Arg Leu Thr Thr Leu Ser Trp
130 135 140

Gln Leu Phe Gln Thr Leu Ser Leu Arg Glu Leu Gln Leu Glu Gln Asn
145 150 155 160

Phe Phe Asn Cys Ser Cys Asp Ile Arg Trp Met Gln Leu Trp Gln Glu
165 170 175

Gln Gly Glu Ala Lys Leu Asn Ser Gln Asn Leu Tyr Cys Ile Asn Ala
180 185 190

Asp Gly Ser Gln Leu Pro Leu Phe Arg Met Asn Ile Ser Gln Cys Asp
195 200 205

Leu Pro Glu Ile Ser Val Ser His Val Asn Leu Thr Val Arg Glu Gly
210 215 220

Asp Asn Ala Val Ile Thr Cys Asn Gly Ser Gly Ser Pro Leu Pro Asp
225 230 235 240

Val Asp Trp Ile Val Thr Gly Leu Gln Ser Ile Asn Thr His Gln Thr
245 250 255

Asn Leu Asn Trp Thr Asn Val His Ala Ile Asn Leu Thr Leu Val Asn
260 265 270

Val Thr Ser Glu Asp Asn Gly Phe Thr Leu Thr Cys Ile Ala Glu Asn
275 280 285

Val Val Gly Met Ser Asn Ala Ser Val Ala Leu Thr Val Tyr Tyr Pro

290 295 300

Pro Arg Val Val Ser Leu Glu Glu Pro Glu Leu Arg Leu Glu His Cys
305 310 315 320

Ile Glu Phe Val Val Arg Gly Asn Pro Pro Pro Thr Leu His Trp Leu
325 330 335

His Asn Gly Gln Pro Leu Arg Glu Ser Lys Ile Ile His Val Glu Tyr
340 345 350

Tyr Gln Glu Gly Glu Ile Ser Glu Gly Cys Leu Leu Phe Asn Lys Pro
355 360 365

Thr His Tyr Asn Asn Gly Asn Tyr Thr Leu Ile Ala Lys Asn Pro Leu
370 375 380

Gly Thr Ala Asn Gln Thr Ile Asn Gly His Phe Leu Lys Glu Pro Phe
385 390 395 400

Pro Glu Ser Thr Asp Asn Phe Ile Leu Phe Asp Glu Val Ser Pro Thr
405 410 415

Pro Pro Ile Thr Val Thr His Lys Pro Glu Glu Asp Thr Phe Gly Val
420 425 430

Ser Ile Ala Val Gly Leu Ala Ala Phe Ala Cys Val Leu Leu Val Val
435 440 445

Leu Phe Val Met Ile Asn Lys Tyr Gly Arg Arg Ser Lys Phe Gly Met
450 455 460

Lys Gly Pro Val Ala Val Ile Ser Gly Glu Glu Asp Ser Ala Ser Pro
465 470 475 480

Leu His His Ile Asn His Gly Ile Thr Thr Pro Ser Ser Leu Asp Ala
485 490 495

Gly Pro Asp Thr Val Val Ile Gly Met Thr Arg Ile Pro Val Ile Glu
500 505 510

Asn Pro Gln Tyr Phe Arg Gln Gly His Asn Cys His Lys Pro Asp Thr
515 520 525

Tyr Val Gln His Ile Lys Arg Arg Asp Ile Val Leu Lys Arg Glu Leu
530 535 540

Gly Glu Gly Ala Phe Gly Lys Val Phe Leu Ala Glu Cys Tyr Asn Leu
545 550 555 560

Ser Pro Thr Lys Asp Lys Met Leu Val Ala Val Lys Ala Leu Lys Asp
565 570 575

Pro Thr Leu Ala Ala Arg Lys Asp Phe Gln Arg Glu Ala Glu Leu Leu
580 585 590

Thr Asn Leu Gln His Glu His Ile Val Lys Phe Tyr Gly Val Cys Gly
595 600 605

Asp Gly Asp Pro Leu Ile Met Val Phe Glu Tyr Met Lys His Gly Asp
610 615 620

Leu Asn Lys Phe Leu Arg Ala His Gly Pro Asp Ala Met Ile Leu Val
625 630 635 640

Asp Gly Gln Pro Arg Gln Ala Lys Gly Glu Leu Gly Leu Ser Gln Met
645 650 655

Leu His Ile Ala Ser Gln Ile Ala Ser Gly Met Val Tyr Leu Ala Ser
660 665 670

Gln His Phe Val His Arg Asp Leu Ala Thr Arg Asn Cys Leu Val Gly
675 680 685

Ala Asn Leu Leu Val Lys Ile Gly Asp Phe Gly Met Ser Arg Asp Val
690 695 700

Tyr Ser Thr Asp Tyr Tyr Arg Leu Phe Asn Pro Ser Gly Asn Asp Phe
705 710 715 720

Cys Ile Trp Cys Glu Val Gly Gly His Thr Met Leu Pro Ile Arg Trp
725 730 735

Met Pro Pro Glu Ser Ile Met Tyr Arg Lys Phe Thr Thr Glu Ser Asp
740 745 750

Val Trp Ser Phe Gly Val Ile Leu Trp Glu Ile Phe Thr Tyr Gly Lys
755 760 765

Gln Pro Trp Phe Gln Leu Ser Asn Thr Glu Val Ile Glu Cys Ile Thr
770 775 780

Gln Gly Arg Val Leu Glu Arg Pro Arg Val Cys Pro Lys Glu Val Tyr
785 790 795 800

Asp Val Met Leu Gly Cys Trp Gln Arg Glu Pro Gln Gln Arg Leu Asn
805 810 815

Ile Lys Glu Ile Tyr Lys Ile Leu His Ala Leu Gly Lys Ala Thr Pro
820 825 830

Ile Tyr Leu Asp Ile Leu Gly
835

<210> 6
<211> 292
<212> PRT
<213> Homo sapiens

<220>
<223> kinase domain of tropomyosin receptor kinase C

<220>
<221> misc_feature
<223> fragment of the polypeptide sequence at SEQ ID NO: 5

