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(54) Title: USE OF A PD-1 ANTAGONIST AND A RAF INHIBITOR IN THE TREATMENT OF CANCER

(57) Abstract: The present disclosure relates to methods and kits for the treatment of cancer. In particular, the disclosure provides methods for treatment of cancers by administering a PD-1 antagonist such as pembrolizumab, nivolumab, atezolizumab, durvalumab and avelumab in combination with a pan-RAF inhibitor such as (R)-2-(1 -(6-amino-5-chloropyrimidine-4- carboxamide)ethyl)-N-(5-chloro-4-(trifluoromethyl)pyridin-2-yl)thiazole-5-carboxamide and N-{7-cyano-6-[4-fluoro-3-({[3- (trifluoromethyl)-phenyl]acetyl}amino)phenoxy]-1,3-benzothiazol-2-yl}cyclopropanecarboxamide.

USE OF A PD-1 ANTAGONIST AND A RAF INHIBITOR IN THE TREATMENT OF CANCER

FIELD

[0001] The present disclosure relates to combination therapies useful for the treatment of cancer. In particular, the disclosure relates to a combination therapy which comprises an antagonist of a Programmed Death 1 protein (PD-1) and a pan-RAF inhibitor.

SEQUENCE LISTING

[0002] This application contains a Sequence Listing which is submitted herewith in electronically readable format. The electronic Sequence Listing file was created on March 21, 2016, is named sequencelisting.txt and has a size of 30 kb. The entire contents of the Sequence Listing in the electronic sequencelisting.txt file are incorporated herein by this reference.

[0003] Programmed cell death 1 (PD-1) is a negative costimulatory receptor expressed primarily on the surface of activated T-cells. PD-1, when engaged by the ligand PD-L1, triggers inhibitory signaling in T-cells. PD-L1 can be constitutively expressed on the surface of cancer cells or can be expressed in response to T cells producing immune-stimulating cytokines such as interferons. PDL-1 can also be expressed on antigen presenting cells (APC) and may have an indirect effect on tumor cells via the T cell. Nivolumab is an anti-PD-1 monoclonal antibody (mAb) that disrupts the PD-1 / PD-L1 interaction with resultant tumor recognition by cytotoxic T cells. Nivolumab has demonstrated long term benefit to a clinically significant but infrequent number of patients with treatment naïve and relapsed/refractory metastatic melanoma. While tumor PD-L1 expression is associated with improved outcomes, benefit was also observed in a subset of patients whose disease was PD-L1 negative/indeterminate. Preliminary studies would indicate that the tumor microenvironment plays a significant role in determining resistance, response, and duration of response to immune checkpoint inhibitor monotherapy. One strategy to increase the long term benefit of immunotherapy to patients would be to focus on the tumor microenvironment role in 1) blocking effector functions (such as antigen specific T-cell recognition and homing to tumor) and 2) reducing T-cell killing capacity.

[0004] Compound A is a potent, small molecule MAPK pathway inhibitor currently being developed for the treatment of solid tumors, both as a single agent and in combination with other agents. As a pan-RAF inhibitor, Compound A has the ability to inhibit both RAF monomer- and dimer-mediated signaling, which is a key feature that distinguishes it from approved BRAF inhibitors (vemurafenib and dabrafenib).

[0005] Though there have been many advances in the treatment of cancer, there remains a need for more effective and/or enhanced treatment of people suffering from the effects of cancer.

BRIEF DESCRIPTION OF THE DRAWINGS

[0006] Figure 1 is a bar graph that shows tumor immunophenotyping results from a CT26 syngeneic mouse model (Example 1).

[0007] Figure 2 is a bar graph that shows MDSC (myeloid-derived suppressor cells) and TAM (tumor associated macrophages) immunophenotyping results of CD11b+Lin cells from a CT26 syngeneic mouse model (Example 1).

DETAILED DESCRIPTION

[0008] The present disclosure relates to new combination therapies for the treatment of cancer. In particular, the present disclosure relates to methods and compositions for treating a subject suffering from a cancer, comprising administering to the subject a pan-RAF inhibitor or a pharmaceutically acceptable salt thereof; and a Programmed Death 1 Protein (PD-1) antagonist; wherein the amount of the pan-RAF inhibitor and PD-1 antagonist is such that the combination thereof is therapeutically effective in the treatment of a cancer. The present disclosure relates to a pan-RAF inhibitor, such as Compound A, having a beneficial effect on the tumor microenvironment by increasing cytotoxic T cell infiltration into the tumor, such effect enhances the efficacy of PD-1 checkpoint blockade in RAF and RAS mutant cancers. It is believed that the combinations of the disclosure utilizing a pan-RAF inhibitor have

advantages over each therapeutic agent administered alone and other combinations involving PD-1 antagonists and other MAPK pathway inhibitors, such as BRAF and MEK inhibitors. Combinations with other MAPK pathway inhibitors, such as BRAF and MEK inhibitors, can suffer from overlapping/additive toxicities as well as drug-mediated CRAF re-activation of the MAPK pathway, which may lead to reduced therapeutic benefit. In contrast, a pan-RAF inhibitor, such as Compound A with its long half-life, may overcome the biological and pharmacological limitations of these other combinations. Thus, a pan-RAF inhibitor, such as Compound A in combination with a PD-1 antagonist can provide a superior clinical benefit with a better safety profile.

[0009] So that the disclosure may be more readily understood, certain technical and scientific terms are specifically defined below. Unless specifically defined elsewhere in this document, all other technical and scientific terms used herein have the meaning commonly understood by one of ordinary skill in the art.

[0010] As used herein, including the claims, the singular forms of words such as "a," "an," and "the," include their corresponding plural references unless the context clearly dictates otherwise.

[0011] The term "subject" includes any organism, preferably an animal, more preferably a mammal (e.g., rat, mouse, dog, cat, rabbit) and most preferably a human.

[0012] As used herein, the term "antibody" refers to any form of antibody that exhibits the desired biological or binding activity. Thus, it is used in the broadest sense and specifically covers, but is not limited to, monoclonal antibodies (including full length monoclonal antibodies), polyclonal antibodies, multispecific antibodies (e.g., bispecific antibodies), humanized, fully human antibodies, chimeric antibodies and camelized single domain antibodies. "Parental antibodies" are antibodies obtained by exposure of an immune system to an antigen prior to modification of the antibodies for an intended use, such as humanization of an antibody for use as a human therapeutic.

[0013] In general, the basic antibody structural unit comprises a tetramer. Each tetramer includes two identical pairs of polypeptide chains, each pair having one "light" (about 25 kDa) and one "heavy" chain (about 50-70 kDa). The amino-terminal portion of each chain includes a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The carboxy-terminal portion of the heavy chain may define a constant region

primarily responsible for effector function. Typically, human light chains are classified as kappa and lambda light chains. Furthermore, human heavy chains are typically classified as mu, delta, gamma, alpha, or epsilon, and define the antibody's isotype as IgM, IgD, IgG, IgA, and IgE, respectively. Within light and heavy chains, the variable and constant regions are joined by a "J" region of about 12 or more amino acids, with the heavy chain also including a "D" region of about 10 more amino acids. See generally, Fundamental Immunology Ch. 7 (Paul, W., ed., 2nd ed. Raven Press, N.Y. (1989).

[0014] The variable regions of each light/heavy chain pair form the antibody binding site. Thus, in general, an intact antibody has two binding sites. Except in bifunctional or bispecific antibodies, the two binding sites are, in general, the same.

[0015] Typically, the variable domains of both the heavy and light chains comprise three hypervariable regions, also called complementarity determining regions (CDRs), which are located within relatively conserved framework regions (FR). The CDRs are usually aligned by the framework regions, enabling binding to a specific epitope. In general, from N-terminal to C- terminal, both light and heavy chains variable domains comprise FR1, CDR1, FR2, CDR2, FR3, CDR3 and FR4. The assignment of amino acids to each domain is, generally, in accordance with the definitions of Sequences of Proteins of Immunological Interest, Kabat, *et al.;* National Institutes of Health, Bethesda, Md. ; 5th ed.; NIH Publ. No. 91-3242 (1991); Kabat (1978) Adv. Prot. Chem. 32: 1-75; Kabat, *et al*, (1977) J. Biol. Chem. 252:6609-6616; Chothia, et al, (1987) J Mol. Biol. 196:901-917 or Chothia, et al, (1989) Nature 342:878-883.

[0016] As used herein, the term "hypervariable region" refers to the amino acid residues of an antibody that are responsible for antigen-binding. The hypervariable region comprises amino acid residues from a "complementarity determining region" or "CDR" (i.e. CDRL1, CDRL2 and CDRL3 in the light chain variable domain and CDRH1, CDRH2 and CDRH3 in the heavy chain variable domain). See Kabat *et al.* (1991) Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (defining the CDR regions of an antibody by sequence); see also Chothia and Lesk (1987) J. Mol. Biol. 196: 901-917 (defining the CDR regions of an antibody by structure). As used herein, the term "framework" or "FR" residues refers to those variable domain residues other than the hypervariable region residues defined herein as CDR residues.