<400> 6

His Ile Lys Arg Arg Asp Ile Val Leu Lys Arg Glu Leu Gly Glu Gly
1 5 10 15

Ala Phe Gly Lys Val Phe Leu Ala Glu Cys Tyr Asn Leu Ser Pro Thr
20 25 30

Lys Asp Lys Met Leu Val Ala Val Lys Ala Leu Lys Asp Pro Thr Leu
35 40 45

Ala Ala Arg Lys Asp Phe Gln Arg Glu Ala Glu Leu Leu Thr Asn Leu
50 55 60

Gln His Glu His Ile Val Lys Phe Tyr Gly Val Cys Gly Asp Gly Asp
65 70 75 80

Pro Leu Ile Met Val Phe Glu Tyr Met Lys His Gly Asp Leu Asn Lys
85 90 95

Phe Leu Arg Ala His Gly Pro Asp Ala Met Ile Leu Val Asp Gly Gln
100 105 110

Pro Arg Gln Ala Lys Gly Glu Leu Gly Leu Ser Gln Met Leu His Ile
115 120 125

Ala Ser Gln Ile Ala Ser Gly Met Val Tyr Leu Ala Ser Gln His Phe
130 135 140

Val His Arg Asp Leu Ala Thr Arg Asn Cys Leu Val Gly Ala Asn Leu
145 150 155 160

Leu Val Lys Ile Gly Asp Phe Gly Met Ser Arg Asp Val Tyr Ser Thr
165 170 175

Asp Tyr Tyr Arg Leu Phe Asn Pro Ser Gly Asn Asp Phe Cys Ile Trp
180 185 190

Cys Glu Val Gly Gly His Thr Met Leu Pro Ile Arg Trp Met Pro Pro
195 200 205

Glu Ser Ile Met Tyr Arg Lys Phe Thr Thr Glu Ser Asp Val Trp Ser
210 215 220

Phe Gly Val Ile Leu Trp Glu Ile Phe Thr Tyr Gly Lys Gln Pro Trp
225 230 235 240

Phe Gln Leu Ser Asn Thr Glu Val Ile Glu Cys Ile Thr Gln Gly Arg
245 250 255

Val Leu Glu Arg Pro Arg Val Cys Pro Lys Glu Val Tyr Asp Val Met
260 265 270

Leu Gly Cys Trp Gln Arg Glu Pro Gln Gln Arg Leu Asn Ile Lys Glu
275 280 285

Ile Tyr Lys Ile
290

<210> 7
<211> 1620
<212> PRT
<213> Homo sapiens

<220>
<223> ALK tyrosine kinase receptor, ALK

<220>
<221> misc_feature
<223> NCBI Accession Number NP_004295

<400> 7

Met Gly Ala Ile Gly Leu Leu Trp Leu Leu Pro Leu Leu Leu Ser Thr
1 5 10 15

Ala Ala Val Gly Ser Gly Met Gly Thr Gly Gln Arg Ala Gly Ser Pro
20 25 30

Ala Ala Gly Pro Pro Leu Gln Pro Arg Glu Pro Leu Ser Tyr Ser Arg
35 40 45

Leu Gln Arg Lys Ser Leu Ala Val Asp Phe Val Val Pro Ser Leu Phe
50 55 60

Arg Val Tyr Ala Arg Asp Leu Leu Leu Pro Pro Ser Ser Ser Glu Leu
65 70 75 80

Lys Ala Gly Arg Pro Glu Ala Arg Gly Ser Leu Ala Leu Asp Cys Ala
85 90 95

Pro Leu Leu Arg Leu Leu Gly Pro Ala Pro Gly Val Ser Trp Thr Ala
100 105 110

Gly Ser Pro Ala Pro Ala Glu Ala Arg Thr Leu Ser Arg Val Leu Lys
115 120 125

Gly Gly Ser Val Arg Lys Leu Arg Arg Ala Lys Gln Leu Val Leu Glu
130 135 140

Leu Gly Glu Glu Ala Ile Leu Glu Gly Cys Val Gly Pro Pro Gly Glu
145 150 155 160

Ala Ala Val Gly Leu Leu Gln Phe Asn Leu Ser Glu Leu Phe Ser Trp
165 170 175

Trp Ile Arg Gln Gly Glu Gly Arg Leu Arg Ile Arg Leu Met Pro Glu
180 185 190

Lys Lys Ala Ser Glu Val Gly Arg Glu Gly Arg Leu Ser Ala Ala Ile
195 200 205

Arg Ala Ser Gln Pro Arg Leu Leu Phe Gln Ile Phe Gly Thr Gly His
210 215 220

Ser Ser Leu Glu Ser Pro Thr Asn Met Pro Ser Pro Ser Pro Asp Tyr
225 230 235 240

Phe Thr Trp Asn Leu Thr Trp Ile Met Lys Asp Ser Phe Pro Phe Leu
245 250 255

Ser His Arg Ser Arg Tyr Gly Leu Glu Cys Ser Phe Asp Phe Pro Cys
260 265 270

Glu Leu Glu Tyr Ser Pro Pro Leu His Asp Leu Arg Asn Gln Ser Trp
275 280 285

Ser Trp Arg Arg Ile Pro Ser Glu Glu Ala Ser Gln Met Asp Leu Leu
290 295 300

Asp Gly Pro Gly Ala Glu Arg Ser Lys Glu Met Pro Arg Gly Ser Phe
305 310 315 320

Leu Leu Leu Asn Thr Ser Ala Asp Ser Lys His Thr Ile Leu Ser Pro
325 330 335

Trp Met Arg Ser Ser Ser Glu His Cys Thr Leu Ala Val Ser Val His
340 345 350

Arg His Leu Gln Pro Ser Gly Arg Tyr Ile Ala Gln Leu Leu Pro His
355 360 365

Asn Glu Ala Ala Arg Glu Ile Leu Leu Met Pro Thr Pro Gly Lys His
370 375 380

Gly Trp Thr Val Leu Gln Gly Arg Ile Gly Arg Pro Asp Asn Pro Phe
385 390 395 400

Arg Val Ala Leu Glu Tyr Ile Ser Ser Gly Asn Arg Ser Leu Ser Ala
405 410 415

Val Asp Phe Phe Ala Leu Lys Asn Cys Ser Glu Gly Thr Ser Pro Gly
420 425 430

Ser Lys Met Ala Leu Gln Ser Ser Phe Thr Cys Trp Asn Gly Thr Val
435 440 445

Leu Gln Leu Gly Gln Ala Cys Asp Phe His Gln Asp Cys Ala Gln Gly
450 455 460

Glu Asp Glu Ser Gln Met Cys Arg Lys Leu Pro Val Gly Phe Tyr Cys

465 470 475 480

Asn Phe Glu Asp Gly Phe Cys Gly Trp Thr Gln Gly Thr Leu Ser Pro
485 490 495

His Thr Pro Gln Trp Gln Val Arg Thr Leu Lys Asp Ala Arg Phe Gln
500 505 510

Asp His Gln Asp His Ala Leu Leu Leu Ser Thr Thr Asp Val Pro Ala
515 520 525

Ser Glu Ser Ala Thr Val Thr Ser Ala Thr Phe Pro Ala Pro Ile Lys
530 535 540

Ser Ser Pro Cys Glu Leu Arg Met Ser Trp Leu Ile Arg Gly Val Leu
545 550 555 560

Arg Gly Asn Val Ser Leu Val Leu Val Glu Asn Lys Thr Gly Lys Glu
565 570 575

Gln Gly Arg Met Val Trp His Val Ala Ala Tyr Glu Gly Leu Ser Leu
580 585 590

Trp Gln Trp Met Val Leu Pro Leu Leu Asp Val Ser Asp Arg Phe Trp
595 600 605

Leu Gln Met Val Ala Trp Trp Gly Gln Gly Ser Arg Ala Ile Val Ala
610 615 620

Phe Asp Asn Ile Ser Ile Ser Leu Asp Cys Tyr Leu Thr Ile Ser Gly
625 630 635 640

Glu Asp Lys Ile Leu Gln Asn Thr Ala Pro Lys Ser Arg Asn Leu Phe
645 650 655

Glu Arg Asn Pro Asn Lys Glu Leu Lys Pro Gly Glu Asn Ser Pro Arg
660 665 670

Gln Thr Pro Ile Phe Asp Pro Thr Val His Trp Leu Phe Thr Thr Cys
675 680 685

Gly Ala Ser Gly Pro His Gly Pro Thr Gln Ala Gln Cys Asn Asn Ala
690 695 700

Tyr Gln Asn Ser Asn Leu Ser Val Glu Val Gly Ser Glu Gly Pro Leu
705 710 715 720

Lys Gly Ile Gln Ile Trp Lys Val Pro Ala Thr Asp Thr Tyr Ser Ile
725 730 735

Ser Gly Tyr Gly Ala Ala Gly Gly Lys Gly Gly Lys Asn Thr Met Met
740 745 750

Arg Ser His Gly Val Ser Val Leu Gly Ile Phe Asn Leu Glu Lys Asp
755 760 765

Asp Met Leu Tyr Ile Leu Val Gly Gln Gln Gly Glu Asp Ala Cys Pro
770 775 780

Ser Thr Asn Gln Leu Ile Gln Lys Val Cys Ile Gly Glu Asn Asn Val
785 790 795 800

Ile Glu Glu Glu Ile Arg Val Asn Arg Ser Val His Glu Trp Ala Gly
805 810 815

Gly Gly Gly Gly Gly Gly Gly Ala Thr Tyr Val Phe Lys Met Lys Asp
820 825 830

Gly Val Pro Val Pro Leu Ile Ile Ala Ala Gly Gly Gly Gly Arg Ala
835 840 845

Tyr Gly Ala Lys Thr Asp Thr Phe His Pro Glu Arg Leu Glu Asn Asn
850 855 860

Ser Ser Val Leu Gly Leu Asn Gly Asn Ser Gly Ala Ala Gly Gly Gly
865 870 875 880

Gly Gly Trp Asn Asp Asn Thr Ser Leu Leu Trp Ala Gly Lys Ser Leu
885 890 895

Gln Glu Gly Ala Thr Gly Gly His Ser Cys Pro Gln Ala Met Lys Lys
900 905 910

Trp Gly Trp Glu Thr Arg Gly Gly Phe Gly Gly Gly Gly Gly Gly Cys
915 920 925

Ser Ser Gly Gly Gly Gly Gly Gly Tyr Ile Gly Gly Asn Ala Ala Ser
930 935 940

Asn Asn Asp Pro Glu Met Asp Gly Glu Asp Gly Val Ser Phe Ile Ser
945 950 955 960

Pro Leu Gly Ile Leu Tyr Thr Pro Ala Leu Lys Val Met Glu Gly His
965 970 975

Gly Glu Val Asn Ile Lys His Tyr Leu Asn Cys Ser His Cys Glu Val
980 985 990

Asp Glu Cys His Met Asp Pro Glu Ser His Lys Val Ile Cys Phe Cys
995 1000 1005

Asp His Gly Thr Val Leu Ala Glu Asp Gly Val Ser Cys Ile Val
1010 1015 1020

Ser Pro Thr Pro Glu Pro His Leu Pro Leu Ser Leu Ile Leu Ser