[0017] As used herein, unless otherwise indicated, "antibody fragment" or "antigen

binding fragment" refers to antigen binding fragments of antibodies, i.e. antibody fragments that retain the ability to bind specifically to the antigen bound by the full-length antibody, e.g. fragments that retain one or more CDR regions. Examples of antibody binding fragments include, but are not limited to, Fab, Fab', $F(ab')_2$, and Fv fragments; diabodies; linear antibodies; single-chain antibody molecules, e.g., sc-Fv; nanobodies and multispecific antibodies formed from antibody fragments.

[0018] An antibody that "specifically binds to" a specified target protein is an antibody that exhibits preferential binding to that target as compared to other proteins, but this specificity does not require absolute binding specificity. An antibody is considered "specific" for its intended target if its binding is determinative of the presence of the target protein in a sample, e.g. without producing undesired results such as false positives. Antibodies, or binding fragments thereof, useful in the present disclosure, will bind to the target protein with an affinity that is at least two fold greater, preferably at least ten times greater, more preferably at least 20- times greater, and most preferably at least 100-times greater than the affinity with non-target proteins. As used herein, an antibody is said to bind specifically to a polypeptide comprising a given amino acid sequence, e.g. the amino acid sequence of a mature human PD-1, or human PD- LI, if it binds to polypeptides comprising that sequence but does not bind to proteins lacking that sequence.

[0019] "Chimeric antibody" refers to an antibody in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in an antibody derived from a particular species (e.g., human) or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in an antibody derived from another species (e.g., mouse) or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity.

[0020] "Human antibody" refers to an antibody that comprises human immunoglobulin protein sequences only. A human antibody may contain murine carbohydrate chains if produced in a mouse, in a mouse cell, or in a hybridoma derived from a mouse cell. Similarly, "mouse antibody" or "rat antibody" refer to an antibody that comprises only mouse or rat immunoglobulin sequences, respectively.

[0021] "Humanized antibody" refers to forms of antibodies that contain sequences from

non-human (e.g., murine) antibodies as well as human antibodies. Such antibodies contain minimal sequence derived from non-human immunoglobulin. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the hypervariable loops correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin sequence. The humanized antibody optionally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. The prefix "hum", "hu" or "h" is added to antibody clone designations when necessary to distinguish humanized antibodies from parental rodent antibodies. The humanized forms of rodent antibodies will generally comprise the same CDR sequences of the parental rodent antibodies, although certain amino acid substitutions may be included to increase affinity, increase stability of the humanized antibody, or for other reasons.

[0022] The terms "cancer", "cancerous", or "malignant" refer to or describe the physiological condition in mammals that is typically characterized by unregulated cell growth. Examples of cancer include but are not limited to, skin cancer, ocular cancer, gastrointestinal cancer, thyroid cancer, breast cancer, ovarian cancer, central nervous system cancer, laryngeal cancer, cervical cancer, lymphatic system cancer, genitourinary tract cancer, bone cancer, biliary tract cancer, endometrial cancer, liver cancer, lung cancer, prostate cancer, pancreatic cancer, and colon cancer. In some embodiments, lung cancer includes non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC). In some embodiments, the cancer is not NSCLC. In some embodiments, the cancer is selected from skin cancer, ocular cancer, gastrointestinal cancer, thyroid cancer, breast cancer, ovarian cancer, brain cancer, laryngeal cancer, cervical cancer, lymphatic system cancer, genitourinary tract cancer, bone cancer, biliary tract cancer, endometrial cancer, uterine cancer, liver cancer, lung cancer, prostate cancer and colon cancer.

In some embodiments, the cancer is a hematological malignancy. In some embodiments, the hematological malignancy is selected from acute myelogenous leukemia (AML), chronic myelogenous leukemia (CML), chronic lymphoblastic leukemia (CLL), myelodysplastic syndrome, B-cell lymphoma, non-Hodgkin's lymphoma (NHL) and Hodgkin's lymphoma. In some embodiments, the cancer is NHL. In some embodiments, the NHL is selected from diffused large B cell lymphoma, follicular lymphoma. and multiple myeloma.

[0023] In some embodiments, the cancer is skin cancer. In some embodiments, the skin cancer is melanoma.

[0024] In some embodiments, the cancer is lung cancer. In some embodiments, the cancer is non-small cell lung cancer (NSCLC). In some embodiments, the cancer is squamous NSCLC. In some embodiments, the cancer is non-squamous NSCLC.

[0025] In some embodiments, the cancer is gastrointestinal cancer. As used herein, "gastrointestinal cancer" includes cancer of the esophagus, stomach (also known as gastric cancer), biliary system, pancreas, small intestine, large intestine, rectum and anus). In some embodiments, the gastrointestinal cancer is adenocarcinoma of the esophagus, adenocarcinoma of the gastroesophageal junction or adenocarcinoma of the stomach. In some embodiments, the gastrointestinal cancer is stomach cancer.

[0026] In some embodiments, the cancer is colon cancer. Colon cancer is also known as colorectal (CRC), bowel, or rectum cancer.

[0027] In some embodiments, the cancer is a central nervous system cancer. In some embodiments, the central nervous system cancer is brain cancer.

[0028] In some embodiments, thyroid cancer is thyroid carcinoma.

[0029] In some embodiments, genitourinary tract cancer is bladder cancer.

[0030] In some embodiments, the hematogical malignancy is selected from acute myelogenous leukemia (AML) and chronic lymphocytic leukemia (CLL).

[0031] In some embodiments, the combinations described herein are used to treat a genetically defined subset of one or more of the cancers described above. In some embodiments, the cancer is BRAF, NRAS, or KRAS mutation positive cancer.

[0032] As used herein, "BRAF" or "BRAF" refers to B-Raf proto-oncogene, serine/threonine kinase, the gene associated with the mRNA sequence assigned as GenBank Accession No. NM_004333, SEQ ID NO:1 (open reading frame is SEQ ID NO:2, nucleotides 62 to 2362 of SEQ ID NO:1), encoding GenPept Accession No. NP_004324, SEQ ID NO:3). Other names for BRAF include rafB1 and Noonan Syndrome 7 (NS7). BRAF functions as a serine/threonine kinase, has a role in regulating the MAP kinase/ERKs signaling pathway and can be found on chromosome 7q.

[0033] As used herein, "KRAS" or "K-Ras" refers to v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog, the gene associated with the mRNA sequence assigned as GenBank

Accession No. NM_004985, SEQ ID NO:4 (open reading frame is SEQ ID NO:5, nucleotides 193 to 759 of SEQ ID NO:4), encoding GenPept Accession No. NP_004976, SEQ ID NO:6, the predominant transcript variant of K-Ras gene on chromosome 12. Other names for K-Ras include KRAS2, and Noonan Syndrome 3 (NS3). K-Ras functions as an oncogene with GTPase activity and can be found on chromosome 12. K-Ras interacts with the cell membrane and various effector proteins, such as Akt and Cdc42, which carry out its signaling function through the cytoskeleton and effects on cell motility (Fotiadou et al. (2007) Mol. Cel. Biol. 27:6742-6755).

[0034] As used herein, "NRAS" or "N-Ras" refers to neuroblastoma RAS viral (v-ras) oncogene homolog, the gene associated with the mRNA sequence assigned as GenBank Accession No. NM_002524, SEQ ID NO:7 (open reading frame is SEQ ID NO:8, nucleotides 255 to 824 of SEQ ID NO:7), encoding GenPept Accession No. NP_002515, SEQ ID NO:9). Other names for N-Ras include Autoimmune Lymphoproliferative Syndrome type IV (ALPS4), NRAS1, and Noonan Syndrome 6 (NS6). N-Ras functions as an oncogene with GTPase activity and can be found on chromosome 1p. NRAS interacts with the cell membrane and various effector proteins, such as RAF and RhoA, which carry out its signaling function through the cytoskeleton and effects on cell adhesion (Fotiadou et al. (2007) Mol. Cel. Biol. 27:6742-6755).

[0035] In some embodiments, the cancer is a RAS mutation positive cancer. RAS mutations include NRAS and KRAS. In some embodiments, the RAS mutation positive cancer is pancreatic cancer, colorectal cancer, non-small cell lung cancer, AML/myeloma or melanoma. In some embodiments, the RAS mutation positive cancer is pancreatic cancer. In some embodiments, the RAS mutation positive cancer is colorectal cancer. In some embodiments, the RAS mutation positive cancer is non-small cell lung cancer. In some embodiments, the RAS mutation positive cancer is AML/myeloma. In some embodiments, the RAS mutation positive cancer is melanoma.

[0036] In some embodiments, the cancer is KRAS mutation positive cancer. In some embodiments, the KRAS mutation positive cancer is pancreatic cancer, colorectal cancer, non-small cell lung cancer, AML/myeloma or melanoma. In some embodiments, the KRAS mutation positive cancer is pancreatic cancer. In some embodiments, the KRAS mutation positive cancer is colorectal cancer. In some embodiments, the KRAS mutation positive cancer

is non-small cell lung cancer. In some embodiments, the KRAS mutation positive cancer is AML/myeloma. In some embodiments, the KRAS mutation positive cancer is melanoma.

[0037] In some embodiments, the cancer is a KRAS exon 2 mutation positive cancer. In some embodiments, one or more of the KRAS exon 2 mutation is in codon 12 or codon 13. In some embodiments, the KRAS exon 2 mutation is in codon 12. In some embodiments, the KRAS exon 2 mutation is in codon 13.