1025 1030 1035

Val Val Thr Ser Ala Leu Val Ala Ala Leu Val Leu Ala Phe Ser
1040 1045 1050

Gly Ile Met Ile Val Tyr Arg Arg Lys His Gln Glu Leu Gln Ala
1055 1060 1065

Met Gln Met Glu Leu Gln Ser Pro Glu Tyr Lys Leu Ser Lys Leu
1070 1075 1080

Arg Thr Ser Thr Ile Met Thr Asp Tyr Asn Pro Asn Tyr Cys Phe
1085 1090 1095

Ala Gly Lys Thr Ser Ser Ile Ser Asp Leu Lys Glu Val Pro Arg
1100 1105 1110

Lys Asn Ile Thr Leu Ile Arg Gly Leu Gly His Gly Ala Phe Gly
1115 1120 1125

Glu Val Tyr Glu Gly Gln Val Ser Gly Met Pro Asn Asp Pro Ser
1130 1135 1140

Pro Leu Gln Val Ala Val Lys Thr Leu Pro Glu Val Cys Ser Glu
1145 1150 1155

Gln Asp Glu Leu Asp Phe Leu Met Glu Ala Leu Ile Ile Ser Lys
1160 1165 1170

Phe Asn His Gln Asn Ile Val Arg Cys Ile Gly Val Ser Leu Gln

1175 1180 1185

Ser Leu Pro Arg Phe Ile Leu Leu Glu Leu Met Ala Gly Gly Asp
1190 1195 1200

Leu Lys Ser Phe Leu Arg Glu Thr Arg Pro Arg Pro Ser Gln Pro
1205 1210 1215

Ser Ser Leu Ala Met Leu Asp Leu Leu His Val Ala Arg Asp Ile
1220 1225 1230

Ala Cys Gly Cys Gln Tyr Leu Glu Glu Asn His Phe Ile His Arg
1235 1240 1245

Asp Ile Ala Ala Arg Asn Cys Leu Leu Thr Cys Pro Gly Pro Gly
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Arg Val Ala Lys Ile Gly Asp Phe Gly Met Ala Arg Asp Ile Tyr
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Arg Ala Ser Tyr Tyr Arg Lys Gly Gly Cys Ala Met Leu Pro Val
1280 1285 1290

Lys Trp Met Pro Pro Glu Ala Phe Met Glu Gly Ile Phe Thr Ser
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Lys Thr Asp Thr Trp Ser Phe Gly Val Leu Leu Trp Glu Ile Phe
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Ser Leu Gly Tyr Met Pro Tyr Pro Ser Lys Ser Asn Gln Glu Val
1325 1330 1335

Leu Glu Phe Val Thr Ser Gly Gly Arg Met Asp Pro Pro Lys Asn
1340 1345 1350

Cys Pro Gly Pro Val Tyr Arg Ile Met Thr Gln Cys Trp Gln His
1355 1360 1365

Gln Pro Glu Asp Arg Pro Asn Phe Ala Ile Ile Leu Glu Arg Ile
1370 1375 1380

Glu Tyr Cys Thr Gln Asp Pro Asp Val Ile Asn Thr Ala Leu Pro
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Ile Glu Tyr Gly Pro Leu Val Glu Glu Glu Glu Lys Val Pro Val
1400 1405 1410

Arg Pro Lys Asp Pro Glu Gly Val Pro Pro Leu Leu Val Ser Gln
1415 1420 1425

Gln Ala Lys Arg Glu Glu Glu Arg Ser Pro Ala Ala Pro Pro Pro
1430 1435 1440

Leu Pro Thr Thr Ser Ser Gly Lys Ala Ala Lys Lys Pro Thr Ala
1445 1450 1455

Ala Glu Ile Ser Val Arg Val Pro Arg Gly Pro Ala Val Glu Gly
1460 1465 1470

Gly His Val Asn Met Ala Phe Ser Gln Ser Asn Pro Pro Ser Glu
1475 1480 1485

Leu His Lys Val His Gly Ser Arg Asn Lys Pro Thr Ser Leu Trp
1490 1495 1500

Asn Pro Thr Tyr Gly Ser Trp Phe Thr Glu Lys Pro Thr Lys Lys
1505 1510 1515

Asn Asn Pro Ile Ala Lys Lys Glu Pro His Asp Arg Gly Asn Leu
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Gly Leu Glu Gly Ser Cys Thr Val Pro Pro Asn Val Ala Thr Gly
1535 1540 1545

Arg Leu Pro Gly Ala Ser Leu Leu Leu Glu Pro Ser Ser Leu Thr
1550 1555 1560

Ala Asn Met Lys Glu Val Pro Leu Phe Arg Leu Arg His Phe Pro
1565 1570 1575

Cys Gly Asn Val Asn Tyr Gly Tyr Gln Gln Gln Gly Leu Pro Leu
1580 1585 1590

Glu Ala Ala Thr Ala Pro Gly Ala Gly His Tyr Glu Asp Thr Ile
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Leu Lys Ser Lys Asn Ser Met Asn Gln Pro Gly Pro
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20 25 30

Pro Ser Pro Leu Gln Val Ala Val Lys Thr Leu Pro Glu Val Cys Ser
35 40 45

Glu Gln Asp Glu Leu Asp Phe Leu Met Glu Ala Leu Ile Ile Ser Lys
50 55 60

Phe Asn His Gln Asn Ile Val Arg Cys Ile Gly Val Ser Leu Gln Ser
65 70 75 80

Leu Pro Arg Phe Ile Leu Leu Glu Leu Met Ala Gly Gly Asp Leu Lys
85 90 95

Ser Phe Leu Arg Glu Thr Arg Pro Arg Pro Ser Gln Pro Ser Ser Leu
100 105 110

Ala Met Leu Asp Leu Leu His Val Ala Arg Asp Ile Ala Cys Gly Cys
115 120 125

Gln Tyr Leu Glu Glu Asn His Phe Ile His Arg Asp Ile Ala Ala Arg
130 135 140

Asn Cys Leu Leu Thr Cys Pro Gly Pro Gly Arg Val Ala Lys Ile Gly
145 150 155 160

Asp Phe Gly Met Ala Arg Asp Ile Tyr Arg Ala Ser Tyr Tyr Arg Lys
165 170 175

Gly Gly Cys Ala Met Leu Pro Val Lys Trp Met Pro Pro Glu Ala Phe
180 185 190

Met Glu Gly Ile Phe Thr Ser Lys Thr Asp Thr Trp Ser Phe Gly Val
195 200 205

Leu Leu Trp Glu Ile Phe Ser Leu Gly Tyr Met Pro Tyr Pro Ser Lys
210 215 220

Ser Asn Gln Glu Val Leu Glu Phe Val Thr Ser Gly Gly Arg Met Asp
225 230 235 240

Pro Pro Lys Asn Cys Pro Gly Pro Val Tyr Arg Ile Met Thr Gln Cys
245 250 255

Trp Gln His Gln Pro Glu Asp Arg Pro Asn Phe Ala Ile Ile Leu Glu
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Arg

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Cys Leu Lys Ser Cys Val Thr Asn Leu Gly Gln Gln Leu Asp Leu Gly
35 40 45