[0038] In some embodiments, the cancer is a KRAS non-exon 2 mutation positive cancer. In some embodiments, the cancer is a KRAS exon 3 or exon 4 mutation positive cancer. In some embodiments, the cancer is a KRAS exon 3 mutation positive cancer. In some embodiments, the KRAS exon 3 mutation is in codon 61. In some embodiments, the cancer is a KRAS exon 4 mutation positive cancer. In some embodiments, the KRAS exon 4 mutation is in codon 117 or codon 146. In some embodiments, the KRAS exon 4 mutation is in codon 117. In some embodiments, the KRAS exon 4 mutation is in codon 146.

[0039] In some embodiments, the cancer is a BRAF mutation positive cancer. In some embodiments, the BRAF mutation positive cancer is melanoma, colorectal cancer, non-small cell lung cancer, or AML/myeloma. In some embodiments, the BRAF mutation positive cancer is melanoma. In some embodiments, the BRAF mutation positive cancer is colorectal. In some embodiments, the BRAF mutation positive cancer is non-small cell lung cancer. In some embodiments, the BRAF mutation positive cancer is AML/myeloma.

[0040] In some embodiments, the cancer is a BRAF V-600 mutation positive cancer. In some embodiments, the BRAF mutation is V600E. In some embodiments, the BRAF mutation is V600G. In some embodiments, the BRAF mutation is V600A. In some embodiments, the BRAF mutation is V600K. In some embodiments, the BRAF mutation is V600M. In some embodiments, the BRAF mutation is V600R. In some embodiments, the BRAF mutation is V600 K.

[0041] In some embodiments, the cancer is an NRAS mutation positive cancer. In some embodiments, the NRAS mutation positive cancer is pancreatic cancer, colorectal cancer, non-small cell lung cancer, AML/myeloma or melanoma. In some embodiments, the NRAS mutation positive cancer is pancreatic cancer. In some embodiments, the NRAS mutation positive cancer is colorectal cancer. In some embodiments, the NRAS mutation positive cancer is non-small cell lung cancer. In some embodiments, the NRAS mutation positive cancer is

AML/myeloma. In some embodiments, the NRAS mutation positive cancer is melanoma.

[0042] In some embodiments, the cancer is an NRAS exon 2, exon 3, or exon 4 mutation positive cancer.

[0043] In some embodiments, the cancer is an NRAS non-exon 2 mutation positive cancer. In some embodiments, the cancer is an NRAS exon 3 mutation positive cancer. In some embodiments, one or more of the NRAS exon 3 mutations is in codon 59 or codon 61. In some embodiments, the NRAS exon 3 mutation is in codon 59. In some embodiments, the NRAS mutation is in codon 61. In some embodiments, the colorectal cancer is an NRAS exon 4 mutation positive colorectal cancer. In some embodiments, the one or more of the NRAS exon 4 mutations is in codon 117 or codon 146. In some embodiments, the NRAS exon 4 mutation is in codon 117. In some embodiments, the NRAS exon 4 mutation is in codon 146.

[0044] In some embodiments, the cancer is an NRAS exon 2 mutation positive cancer. In some embodiments, the one or more of the NRAS exon 2 mutations is in codon 12 or codon 13. In some embodiments, the NRAS exon 2 mutation is in codon 12. In some embodiments, that NRAS exon 13.

[0045] In some embodiments, the cancer is relapsed, refractory, or advanced cancer. In some embodiments, the cancer is refractory. In one aspect, refractory cancer does not respond to treatment; it is also known as resistant cancer. In some embodiments, the tumor is unresectable. In one aspect, an unresectable tumor is unable to be removed by surgery. In some embodiments, the cancer has not been previously treated. In some embodiments, the cancer is locally advanced. In one aspect, "locally advanced" refers to a cancer that is somewhat extensive but still confined to one area. In some instances, "locally advanced" can refer to a small tumor that hasn't spread but has invaded nearby organs or tissues that make it difficult to remove with surgery alone. In some embodiments, the cancer is metastatic. In one aspect, metastatic cancer is a cancer that has spread from the part of the body where it started (the primary site) to other parts of the body.

[0046] In some embodiments, the cancer that may be treated in accordance with the disclosed combination includes one or more cancers characterized by elevated expression of PD-1, PD-L1, and/or PD-L2 in tested tissue samples. In some embodiments, the cancer that may be treated in accordance with the disclosed combination includes one or more cancers characterized by decreased expression of PD-1, PD-L1, and/or PD-L2 in tested tissue samples.

In some embodiments, the cancer that may be treated in accordance with the disclosed combination is PD-L1 positive. In some embodiments, the cancer that may be treated in accordance with the disclosed combination is PD-L1 negative.

[0047] "Biotherapeutic agent" means a biological molecule, such as an antibody or fusion protein, that blocks ligand / receptor signaling in any biological pathway that supports tumor maintenance and/or growth or suppresses the anti-tumor immune response.

[0048] "CDR" or "CDRs" as used herein means complementarity determining region(s) in a immunoglobulin variable region, defined using the Kabat numbering system, unless otherwise indicated.

[0049] "Chemotherapeutic agent" is a chemical compound useful in the treatment of cancer. Classes of chemotherapeutic agents include, but are not limited to: alkylating agents, antimetabolites, kinase inhibitors, spindle poison plant alkaloids, cytoxic/antitumor antibiotics, topisomerase inhibitors, photosensitizers, anti-estrogens and selective estrogen receptor modulators (SERMs), anti-progesterones,estrogen receptor down-regulators (ERDs), estrogen receptor antagonists, leutinizing hormone-releasing hormone agonists, anti-androgens, aromatase inhibitors, EGFR inhibitors, VEGF inhibitors, anti-sense oligonucleotides that that inhibit expression of genes implicated in abnormal cell proliferation or tumor growth. Chemotherapeutic agents useful in the treatment methods of the present disclosure include cytostatic and/or cytotoxic agents.

[0050] "Clothia" as used herein means an antibody numbering system described in Al-Lazikani et al, JMB 273:927-948 (1997).

[0051] "Conservatively modified variants" or "conservative substitution" refers to substitutions of amino acids in a protein with other amino acids having similar characteristics (e.g. charge, side-chain size, hydrophobicity/hydrophilicity, backbone conformation and rigidity, etc.), such that the changes can frequently be made without altering the biological activity or other desired property of the protein, such as antigen affinity and/or specificity. Those of skill in this art recognize that, in general, single amino acid substitutions in non-essential regions of a polypeptide do not substantially alter biological activity (see, e.g., Watson *et al.* (1987) Molecular Biology of the Gene, The Benjamin/Cummings Pub. Co., p. 224 (4th Ed.)). In addition, substitutions of structurally or functionally similar amino acids are less likely to disrupt biological activity.

[0052] "Consists essentially of," and variations such as "consist essentially of or "consisting essentially of," as used throughout the specification and claims, indicate the inclusion of any recited elements or group of elements, and the optional inclusion of other elements, of similar or different nature than the recited elements, that do not materially change the basic or novel properties of the specified dosage regimen, method, or composition. As a non-limiting example, a PD-1 antagonist that consists essentially of a recited amino acid sequence may also include one or more amino acids, including substitutions of one or more amino acid residues, which do not materially affect the properties of the binding compound.

[0053] The term "about" is used herein to mean approximately, in the region of, roughly, or around. When the term "about" is used in conjunction with a numerical range, it modifies that range by extending the boundaries above and below the numerical values set forth. In general, the term "about" is used herein to modify a numerical value above and below the stated value by a variance of 10%.

[0054] As used herein, the term "comprises" means "includes, but is not limited to."

[0055] As used herein, the term "Raf kinase" or "RAF kinase" refers to any one of a family of serine/threonine-protein kinases. The family consists of three isoform members (BRAF, C-Raf (Raf-1), and A-Raf). Raf protein kinases are involved in the MAPK signaling pathway consisting of a kinase cascade that relays extracellular signals to the nucleus to regulate gene expression and key cellular functions. Unless otherwise indicated by context, the term "Raf kinase" or "RAF kinase" is meant to refer to any Raf kinase protein from any species, including, without limitation. In one aspect, the Raf kinase is a human Raf kinase.

[0056] The term "Raf inhibitor", "RAF inhibitor", "inhibitor of Raf" "inhibitor of RAF", "RAF kinase inhibitor" or "Raf kinase inhibitor" is used to signify a compound which is capable of interacting with one or more isoform members (BRAF, C-Raf (Raf-1) and/or A-Raf) of the serine/threonine-protein kinase, Raf including mutant forms. Some examples of BRAF mutant forms include BRAF V600E, BRAF V600D, and BRAF V600K.

[0057] In some embodiments, the one or more isoforms of RAF protein kinase is at least about 50% inhibited, at least about 75% inhibited, at least about 90% inhibited, at least about 95% inhibited, at least about 98% inhibited, or at least about 99% inhibited. In some embodiments, the concentration of RAF kinase inhibitor required to reduce RAF kinase activity

by 50% is less than about 1 μM, less than about 500 nM, less than about 100 nM, less than about 50 nM, less than about 25 nM, less than about 10 nM, less than about 5 nM, or less than about 1 nM.