Thr Pro His Asn Leu Ser Glu Pro Cys Ile Gln Gly Cys His Phe Trp
50 55 60

Asn Ser Val Asp Gln Lys Asn Cys Ala Leu Lys Cys Arg Glu Ser Cys
65 70 75 80

Glu Val Gly Cys Ser Ser Ala Glu Gly Ala Tyr Glu Glu Glu Val Leu
85 90 95

Glu Asn Ala Asp Leu Pro Thr Ala Pro Phe Ala Ser Ser Ile Gly Ser
100 105 110

His Asn Met Thr Leu Arg Trp Lys Ser Ala Asn Phe Ser Gly Val Lys
115 120 125

Tyr Ile Ile Gln Trp Lys Tyr Ala Gln Leu Leu Gly Ser Trp Thr Tyr
130 135 140

Thr Lys Thr Val Ser Arg Pro Ser Tyr Val Val Lys Pro Leu His Pro
145 150 155 160

Phe Thr Glu Tyr Ile Phe Arg Val Val Trp Ile Phe Thr Ala Gln Leu
165 170 175

Gln Leu Tyr Ser Pro Pro Ser Pro Ser Tyr Arg Thr His Pro His Gly
180 185 190

Val Pro Glu Thr Ala Pro Leu Ile Arg Asn Ile Glu Ser Ser Ser Pro
195 200 205

Asp Thr Val Glu Val Ser Trp Asp Pro Pro Gln Phe Pro Gly Gly Pro
210 215 220

Ile Leu Gly Tyr Asn Leu Arg Leu Ile Ser Lys Asn Gln Lys Leu Asp
225 230 235 240

Ala Gly Thr Gln Arg Thr Ser Phe Gln Phe Tyr Ser Thr Leu Pro Asn
245 250 255

Thr Ile Tyr Arg Phe Ser Ile Ala Ala Val Asn Glu Val Gly Glu Gly
260 265 270

Pro Glu Ala Glu Ser Ser Ile Thr Thr Ser Ser Ser Ala Val Gln Gln
275 280 285

Glu Glu Gln Trp Leu Phe Leu Ser Arg Lys Thr Ser Leu Arg Lys Arg
290 295 300

Ser Leu Lys His Leu Val Asp Glu Ala His Cys Leu Arg Leu Asp Ala
305 310 315 320

Ile Tyr His Asn Ile Thr Gly Ile Ser Val Asp Val His Gln Gln Ile
325 330 335

Val Tyr Phe Ser Glu Gly Thr Leu Ile Trp Ala Lys Lys Ala Ala Asn
340 345 350

Met Ser Asp Val Ser Asp Leu Arg Ile Phe Tyr Arg Gly Ser Gly Leu
355 360 365

Ile Ser Ser Ile Ser Ile Asp Trp Leu Tyr Gln Arg Met Tyr Phe Ile
370 375 380

Met Asp Glu Leu Val Cys Val Cys Asp Leu Glu Asn Cys Ser Asn Ile
385 390 395 400

Glu Glu Ile Thr Pro Pro Ser Ile Ser Ala Pro Gln Lys Ile Val Ala
405 410 415

Asp Ser Tyr Asn Gly Tyr Val Phe Tyr Leu Leu Arg Asp Gly Ile Tyr
420 425 430

Arg Ala Asp Leu Pro Val Pro Ser Gly Arg Cys Ala Glu Ala Val Arg
435 440 445

Ile Val Glu Ser Cys Thr Leu Lys Asp Phe Ala Ile Lys Pro Gln Ala
450 455 460

Lys Arg Ile Ile Tyr Phe Asn Asp Thr Ala Gln Val Phe Met Ser Thr
465 470 475 480

Phe Leu Asp Gly Ser Ala Ser His Leu Ile Leu Pro Arg Ile Pro Phe
485 490 495

Ala Asp Val Lys Ser Phe Ala Cys Glu Asn Asn Asp Phe Leu Val Thr
500 505 510

Asp Gly Lys Val Ile Phe Gln Gln Asp Ala Leu Ser Phe Asn Glu Phe
515 520 525

Ile Val Gly Cys Asp Leu Ser His Ile Glu Glu Phe Gly Phe Gly Asn
530 535 540

Leu Val Ile Phe Gly Ser Ser Ser Gln Leu His Pro Leu Pro Gly Arg
545 550 555 560

Pro Gln Glu Leu Ser Val Leu Phe Gly Ser His Gln Ala Leu Val Gln

565 570 575

Trp Lys Pro Pro Ala Leu Ala Ile Gly Ala Asn Val Ile Leu Ile Ser
580 585 590

Asp Ile Ile Glu Leu Phe Glu Leu Gly Pro Ser Ala Trp Gln Asn Trp
595 600 605

Thr Tyr Glu Val Lys Val Ser Thr Gln Asp Pro Pro Glu Val Thr His
610 615 620

Ile Phe Leu Asn Ile Ser Gly Thr Met Leu Asn Val Pro Glu Leu Gln
625 630 635 640

Ser Ala Met Lys Tyr Lys Val Ser Val Arg Ala Ser Ser Pro Lys Arg
645 650 655

Pro Gly Pro Trp Ser Glu Pro Ser Val Gly Thr Thr Leu Val Pro Ala
660 665 670

Ser Glu Pro Pro Phe Ile Met Ala Val Lys Glu Asp Gly Leu Trp Ser
675 680 685

Lys Pro Leu Asn Ser Phe Gly Pro Gly Glu Phe Leu Ser Ser Asp Ile
690 695 700

Gly Asn Val Ser Asp Met Asp Trp Tyr Asn Asn Ser Leu Tyr Tyr Ser
705 710 715 720

Asp Thr Lys Gly Asp Val Phe Val Trp Leu Leu Asn Gly Thr Asp Ile
725 730 735

Ser Glu Asn Tyr His Leu Pro Ser Ile Ala Gly Ala Gly Ala Leu Ala
740 745 750

Phe Glu Trp Leu Gly His Phe Leu Tyr Trp Ala Gly Lys Thr Tyr Val
755 760 765

Ile Gln Arg Gln Ser Val Leu Thr Gly His Thr Asp Ile Val Thr His
770 775 780

Val Lys Leu Leu Val Asn Asp Met Val Val Asp Ser Val Gly Gly Tyr
785 790 795 800

Leu Tyr Trp Thr Thr Leu Tyr Ser Val Glu Ser Thr Arg Leu Asn Gly
805 810 815

Glu Ser Ser Leu Val Leu Gln Thr Gln Pro Trp Phe Ser Gly Lys Lys
820 825 830

Val Ile Ala Leu Thr Leu Asp Leu Ser Asp Gly Leu Leu Tyr Trp Leu
835 840 845

Val Gln Asp Ser Gln Cys Ile His Leu Tyr Thr Ala Val Leu Arg Gly
850 855 860

Gln Ser Thr Gly Asp Thr Thr Ile Thr Glu Phe Ala Ala Trp Ser Thr
865 870 875 880

Ser Glu Ile Ser Gln Asn Ala Leu Met Tyr Tyr Ser Gly Arg Leu Phe
885 890 895

Trp Ile Asn Gly Phe Arg Ile Ile Thr Thr Gln Glu Ile Gly Gln Lys
900 905 910

Thr Ser Val Ser Val Leu Glu Pro Ala Arg Phe Asn Gln Phe Thr Ile
915 920 925

Ile Gln Thr Ser Leu Lys Pro Leu Pro Gly Asn Phe Ser Phe Thr Pro
930 935 940

Lys Val Ile Pro Asp Ser Val Gln Glu Ser Ser Phe Arg Ile Glu Gly
945 950 955 960

Asn Ala Ser Ser Phe Gln Ile Leu Trp Asn Gly Pro Pro Ala Val Asp
965 970 975

Trp Gly Val Val Phe Tyr Ser Val Glu Phe Ser Ala His Ser Lys Phe
980 985 990

Leu Ala Ser Glu Gln His Ser Leu Pro Val Phe Thr Val Glu Gly Leu
995 1000 1005

Glu Pro Tyr Ala Leu Phe Asn Leu Ser Val Thr Pro Tyr Thr Tyr
1010 1015 1020

Trp Gly Lys Gly Pro Lys Thr Ser Leu Ser Leu Arg Ala Pro Glu
1025 1030 1035

Thr Val Pro Ser Ala Pro Glu Asn Pro Arg Ile Phe Ile Leu Pro
1040 1045 1050

Ser Gly Lys Cys Cys Asn Lys Asn Glu Val Val Val Glu Phe Arg
1055 1060 1065

Trp Asn Lys Pro Lys His Glu Asn Gly Val Leu Thr Lys Phe Glu
1070 1075 1080

Ile Phe Tyr Asn Ile Ser Asn Gln Ser Ile Thr Asn Lys Thr Cys
1085 1090 1095

Glu Asp Trp Ile Ala Val Asn Val Thr Pro Ser Val Met Ser Phe
1100 1105 1110

Gln Leu Glu Gly Met Ser Pro Arg Cys Phe Ile Ala Phe Gln Val
1115 1120 1125

Arg Ala Phe Thr Ser Lys Gly Pro Gly Pro Tyr Ala Asp Val Val
1130 1135 1140

Lys Ser Thr Thr Ser Glu Ile Asn Pro Phe Pro His Leu Ile Thr
1145 1150 1155

Leu Leu Gly Asn Lys Ile Val Phe Leu Asp Met Asp Gln Asn Gln
1160 1165 1170

Val Val Trp Thr Phe Ser Ala Glu Arg Val Ile Ser Ala Val Cys
1175 1180 1185

Tyr Thr Ala Asp Asn Glu Met Gly Tyr Tyr Ala Glu Gly Asp Ser
1190 1195 1200

Leu Phe Leu Leu His Leu His Asn Arg Ser Ser Ser Glu Leu Phe
1205 1210 1215

Gln Asp Ser Leu Val Phe Asp Ile Thr Val Ile Thr Ile Asp Trp
1220 1225 1230

Ile Ser Arg His Leu Tyr Phe Ala Leu Lys Glu Ser Gln Asn Gly
1235 1240 1245

Met Gln Val Phe Asp Val Asp Leu Glu His Lys Val Lys Tyr Pro
1250 1255 1260

Arg Glu Val Lys Ile His Asn Arg Asn Ser Thr Ile Ile Ser Phe

1265 1270 1275

Ser Val Tyr Pro Leu Leu Ser Arg Leu Tyr Trp Thr Glu Val Ser
1280 1285 1290

Asn Phe Gly Tyr Gln Met Phe Tyr Tyr Ser Ile Ile Ser His Thr
1295 1300 1305

Leu His Arg Ile Leu Gln Pro Thr Ala Thr Asn Gln Gln Asn Lys
1310 1315 1320

Arg Asn Gln Cys Ser Cys Asn Val Thr Glu Phe Glu Leu Ser Gly
1325 1330 1335

Ala Met Ala Ile Asp Thr Ser Asn Leu Glu Lys Pro Leu Ile Tyr
1340 1345 1350

Phe Ala Lys Ala Gln Glu Ile Trp Ala Met Asp Leu Glu Gly Cys
1355 1360 1365

Gln Cys Trp Arg Val Ile Thr Val Pro Ala Met Leu Ala Gly Lys
1370 1375 1380

Thr Leu Val Ser Leu Thr Val Asp Gly Asp Leu Ile Tyr Trp Ile
1385 1390 1395

Ile Thr Ala Lys Asp Ser Thr Gln Ile Tyr Gln Ala Lys Lys Gly
1400 1405 1410

Asn Gly Ala Ile Val Ser Gln Val Lys Ala Leu Arg Ser Arg His
1415 1420 1425

Ile Leu Ala Tyr Ser Ser Val Met Gln Pro Phe Pro Asp Lys Ala
1430 1435 1440

Phe Leu Ser Leu Ala Ser Asp Thr Val Glu Pro Thr Ile Leu Asn
1445 1450 1455

Ala Thr Asn Thr Ser Leu Thr Ile Arg Leu Pro Leu Ala Lys Thr
1460 1465 1470

Asn Leu Thr Trp Tyr Gly Ile Thr Ser Pro Thr Pro Thr Tyr Leu
1475 1480 1485

Val Tyr Tyr Ala Glu Val Asn Asp Arg Lys Asn Ser Ser Asp Leu
1490 1495 1500

Lys Tyr Arg Ile Leu Glu Phe Gln Asp Ser Ile Ala Leu Ile Glu
1505 1510 1515

Asp Leu Gln Pro Phe Ser Thr Tyr Met Ile Gln Ile Ala Val Lys
1520 1525 1530

Asn Tyr Tyr Ser Asp Pro Leu Glu His Leu Pro Pro Gly Lys Glu
1535 1540 1545

Ile Trp Gly Lys Thr Lys Asn Gly Val Pro Glu Ala Val Gln Leu
1550 1555 1560

Ile Asn Thr Thr Val Arg Ser Asp Thr Ser Leu Ile Ile Ser Trp
1565 1570 1575

Arg Glu Ser His Lys Pro Asn Gly Pro Lys Glu Ser Val Arg Tyr
1580 1585 1590

Gln Leu Ala Ile Ser His Leu Ala Leu Ile Pro Glu Thr Pro Leu
1595 1600 1605

Arg Gln Ser Glu Phe Pro Asn Gly Arg Leu Thr Leu Leu Val Thr
1610 1615 1620

Arg Leu Ser Gly Gly Asn Ile Tyr Val Leu Lys Val Leu Ala Cys
1625 1630 1635

His Ser Glu Glu Met Trp Cys Thr Glu Ser His Pro Val Thr Val
1640 1645 1650

Glu Met Phe Asn Thr Pro Glu Lys Pro Tyr Ser Leu Val Pro Glu
1655 1660 1665

Asn Thr Ser Leu Gln Phe Asn Trp Lys Ala Pro Leu Asn Val Asn
1670 1675 1680

Leu Ile Arg Phe Trp Val Glu Leu Gln Lys Trp Lys Tyr Asn Glu
1685 1690 1695

Phe Tyr His Val Lys Thr Ser Cys Ser Gln Gly Pro Ala Tyr Val
1700 1705 1710

Cys Asn Ile Thr Asn Leu Gln Pro Tyr Thr Ser Tyr Asn Val Arg
1715 1720 1725

Val Val Val Val Tyr Lys Thr Gly Glu Asn Ser Thr Ser Leu Pro
1730 1735 1740

Glu Ser Phe Lys Thr Lys Ala Gly Val Pro Asn Lys Pro Gly Ile