[0058] In some embodiments, such inhibition is selective for one or more Raf isoforms, i.e., the Raf inhibitor or a pharmaceutically acceptable salt thereof is selective for one or more of BRAF (wild type), mutant BRAF, A-Raf, and C-Raf kinase. In some embodiments, the Raf inhibitor or a pharmaceutically acceptable salt thereof is selective for BRAF (wild type), BRAF V600E, A-Raf and C-Raf. In some embodiments, the Raf inhibitor or a pharmaceutically acceptable salt thereof is selective for BRAF (wild type), BRAF V600E, A-Raf and C-Raf. In some embodiments, the Raf inhibitor or a pharmaceutically acceptable salt thereof is selective for BRAF (wild type), BRAF V600D, A-Raf and C-Raf. In some embodiments, the Raf inhibitor or a pharmaceutically acceptable salt thereof is selective for BRAF (wild type), BRAF V600K, and C-Raf. In some embodiments, the Raf inhibitor or a pharmaceutically acceptable salt thereof is selective for more than BRAF V600. In some embodiments, the Raf inhibitor or a pharmaceutically acceptable salt thereof is selective for more than BRAF V600E.

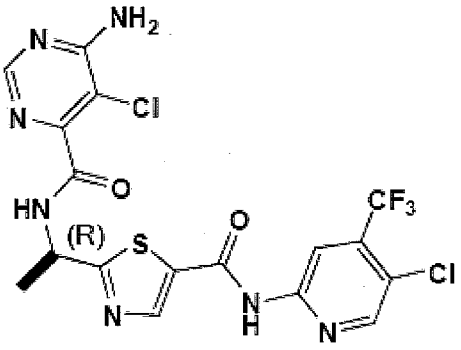
[0059] In some embodiments, the Raf inhibitor or a pharmaceutically acceptable salt thereof is selective for BRAF and C-Raf kinases. In some embodiments, the Raf inhibitor or a pharmaceutically acceptable salt thereof is selective for BRAF (wild type), BRAF V600E and C-Raf. In some embodiments, the Raf inhibitor or a pharmaceutically acceptable salt thereof is selective for BRAF (wild type), BRAF V600D and C-Raf. In some embodiments, the Raf inhibitor or a pharmaceutically acceptable salt thereof is selective for BRAF (wild type), BRAF V600K and C-Raf. In some embodiments, the Raf inhibitor or a pharmaceutically acceptable salt thereof is selective for mutant BRAF. In some embodiments, the Raf inhibitor or a pharmaceutically acceptable salt thereof is selective for mutant BRAF V600E. In some embodiments, the Raf inhibitor or a pharmaceutically acceptable salt thereof is selective for mutant BRAF V600D. In some embodiments, the Raf inhibitor or a pharmaceutically acceptable salt thereof is selective for mutant BRAF V600K.

[0060] The term "wild type" refers to a polypeptide or polynucleotide sequence that occurs in a native population without genetic modification. As it is used, a "mutant" includes a polypeptide or polynucleotide having at least one modification to an amino acid or nucleic acid compared to the corresponding amino acid or nucleic acid found in a wild type polypeptide or

polynucleotide. Included in the term mutant is Single Nucleotide Polymorphism (SNP) where a single base pair distinction exists in the sequence of a nucleic acid strand compared to the most prevalently found (wild type) nucleic acid strand.

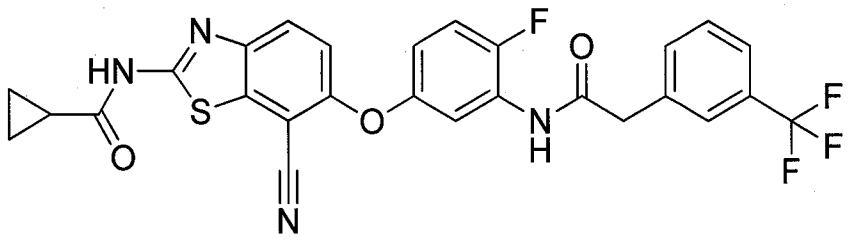
[0061] The term "pan-RAF inhibitor" refers to a Raf inhibitor that inhibits more Raf kinases than BRAF (wild type) and/or the mutant isoform BRAF V600. In some embodiments, the pan-RAF inhibitor is selected from (R)-2-(1-(6-amino-5-chloropyrimidine-4-carboxamide)ethyl)-N-(5-chloro-4-(trifluoromethyl)pyridin-2-yl)thiazole-5-carboxamide (Compound A) , N-{7-cyano-6-[4-fluoro-3-({[3-(trifluoromethyl)-phenyl] -acetyl}-amino) -phenoxy]-1,3-benzothiazol-2-yl}cyclopropanecarboxamide (Compound B) or a pharmaceutically acceptable salt thereof.

[0062] In some embodiments, the pan-RAF inhibitor is (R)-2-(1-(6-amino-5-chloropyrimidine-4-carboxamide)ethyl)-N-(5-chloro-4-(trifluoromethyl)pyridin-2-yl)thiazole-5-carboxamide (Compound A) or a pharmaceutically acceptable salt thereof:



(Compound A). Compound A is described in WO 2009/006389 and WO 2015/148828.

[0063] In some embodiments, the pan-RAF inhibitor is N-{7-cyano-6-[4-fluoro-3-({[3-(trifluoromethyl)-phenyl] -acetyl}-amino) -phenoxy]-1,3-benzothiazol-2-yl}cyclopropanecarboxamide (Compound B) or a pharmaceutically acceptable salt thereof;



(Compound B). Compound B is described in WO 2010/064722. Pan-RAF inhibitors, such as Compound A and Compound

B, that can inhibit more isoforms of Raf kinase proteins than BRAF V600 have the ability to inhibit both Raf monomer and dimer-mediated signaling, which is a key feature that distinguishes these pan-Raf inhibitors from recently approved BRAF specific inhibitors (vemurafenib and dabrafenib).

[0064] As used herein, the term "pharmaceutically acceptable salt" refers to those salts which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of humans and lower animals without undue toxicity, irritation, allergic response and the like, and are commensurate with a reasonable benefit/risk ratio. A "pharmaceutically acceptable salt" means any non-toxic salt of a compound disclosed herein that, upon administration to a recipient, is capable of providing, either directly or indirectly, the compound or an active metabolite or residue thereof.

[0065] Pharmaceutically acceptable salts are well known in the art. For example, S. M. Berge et al., describe pharmaceutically acceptable salts in detail in J. Pharmaceutical Sciences, 1977, 66, 1-19, incorporated herein by reference. Pharmaceutically acceptable salts of compounds described herein include those derived from suitable inorganic and organic acids and bases. Examples of pharmaceutically acceptable, nontoxic acid addition salts are salts of an amino group formed with inorganic acids such as hydrochloric acid, hydrobromic acid, phosphoric acid, sulfuric acid and perchloric acid or with organic acids such as acetic acid, oxalic acid, maleic acid, tartaric acid, citric acid, succinic acid or malonic acid or by using other methods used in the art such as ion exchange. Other pharmaceutically acceptable salts include adipate, alginate, ascorbate, aspartate, benzenesulfonate, benzoate, bisulfate, borate, butyrate, camphorate, camphorsulfonate, citrate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, formate, fumarate, glucoheptonate, glycerophosphate, gluconate, hemisulfate, heptanoate, hexanoate, hydroiodide, 2-hydroxy-ethanesulfonate, lactobionate, lactate, laurate, lauryl sulfate, malate, maleate, malonate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, oleate, oxalate, palmitate, pamoate, pectinate, persulfate, 3-phenylpropionate, phosphate, picrate, pivalate, propionate, stearate, succinate, sulfate, tartrate, thiocyanate, p-toluenesulfonate, undecanoate, valerate salts, and the like. Salts derived from appropriate bases include alkali metal, alkaline earth metal, ammonium and N+(C1-4alkyl)4 salts. This disclosure also provides the quaternization of any basic nitrogen-containing groups. Water or oil-soluble or dispersable products may be obtained by such quaternization. Representative

alkali or alkaline earth metal salts include sodium, lithium, potassium, calcium, magnesium, and the like. Further pharmaceutically acceptable salts include, when appropriate, nontoxic ammonium, quaternary ammonium, and amine cations formed using counterions such as halide, hydroxide, carboxylate, sulfate, phosphate, nitrate, loweralkyl sulfonate and aryl sulfonate.

[0066] Compounds capable of inhibiting the activity of various RAF kinases beyond BRAF (wild type) and/or the mutant isoform BRAF V600 i.e., pan-RAF inhibitors may be used in the methods of the instant disclosure. In particular, these pan-RAF inhibitors include for example, Compound A, Compound B, compounds disclosed in WO 2009/006389, and US 2013/0252977 (DP-4978/ LY3009120), and including but not limited to compounds RAF-265, ARQ-736, CEP-32496, CCT 196969, CCT 241161, and REDX-04988.

[0067] Pan-RAF inhibitors or a pharmaceutically acceptable salts thereof can be assayed in vitro or in vivo for their ability to bind to and/or inhibit Raf kinases. In vitro assays include biochemical FRET assays to measure the phophorylation of MEK by Raf kinases as a method for quantifying the ability of compounds to inhibit the enzymatic activity of Raf kinases. The compounds also can be assayed for their ability to affect cellular or physiological functions mediated by Raf kinase activity. For example in vitro assays quantitate the amount of phosphor-ERK in colorectal cancer cells. Assays for each of these activities are known in the art.