1745 1750 1755

Pro Lys Leu Leu Glu Gly Ser Lys Asn Ser Ile Gln Trp Glu Lys
1760 1765 1770

Ala Glu Asp Asn Gly Cys Arg Ile Thr Tyr Tyr Ile Leu Glu Ile
1775 1780 1785

Arg Lys Ser Thr Ser Asn Asn Leu Gln Asn Gln Asn Leu Arg Trp
1790 1795 1800

Lys Met Thr Phe Asn Gly Ser Cys Ser Ser Val Cys Thr Trp Lys
1805 1810 1815

Ser Lys Asn Leu Lys Gly Ile Phe Gln Phe Arg Val Val Ala Ala
1820 1825 1830

Asn Asn Leu Gly Phe Gly Glu Tyr Ser Gly Ile Ser Glu Asn Ile
1835 1840 1845

Ile Leu Val Gly Asp Asp Phe Trp Ile Pro Glu Thr Ser Phe Ile
1850 1855 1860

Leu Thr Ile Ile Val Gly Ile Phe Leu Val Val Thr Ile Pro Leu
1865 1870 1875

Thr Phe Val Trp His Arg Arg Leu Lys Asn Gln Lys Ser Ala Lys
1880 1885 1890

Glu Gly Val Thr Val Leu Ile Asn Glu Asp Lys Glu Leu Ala Glu
1895 1900 1905

Leu Arg Gly Leu Ala Ala Gly Val Gly Leu Ala Asn Ala Cys Tyr
1910 1915 1920

Ala Ile His Thr Leu Pro Thr Gln Glu Glu Ile Glu Asn Leu Pro
1925 1930 1935

Ala Phe Pro Arg Glu Lys Leu Thr Leu Arg Leu Leu Leu Gly Ser

1940 1945 1950

Gly Ala Phe Gly Glu Val Tyr Glu Gly Thr Ala Val Asp Ile Leu
1955 1960 1965

Gly Val Gly Ser Gly Glu Ile Lys Val Ala Val Lys Thr Leu Lys
1970 1975 1980

Lys Gly Ser Thr Asp Gln Glu Lys Ile Glu Phe Leu Lys Glu Ala
1985 1990 1995

His Leu Met Ser Lys Phe Asn His Pro Asn Ile Leu Lys Gln Leu
2000 2005 2010

Gly Val Cys Leu Leu Asn Glu Pro Gln Tyr Ile Ile Leu Glu Leu
2015 2020 2025

Met Glu Gly Gly Asp Leu Leu Thr Tyr Leu Arg Lys Ala Arg Met
2030 2035 2040

Ala Thr Phe Tyr Gly Pro Leu Leu Thr Leu Val Asp Leu Val Asp
2045 2050 2055

Leu Cys Val Asp Ile Ser Lys Gly Cys Val Tyr Leu Glu Arg Met
2060 2065 2070

His Phe Ile His Arg Asp Leu Ala Ala Arg Asn Cys Leu Val Ser
2075 2080 2085

Val Lys Asp Tyr Thr Ser Pro Arg Ile Val Lys Ile Gly Asp Phe
2090 2095 2100

Gly Leu Ala Arg Asp Ile Tyr Lys Asn Asp Tyr Tyr Arg Lys Arg
2105 2110 2115

Gly Glu Gly Leu Leu Pro Val Arg Trp Met Ala Pro Glu Ser Leu
2120 2125 2130

Met Asp Gly Ile Phe Thr Thr Gln Ser Asp Val Trp Ser Phe Gly
2135 2140 2145

Ile Leu Ile Trp Glu Ile Leu Thr Leu Gly His Gln Pro Tyr Pro
2150 2155 2160

Ala His Ser Asn Leu Asp Val Leu Asn Tyr Val Gln Thr Gly Gly
2165 2170 2175

Arg Leu Glu Pro Pro Arg Asn Cys Pro Asp Asp Leu Trp Asn Leu
2180 2185 2190

Met Thr Gln Cys Trp Ala Gln Glu Pro Asp Gln Arg Pro Thr Phe
2195 2200 2205

His Arg Ile Gln Asp Gln Leu Gln Leu Phe Arg Asn Phe Phe Leu
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Asn Ser Ile Tyr Lys Ser Arg Asp Glu Ala Asn Asn Ser Gly Val
2225 2230 2235

Ile Asn Glu Ser Phe Glu Gly Glu Asp Gly Asp Val Ile Cys Leu
2240 2245 2250

Asn Ser Asp Asp Ile Met Pro Val Ala Leu Met Glu Thr Lys Asn
2255 2260 2265

Arg Glu Gly Leu Asn Tyr Met Val Leu Ala Thr Glu Cys Gly Gln
2270 2275 2280

Gly Glu Glu Lys Ser Glu Gly Pro Leu Gly Ser Gln Glu Ser Glu
2285 2290 2295

Ser Cys Gly Leu Arg Lys Glu Glu Lys Glu Pro His Ala Asp Lys
2300 2305 2310

Asp Phe Cys Gln Glu Lys Gln Val Ala Tyr Cys Pro Ser Gly Lys
2315 2320 2325

Pro Glu Gly Leu Asn Tyr Ala Cys Leu Thr His Ser Gly Tyr Gly
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Asp Gly Ser Asp
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35 40 45

Thr Asp Gln Glu Lys Ile Glu Phe Leu Lys Glu Ala His Leu Met Ser
50 55 60

Lys Phe Asn His Pro Asn Ile Leu Lys Gln Leu Gly Val Cys Leu Leu
65 70 75 80

Asn Glu Pro Gln Tyr Ile Ile Leu Glu Leu Met Glu Gly Gly Asp Leu
85 90 95

Leu Thr Tyr Leu Arg Lys Ala Arg Met Ala Thr Phe Tyr Gly Pro Leu
100 105 110

Leu Thr Leu Val Asp Leu Val Asp Leu Cys Val Asp Ile Ser Lys Gly
115 120 125

Cys Val Tyr Leu Glu Arg Met His Phe Ile His Arg Asp Leu Ala Ala
130 135 140

Arg Asn Cys Leu Val Ser Val Lys Asp Tyr Thr Ser Pro Arg Ile Val
145 150 155 160

Lys Ile Gly Asp Phe Gly Leu Ala Arg Asp Ile Tyr Lys Asn Asp Tyr
165 170 175

Tyr Arg Lys Arg Gly Glu Gly Leu Leu Pro Val Arg Trp Met Ala Pro
180 185 190

Glu Ser Leu Met Asp Gly Ile Phe Thr Thr Gln Ser Asp Val Trp Ser
195 200 205

Phe Gly Ile Leu Ile Trp Glu Ile Leu Thr Leu Gly His Gln Pro Tyr
210 215 220

Pro Ala His Ser Asn Leu Asp Val Leu Asn Tyr Val Gln Thr Gly Gly
225 230 235 240

Arg Leu Glu Pro Pro Arg Asn Cys Pro Asp Asp Leu Trp Asn Leu Met
245 250 255

Thr Gln Cys Trp Ala Gln Glu Pro Asp Gln Arg Pro Thr Phe His Arg
260 265 270

Ile Gln Asp Gln
275