[0068] "Framework region" or "FR" as used herein means the immunoglobulin variable regions excluding the CDR regions.

[0069] "Homology" refers to sequence similarity between two polypeptide sequences when they are optimally aligned. When a position in both of the two compared sequences is occupied by the same amino acid monomer subunit, e.g., if a position in a light chain CDR of two different Abs is occupied by alanine, then the two Abs are homologous at that position. The percent of homology is the number of homologous positions shared by the two sequences divided by the total number of positions compared x 100. For example, if 8 of 10 of the positions in two sequences are matched or homologous when the sequences are optimally aligned then the two sequences are 80% homologous. Generally, the comparison is made when two sequences are aligned to give maximum percent homology. For example, the comparison can be performed by a BLAST algorithm wherein the parameters of the algorithm are selected

to give the largest match between the respective sequences over the entire length of the respective reference sequences.

[0070] The following references relate to BLAST algorithms often used for sequence analysis: BLAST ALGORITHMS: Altschul, S.F., *et al,* (1990) J. Mol. Biol. 215:403-410; Gish, W., *et al,* (1993) Nature Genet. 3:266-272; Madden, T.L., *et al,* (1996) Meth. Enzymol. 266:131-141; Altschul, S.F., *et al,* (1997) Nucleic Acids Res. 25:3389-3402; Zhang, J., *et al,* (1997) Genome Res. 7:649-656; Wootton, J.C., *et al,* (1993) Comput. Chem. 17: 149-163; Hancock, J.M. *et al,* (1994) Comput. Appl. Biosci. 10:67-70; ALIGNMENT SCORING SYSTEMS: Dayhoff, M.O., *et al,* "A model of evolutionary change in proteins." in Atlas of Protein Sequence and Structure, (1978) vol. 5, suppl. 3. M.O. Dayhoff (ed.), pp. 345-352, Natl. Biomed. Res. Found., Washington, DC; Schwartz, R.M., *et al,* "Matrices for detecting distant relationships." in Atlas of Protein Sequence and Structure, (1978) vol. 5, suppl. 3." M.O. Dayhoff (ed.), pp. 353-358, Natl. Biomed. Res. Found., Washington, DC; Altschul, S.F., (1991) J. Mol. Biol. 219:555-565; States, D.J., *et al,* (1991) Methods 3:66-70; Henikoff, S., *et al,* (1992) Proc. Natl. Acad. Sci. USA 89: 10915-10919; Altschul, S.F., *et al,* (1993) J. Mol. Evol. 36:290-300; ALIGNMENT STATISTICS: Karlin, S., *et al,* (1990) Proc. Natl. Acad. Sci. USA 87:2264-2268; Karlin, S., *et al,* (1993) Proc. Natl. Acad. Sci. USA 90:5873-5877; Dembo, A., *et al,* (1994) Ann. Prob. 22:2022-2039; and Altschul, S.F. "Evaluating the statistical significance of multiple distinct local alignments." in Theoretical and Computational Methods in Genome Research (S. Suhai, ed.), (1997) pp. 1-14, Plenum, New York.

[0071] "Isolated antibody" and "isolated antibody fragment" refers to the purification status and in such context means the named molecule is substantially free of other biological molecules such as nucleic acids, proteins, lipids, carbohydrates, or other material such as cellular debris and growth media. Generally, the term "isolated" is not intended to refer to a complete absence of such material or to an absence of water, buffers, or salts, unless they are present in amounts that substantially interfere with experimental or therapeutic use of the binding compound as described herein.

[0072] "Kabat" as used herein means an immunoglobulin alignment and numbering system pioneered by Elvin A. Kabat ((1991) Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md.).

[0073] "Monoclonal antibody" or "mAb" or "Mab", as used herein, refers to a

population of substantially homogeneous antibodies, i.e., the antibody molecules comprising the population are identical in amino acid sequence except for possible naturally occurring mutations that may be present in minor amounts. In contrast, conventional (polyclonal) antibody preparations typically include a multitude of different antibodies having different amino acid sequences in their variable domains, particularly their CDRs, which are often specific for different epitopes. The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present disclosure may be made by the hybridoma method first described by Kohler *et al.* (1975) Nature 256: 495, or may be made by recombinant DNA methods (see, e.g., U.S. Pat. No. 4,816,567). The "monoclonal antibodies" may also be isolated from phage antibody libraries using the techniques described in Clackson *et al.* (1991) Nature 352: 624-628 and Marks *et al.* (1991) J. Mol. Biol. 222: 581-597, for example. See also Presta (2005) J. Allergy Clin. Immunol. 1 16:731.

[0074] "PD-1 antagonist" means any chemical compound or biological molecule that blocks binding of PD-Ll expressed on a cancer cell or an antigen presenting cell (APC) to PD-1 expressed on an immune cell (T cell, B cell or NKT cell) and could also apply to inhibitors that block binding of PD-L2 expressed on a cancer cell to the immune-cell expressed PD-1. Alternative names or synonyms for PD-1 and its ligands include: PDCD1, PD1, CD279 and SLEB2 for PD-1; PDCD1L1, PDL1, B7H1, B7-4, CD274 and B7-H for PD-Ll; and PDCD1L2, PDL2, B7-DC, Btdc and CD273 for PD-L2. In any of the treatment methods, pharmaceutical compositions, and uses of the present disclosure in which a human subject is being treated, the PD-1 antagonist blocks binding of human PD-Ll to human PD-1, and may block binding of both human PD-Ll and PD-L2 to human PD-1. Human PD-1 amino acid sequences can be found in NCBI Locus No.: NP 005009. Human PD-Ll and PD-L2 amino acid sequences can be found in NCBI Locus No.: NP_054862 and NP_079515, respectively.

[0075] PD-1 antagonists useful in any of the treatment methods, pharmaceutical compositions and uses of the present disclosure include a monoclonal antibody (mAb), or antigen binding fragment thereof, which specifically binds to PD-1 or PD-Ll, and preferably specifically binds to human PD-1 or human PD-Ll. The mAb may be a human antibody, a

humanized antibody or a chimeric antibody, and may include a human constant region. In some embodiments the human constant region is selected from the group consisting of IgGl, IgG2, IgG3 and IgG4 constant regions, and in embodiments, the human constant region is an IgGl or IgG4 constant region. In some embodiments, the antigen binding fragment is selected from the group consisting of Fab, Fab'-SH, F(ab')2, scFv and Fv fragments.

[0076] Examples of mAbs that bind to human PD-1, and useful in the treatment methods, pharmaceutical compositions and uses of the present disclosure, are described in US7521051, US8779105, US8008449, US8900587, US8952136, US8354509, US8735553, US9102728, US8993731, US9102727, US9181342, US8927697, US8945561, US748802, US7322582, US7524498 and US9205148. Specific anti-human PD-1 mAbs useful as the PD-1 antagonist in the treatment methods, pharmaceutical compositions and uses of the present disclosure include, but are not limited to: pembrolizumab (formerly MK-3475 and lambrolizumab), marketed in the USA under the tradename KEYTRUDA®, a humanized IgG4 mAb with the structure described in WHO Drug Information, Vol. 27, No. 2, pages 161-162 (2013); nivolumab (formerly ONO-4538, MDX1106 or BMS-936558), marketed in the USA under the tradename OPDIVO®, a human IgG4 mAb with the structure described in WHO Drug Information, Vol. 27, No. 1, pages 68-69 (2013); and the humanized antibodies h409Al l, h409A16 and h409A17, which are described in WO2008/156712; PDR-100; SHR-1210; REGN-2810; MEDI-0680; BGB-108; and PF-06801591.

[0077] Examples of mAbs that bind to human PD-L1, and useful in the treatment methods, pharmaceutical compositions and uses of the present disclosure, are described in WO2013/019906, W02010/077634 Al and US8383796. Specific anti-human PD-L1 mAbs useful as the PD-1 antagonist in the treatment methods, pharmaceutical compositions and uses of the present disclosure include, but are not limited to atezolizumab (MPDL3280A), BMS-936559, durvalumab (MEDI4736), and avelumab (MSB0010718C).

[0078] Other PD- 1 antagonists useful in the any of the treatment methods, pharmaceutical compositions and uses of the present disclosure include an immunoadhesion that specifically binds to PD-1 or PD-L1, and preferably specifically binds to human PD-1 or human PD-L1, e.g., a fusion protein containing the extracellular or PD-1 binding portion of PD-L1 or PD-L2 fused to a constant region such as an Fc region of an immunoglobulin molecule. Examples of immunoadhesion molecules that specifically bind to PD-1 are described

in WO2010/027827 and WO2011/066342. Specific fusion proteins useful as the PD-1 antagonist in the treatment methods, pharmaceutical compositions and uses of the present disclosure include AMP-224 (also known as B7- DCIg), which is a PD-L2-FC fusion protein and binds to human PD-1.

[0079] In some embodiments, the PD-1 antagonist is selected from nivolumab, pembrolizumab, PDR-001, SHR-1210, AMP-224, REGN-2810, MEDI-0680, BGB-108, PF-06801591, atezolizumab, durvalumab, and BMS-936559, and AMP-224.

[0080] In some embodiments, the PD-1 antagonist is selected from nivolumab, pembrolizumab, PDR-001, SHR-1210, AMP-224, REGN-2810, MEDI-0680, BGB-108, and PF-06801591, and AMP-224.

[0081] In some embodiments, the PD-1 antagonist is selected from pembrolizumab and nivolumab.

[0082] In some embodiments, the PD-1 antagonist is nivolumab.

[0083] In some embodiments, the PD-1 antagonist is pembrolizumab.

[0084] In some embodiments, the PD-1 antagonist is selected from atezolizumab, durvalumab, avelumab and BMS-936559.

[0085] In some embodiments of the treatment methods, pharmaceutical compositions and uses of the present disclosure, the PD-1 antagonist is a monoclonal antibody, or antigen binding fragment thereof, which comprises nivolumab.

[0086] In some embodiments of the treatment methods, pharmaceutical compositions and uses of the present disclosure, the PD-1 antagonist is a monoclonal antibody, or antigen binding fragment thereof, which comprises pembrolizumab.

[0087] In other embodiments of the treatment methods, pharmaceutical compositions and uses of the present disclosure, the PD-1 antagonist is a monoclonal antibody, or antigen binding fragment thereof, which specifically binds to human PD-1 and comprises (a) a heavy chain variable region of an antibody described herein or a variant thereof, and (b) a light chain variable region of an antibody described herein or a variant thereof. A variant of a heavy chain variable region sequence is identical to the reference sequence except having up to seventeen conservative amino acid substitutions in the framework region (i.e., outside of the CDRs), and preferably has less than ten, nine, eight, seven, six or five conservative amino acid substitutions in the framework region. A variant of a light chain variable region sequence is identical to the

reference sequence except having up to five conservative amino acid substitutions in the framework region (i.e., outside of the CDRs), and preferably has less than four, three or two conservative amino acid substitution in the framework region.

[0088] "PD-Ll" or "PD-L2" expression as used herein means any detectable level of expression of the designated PD-L protein on the cell surface or of the designated PD-L mRNA within a cell or tissue. PD-L protein expression may be detected with a diagnostic PD-L antibody in an IHC assay of a tumor tissue section or by flow cytometry. Alternatively, PD-L protein expression by tumor cells may be detected by PET imaging, using a binding agent (e.g., antibody fragment, affibody and the like) that specifically binds to the desired PD-L target, e.g., PD-Ll or PD-L2. Techniques for detecting and measuring PD-L mRNA expression include RT-PCR and realtime quantitative RT-PCR.

[0089] "Tissue Section" refers to a single part or piece of a tissue sample, e.g., a thin slice of tissue cut from a sample of a normal tissue or of a tumor.

[0090] "Treat" or "treating" a cancer as used herein means to administer a combination therapy comprising a PD-1 antagonist and a pan-RAF inhibitor to a subject having a cancer, or diagnosed with a cancer, to achieve at least one positive therapeutic effect, such as for example, reduced number of cancer cells, reduced tumor size, reduced rate of cancer cell infiltration into peripheral organs, or reduced rate of tumor metastasis or tumor growth. Positive therapeutic effects in cancer can be measured in a number of ways (See, W. A. Weber, J. Nucl. Med. 50: 1S- 10S (2009)). For example, with respect to tumor growth inhibition, according to NCI standards, a T/C $\leq$42% is the minimum level of anti-tumor activity. A T/C < 10% is considered a high anti-tumor activity level, with T/C (%) = Median tumor volume of the treated/Median tumor volume of the control x 100. In some embodiments, the treatment achieved by a therapeutically effective amount is any of progression free survival (PFS), disease free survival (DFS) or overall survival (OS). PFS, also referred to as "Time to Tumor Progression" indicates the length of time during and after treatment that the cancer does not grow, and includes the amount of time patients have experienced a complete response or a partial response, as well as the amount of time patients have experienced stable disease. DFS refers to the length of time during and after treatment that the patient remains free of disease. OS refers to a prolongation in life expectancy as compared to naive or untreated subjects or patients. The dosage regimen of a combination therapy described herein that is effective to treat a cancer patient may vary

according to factors such as the disease state, age, and weight of the patient, and the ability of the therapy to elicit an anti-cancer response in the subject. While an embodiment of the treatment method, pharmaceutical compositions and uses of the present disclosure may not be effective in achieving a positive therapeutic effect in every subject, it should do so in a statistically significant number of subjects as determined by any statistical test known in the art such as the Student's t-test, the chi2-test, the U-test according to Mann and Whitney, the Kruskal-Wallis test (H-test), Jonckheere-Terpstra- test and the Wilcoxon-test.

[0091] "Tumor" as it applies to a subject diagnosed with, or suspected of having, a cancer refers to a malignant or potentially malignant neoplasm or tissue mass of any size, and includes primary tumors and secondary neoplasms. A solid tumor is an abnormal growth or mass of tissue that usually does not contain cysts or liquid areas. Different types of solid tumors are named for the type of cells that form them. Examples of solid tumors are sarcomas, carcinomas, and lymphomas. Leukemias (cancers of the blood) generally do not form solid tumors (National Cancer Institute, Dictionary of Cancer Terms).

[0092] "Tumor burden" also referred to as "tumor load", refers to the total amount of tumor material distributed throughout the body. Tumor burden refers to the total number of cancer cells or the total size of tumor(s), throughout the body, including lymph nodes and bone narrow. Tumor burden can be determined by a variety of methods known in the art, such as, e.g. by measuring the dimensions of tumor(s) upon removal from the subject, e.g., using calipers, or while in the body using imaging techniques, e.g., ultrasound, bone scan, computed tomography (CT) or magnetic resonance imaging (MRI) scans.

[0093] The term "tumor size" refers to the total size of the tumor which can be measured as the length and width of a tumor. Tumor size may be determined by a variety of methods known in the art, such as, e.g. by measuring the dimensions of tumor(s) upon removal from the subject, e.g., using calipers, or while in the body using imaging techniques, e.g., bone scan, ultrasound, CT or MRI scans.

[0094] "Variable regions" or "V region" as used herein means the segment of IgG chains which is variable in sequence between different antibodies. It extends to Kabat residue 109 in the light chain and 1 13 in the heavy chain.

[0095] In one aspect, the disclosure provides a method for treating a cancer in a subject comprising administering to the subject a combination therapy which comprises a PD-1

antagonist and a pan-RAF inhibitor.

[0096] The combination therapy may also comprise one or more additional therapeutic agents. In some embodiments, the combination therapy comprises the additional therapeutic agents is vedolizumab and ipilimumab.

[0097] Each therapeutic agent in a combination therapy of the disclosure may be administered either alone or in a pharmaceutical composition which comprises the therapeutic agent and one or more pharmaceutically acceptable carriers, excipients and diluents, according to standard pharmaceutical practice.

[0098] Each therapeutic agent in a combination therapy of the disclosure may be administered simultaneously (i.e., in the same pharmaceutical composition), concurrently (i.e., in separate pharmaceutical compositions administered one right after the other in any order) or sequentially in any order. Sequential administration is particularly useful when the therapeutic agents in the combination therapy are in different dosage forms (one agent is a tablet or capsule and another agent is a sterile liquid) and/or are administered on different dosing schedules.

[0099] In some embodiments, the PD-1 antagonist is administered before administration of the pan-RAF inhibitor, while in other embodiments, the PD-1 antagonist is administered after administration of the pan-RAF inhibitor. In some embodiments, the PD-1 antagonist is administered at least 1 hour after administration of the pan-RAF inhibitor. In some embodiments, nivolumab is administered at least 1 hour after Compound A oral dosing. In some embodiments, the PD-1 antagonist and pan-RAF inhibitor are administered concomitantly.

[00100] In some embodiments, at least one of the therapeutic agents in the combination therapy is administered using the same dosage regimen (dose, frequency and duration of treatment) that is typically employed when the agent is used as monotherapy for treating the same cancer. In other embodiments, the subject receives a lower total amount of at least one of the therapeutic agents in the combination therapy than when the agent is used as monotherapy, e.g., smaller doses, less frequent doses, and/or shorter treatment duration.

[00101] Each therapeutic agent in a combination therapy of the disclosure can be administered orally or parenterally, including the intravenous, intramuscular, intraperitoneal, subcutaneous, rectal, topical, and transdermal routes of administration.

[00102] A combination therapy of the disclosure may be used prior to or following

surgery to remove a tumor and may be used prior to, during or after radiation therapy.

[00103] In some embodiments, a combination therapy of the disclosure is administered to a subject who has not been previously treated with a biotherapeutic or chemotherapeutic agent, i.e., is treatment-naive. In other embodiments, the combination therapy is administered to a subject who failed to achieve a sustained response after prior therapy with a biotherapeutic or chemotherapeutic agent, i.e., is treatment-experienced.

[00104] In some embodiments, a combination therapy of the disclosure is typically used to treat a tumor that is large enough to be found by palpation or by imaging techniques well known in the art, such as MRI, ultrasound, or CAT scan. In some embodiments, a combination therapy of the disclosure is used to treat an advanced stage tumor having dimensions of at least about 200 mm^3 300 mm^3, 400 mm^3, 500 mm^3, 750 mm^3, or up to 1000 mm^3.

[00105] Selecting a dosage regimen (also referred to herein as an administration regimen) for a combination therapy of the disclosure depends on several factors, including the serum or tissue turnover rate of the entity, the level of symptoms, the immunogenicity of the entity, and the accessibility of the target cells, tissue or organ in the subject being treated. Preferably, a dosage regimen maximizes the amount of each therapeutic agent delivered to the subject consistent with an acceptable level of side effects. Accordingly, the dose amount and dosing frequency of each agent in the combination depends in part on the particular therapeutic agent, the severity of the cancer being treated, and subject characteristics. Determination of the appropriate dosage regimen may be made by the clinician, e.g., using parameters or factors known or suspected in the art to affect treatment or predicted to affect treatment, and will depend, for example, on the subject's clinical history (e.g., previous therapy), the type and stage of the cancer to be treated and biomarkers of response to one or more of the therapeutic agents in the combination therapy.

[00106] Biotherapeutic agents in a combination therapy of described in the disclosure may be administered by continuous infusion, or by doses at intervals of, e.g., daily, every other day, three times per week, or one time each week, two weeks, three weeks, monthly, bimonthly, etc. A total weekly dose is generally at least 0.05 μg/kg, 0.2 μg/kg, 0.5 μg/kg, 1 μg/kg, 10 μg/kg, 100 μg/kg, 0.2 mg/kg, 1.0 mg/kg, 2.0 mg/kg, 10 mg/kg, 25 mg/kg, 50 mg/kg body weight or more. See, e.g., Yang *et al.* (2003) New Engl. J. Med. 349:427-434; Herold *et al.* (2002) New Engl. J. Med. 346: 1692-1698; Liu *et al.* (1999) J. Neurol. Neurosurg. Psych.

67:451-456; Portielji *et al.* (20003) Cancer Immunol. Immunother. 52: 133-144.

[00107] In some embodiments that employ an anti-human PD-1 mAb as the PD-1 antagonist in the combination therapy, the dosing regimen will comprise administering the anti-human PD-1 mAb at a dose of 1, 2, 3, 5 or l0mg/kg at intervals of about 14 days (± 2 days) or about 21 days (± 2 days) or about 30 days (± 2 days) throughout the course of treatment.

[00108] In certain embodiments, a subject will be administered an intravenous (IV) infusion of a pharmaceutical composition comprising any of the PD-1 antagonists described herein.

[00109] In one embodiment of the disclosure, the PD-1 antagonist in the combination therapy is nivolumab, which is administered intravenously at a dose selected from the group consisting of: 1 mg/kg Q2W, 2 mg/kg Q2W, 3 mg/kg Q2W, 5 mg/kg Q2W, 10 mg Q2W, 1 mg/kg Q3W, 2 mg/kg Q3W, 3 mg/kg Q3W, 5 mg/kg Q3W, and 10 mg Q3W.

[00110] In another embodiment of the disclosure, the PD-1 antagonist in the combination therapy is pembrolizumab, which is administered intravenously at a dose selected from the group consisting of 1 mg/kg Q2W, 2 mg/kg Q2W, 3 mg/kg Q2W, 5 mg/kg Q2W, 10 mg Q2W, 1 mg/kg Q3W, 2 mg/kg Q3W, 3 mg/kg Q3W, 5 mg/kg Q3W, and 10 mg Q3W. In some embodiments, pembrolizumab is administered intravenously which comprises 25 mg/ml pembrolizumab, 7% (w/v) sucrose, 0.02% (w/v) polysorbate 80 in 10 mM histidine buffer pH 5.5, and the selected dose of the pharmaceutical composition is administered by IV infusion over a time period of 30 minutes. The optimal dose for pembrolizumab in combination with a pan-RAF inhibitor may be identified by dose escalation starting with 2 mg/kg and going up to 10 mg/kg with the frequency of administration matched to that selected for the pan-RAF inhibitor, e.g., Compound A.

[00111] In some embodiments, a pharmaceutical composition comprising an anti-PD-1 antibody as the PD-1 antagonist may be provided as a liquid formulation or prepared by reconstituting a lyophilized powder with sterile water for injection prior to use. WO 2012/135408 describes the preparation of liquid and lyophilized pharmaceutical compositions comprising pembrolizumab that are suitable for use as described in the present disclosure. In some embodiments, a pharmaceutical composition comprising pembrolizumab is provided in a glass vial which contains about 50 mg of pembrolizumab. In some embodiments, a pharmaceutical composition comprising nivolumab is provided in a glass vial which contains

about 40 mg/4mL or 100 mg/10mL of nivolumab.

[00112] Formulation of an antibody or fragment to be administered will vary according to the route of administration and formulation (e.g., solution, emulsion, capsule) selected. An appropriate pharmaceutical composition comprising an antibody or functional fragment thereof to be administered can be prepared in a physiologically acceptable vehicle or carrier. A mixture of antibodies and/or fragments can also be used. For solutions or emulsions, suitable carriers include, for example, aqueous or alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles can include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's or fixed oils. A variety of appropriate aqueous carriers are known to the skilled artisan, including water, buffered water, buffered saline, polyols (e.g., glycerol, propylene glycol, liquid polyethylene glycol), dextrose solution and glycine. Intravenous vehicles can include various additives, preservatives, or fluid, nutrient or electrolyte replenishers (See, generally, *Remington's Pharmaceutical Science*, 16th Edition, Mack, Ed. 1980). The compositions can optionally contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such as pH adjusting and buffering agents and toxicity adjusting agents, for example, sodium acetate, sodium chloride, potassium chloride, calcium chloride and sodium lactate. The antibodies and fragments described in this disclosure can be lyophilized for storage and reconstituted in a suitable carrier prior to use according to art-known lyophilization and reconstitution techniques. The optimum concentration of the active ingredient(s) in the chosen medium can be determined empirically, according to procedures well known to the skilled artisan, and will depend on the ultimate pharmaceutical formulation desired. For inhalation, the antibody or fragment can be solubilized and loaded into a suitable dispenser for administration (e.g., an atomizer, nebulizer or pressurized aerosol dispenser).

[00113] The antibody or fragment can be administered in a single dose or multiple doses. The dosage can be determined by methods known in the art and is dependent, for example, upon the antibody or fragment chosen, the subject's age, sensitivity and tolerance to drugs, and overall well-being. Antibodies and antigen-binding fragments thereof, such as human, humanized and chimeric antibodies and antigen-binding fragments can often be administered with less frequency than other types of therapeutics. For example, an effective amount of an antibody can range from about 0.01 mg/kg to about 5 or 10 mg/kg administered daily, weekly,

biweekly or monthly.

[00114] Compositions for use in the method of the disclosure may be formulated in unit dosage form for ease of administration and uniformity of dosage. The expression "unit dosage form" as used herein refers to a physically discrete unit of agent appropriate for the subject to be treated. It will be understood, however, that the total daily usage of the therapeutic agents of the combination will be decided by the attending physician within the scope of sound medical judgment. A unit dosage form for parenteral administration may be in ampoules or in multi-dose containers.

[00115] The pan-RAF inhibitor and PD-1 antagonist are administered in such a way that they provide a beneficial effect in the treatment of a cancer. Administration can be by any suitable means provided that the administration provides the desired therapeutic effect, i.e., additivity or synergism. In some embodiments, the pan-RAF inhibitor and PD-1 antagonist are administered during the same cycle of therapy, e.g., during one cycle of therapy, both the RAF kinse inhibitor and PD-1 antagonist are administered to the subject.

[00116] In some embodiments, the pan-RAF inhibitor and PD-1 antagonist are cyclically administered to a subject. Cycling therapy involves the administration of a first agent (e.g., a first prophylactic or therapeutic agent) for a period of time, followed by the administration of a second agent and/or third agent (e.g., a second and/or third prophylactic or therapeutic agent) for a period of time and repeating this sequential administration. Cycling therapy can reduce the development of resistance to one or more of the therapies, avoid or reduce the side effects of one of the therapies, and/or improve the efficacy of the treatment.

[00117] In some embodiments, the treatment period during which an agent is administered is then followed by a non-treatment period of particular time duration, during which the therapeutic agents are not administered to the subject. This non-treatment period can then be followed by a series of subsequent treatment and non-treatment periods of the same or different frequencies for the same or different lengths of time. In some embodiments, the treatment and non-treatment periods are alternated. It will be understood that the period of treatment in cycling therapy may continue until the subject has achieved a complete response or a partial response, at which point the treatment may be stopped. Alternatively, the period of treatment in cycling therapy may continue until the subject has achieved a complete response or a partial response, at which point the period of treatment may continue for a particular number

of cycles. In some embodiments, the length of the period of treatment may be a particular number of cycles, regardless of subject response. In some other embodiments, the length of the period of treatment may continue until the subject relapses.

[00118] The amounts or suitable dosages of the pan-RAF inhibitor depends upon a number of factors, including the nature of the severity of the condition to be treated, the particular inhibitor, the route of administration and the age, weight, general health, and response of the individual subject. In some embodiments, the suitable dose level is one that achieves a therapeutic response as measured by tumor regression, or other standard measures of disease progression, progression free survival or overall survival. In some embodiments, the suitable dose level is one that achieves this therapeutic response and also minimizes any side effects associated with the administration of the therapeutic agent.

[00119] Suitable daily dosages of inhibitors of Raf kinases can generally range, in single or divided or multiple doses, from about 10% to about 100% of the maximum tolerated dose as a single agent. In some embodiments, the suitable dosages are from about 15% to about 100% of the maximum tolerated dose as a single agent. In some some embodiments, the suitable dosages are from about 25% to about 90% of the maximum tolerated dose as a single agent. In some other embodiments, the suitable dosages are from about 30% to about 80% of the maximum tolerated dose as a single agent. In some other embodiments, the suitable dosages are from about 40% to about 75% of the maximum tolerated dose as a single agent. In some other embodiments, the suitable dosages are from about 45% to about 60% of the maximum tolerated dose as a single agent. In some embodiments, suitable dosages are 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 95%, about 100%, about 105%, or about 110% of the maximum tolerated dose as a single agent.

[00120] It will be understood that a suitable dosage of a pan-RAF inhibitor may be taken at any time of the day or night. In some embodiments, a suitable dosage of a selective inhibitor of pan-RAF inhibitor is taken in the morning. In some other embodiments, a suitable dosage of a pan-RAF inhibitor is taken in the evening. In some other embodiments, a suitable dosage of a pan-RAF inhibitor is taken both in the morning and the evening. It will be understood that a suitable dosage of a pan-RAF inhibitor may be taken with or without food. In some

embodiments a suitable dosage of a pan-RAF inhibitor is taken with a meal. In some embodiments a suitable dosage of a pan-RAF inhibitor is taken while fasting. In some embodiments a suitable dosage of a pan-RAF inhibitor is taken on an empty stomach with subjects remaining nothing by mouth except for water for 2 hours before and 1 hour after each dose.

[00121] The present disclosure provides a method of treating a subject suffering from a cancer, comprising administering to the subject: (i) Compound A or a pharmaceutically acceptable salt thereof; and (ii) a PD-1 antagonist; the amount of (i) and (ii) being such that the combination thereof is therapeutically effective in the treatment of a cancer. Compound A is a pan-RAF inhibitor with a long half life which can support once weekly dosing (QW). In some embodiments, Compound A is administered once weekly with a rest period of 6 days between each administration. Suitable weekly dosages of Compound A can generally range, in single or divided or multiple doses, from about 300 mg to about 600 mg once weekly (QW). Compound A can generally range, in single or divided or multiple doses, from 300 mg to 600 mg once weekly (QW). In some embodiments, Compound A is administered from about 300 to about 600 mg once weekly. In some embodiments, Compound A is administered from up to 300 to 600 mg once weekly. Once weekly means with a rest period of 6 days between each administration. In some embodiments, Compound A is administered as a single dose. In some embodiments, Compound A is administered QW in an amount of 300 mg to 600 mg per dose. In some embodiments, Compound A is administered in an amount of 300 mg to 600 mg per dose on starting on day 1, week 1 and is administered once weekly with a rest period of 6 days between each administration.

[00122] In some embodiments, Compound A is administered as a divided dose. In some embodiments, Compound A is administered as a divided dose on the same day. In some embodiments, Compound A is administered in multiple doses. In some other embodiments, the suitable weekly dosage is from about 400 mg to about 600 mg per dose once a week. In some other embodiments, the suitable weekly dosage is from about 200 mg to about 500 mg per dose once a week. In some other embodiments, the suitable weekly dosage is from about 200 mg to about 300 mg per dose once a week. In some embodiments, suitable weekly dosages are about 200 mg, 300 mg, about 400 mg, about 500 mg, about 600 mg, about 700 mg, about 800 mg, or about 900 mg per dose once a week. In some embodiments, the QW dosing schedule

differentiates the combination of Compound A and PD-1 antagonist based on superior safety from other available therapies. In some embodiments, the QW dosing schedule differentiates the combination of Compound A and PD-1 antagonist based on superior efficacy from other available therapies.

[00123] The dosage of the pan-RAF inhibitor administered to a subject will also depend on frequency of administration. In some embodiments, Compound A is administered once weekly (QW) in an amount of 300 mg to 600 mg per dose with a rest period of 6 days between each administration. In some embodiments, Compound A is in an amount of 300 mg to 600 mg per dose administered starting on day 1, week 1.

[00124] In some embodiments, Compound A is administered every other day. In some embodiments, Compound A is administered every other day (QOD) in an amount of from about 100 mg to about 200 mg per dose. In some some embodiments, Compound A is administered QOD in an amount of about 100 mg per dose. In some embodiments, Compound A is administered QOD in an amount of about 200 mg per dose.

[00125] In some embodiments, Compound A is administered from up to about 200 mg per dose. In some embodiments, Compound A is administered from up to 200 mg per dose. Suitable QOD dosages of a pan-RAF inhibitor e.g., Compound A can generally range, in single or divided or multiple doses, from up to about 200 mg per dose. In some embodiments, Compound A is administered as a single dose. In some embodiments, Compound A is administered as a divided dose. In some embodiments, Compound A is administered in multiple doses. Other suitable dosages of Compound A can generally range, in single or divided or multiple doses, from about 50 mg to about 200 mg per dose. Other suitable dosages of Compound A can generally range, in single or divided or multiple doses, from about 75 mg to about 200 mg per dose. In some embodiments, the suitable dosages are from about 100 mg to about 200 mg per dose. In some other embodiments, the suitable dosages are from about 150 mg to about 200 mg twice daily. In some embodiments, suitable dosages are about 20 mg, about 25 mg, about 30 mg, about 35 mg, about 40 mg, about 45 mg, about 50 mg, about 55 mg, about 60 mg, about 65 mg, about 70 mg, about 75 mg, about 80 mg, about 85 mg, about 90 mg, about 95 mg, about 100 mg, about 105 mg, about 110 mg, about 115 mg, about 120 mg, about 125 mg, about 130 mg, about 135 mg, about 140 mg, about 145 mg, about 150 mg, about 155 mg, about 160 mg, about 165 mg, about 170 mg, about 175 mg, about 180 mg, about 185 mg,

about 190 mg, about 195 mg, or about 200 mg per dose. In some embodiments, the suitable dosage of Compound A is from about 100 mg to about 200 mg per dose.

[00126] The term "pharmaceutically acceptable carrier" is used herein to refer to a material that is compatible with a recipient subject. In one aspect, the subject is a mammal. In one aspect, the subject is a human. In one aspect, the material is suitable for delivering active therapeutic agent to the target site without terminating the activity of the agent. The toxicity or adverse effects, if any, associated with the carrier preferably are commensurate with a reasonable risk/benefit ratio for the intended use of the therapeutic agent.

[00127] The terms "carrier", "adjuvant", or "vehicle" are used interchangeably herein, and include any and all solvents, diluents, and other liquid vehicles, dispersion or suspension aids, surface active agents, isotonic agents, thickening or emulsifying agents, preservatives, solid binders, lubricants and the like, as suited to the particular dosage form desired. *Remington: The Science and Practice of Pharmacy, 20th Ed.*, ed. A. Gennaro, Lippincott Williams & Wilkins, 2000 discloses various carriers used in formulating pharmaceutically acceptable compositions and known techniques for the preparation thereof. Except insofar as any conventional carrier medium is incompatible with the compounds of the disclosure, such as by producing any undesirable biological effect or otherwise interacting in a deleterious manner with any other component(s) of the pharmaceutically acceptable composition, its use is contemplated to be within the scope of this disclosure. Some examples of materials which can serve as pharmaceutically acceptable carriers include, but are not limited to, ion exchangers, alumina, aluminum stearate, lecithin, serum proteins, such as human serum albumin, buffer substances such as disodium hydrogen phosphate, potassium hydrogen phosphate, sodium carbonate, sodium bicarbonate, potassium carbonate, potassium bicarbonate, magnesium hydroxide and aluminum hydroxide, glycine, sorbic acid, or potassium sorbate, partial glyceride mixtures of saturated vegetable fatty acids, water, pyrogen-free water, salts or electrolytes such as protamine sulfate, disodium hydrogen phosphate, potassium hydrogen phosphate, sodium chloride, and zinc salts, colloidal silica, magnesium trisilicate, polyvinyl pyrrolidone, polyacrylates, waxes, polyethylene-polyoxypropylene-block polymers, wool fat, sugars such as lactose, glucose, sucrose, starches such as corn starch and potato starch, cellulose and its derivatives such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate, powdered tragacanth; malt, gelatin, talc, excipients such as cocoa butter and

suppository waxes, oils such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil, glycols such as propylene glycol and polyethylene glycol, esters such as ethyl oleate and ethyl laurate, agar, alginic acid, isotonic saline, Ringer's solution, alcohols such as ethanol, isopropyl alcohol, hexadecyl alcohol, and glycerol, cyclodextrins, lubricants such as sodium lauryl sulfate and magnesium stearate, petroleum hydrocarbons such as mineral oil and petrolatum. Coloring agents, releasing agents, coating agents, sweetening, flavoring and perfuming agents, preservatives and antioxidants can also be present in the composition, according to the judgment of the formulator.

[00128] The pharmaceutical compositions of the disclosure can be manufactured by methods well known in the art such as conventional granulating, mixing, dissolving, encapsulating, lyophilizing, or emulsifying processes, among others. Compositions may be produced in various forms, including granules, precipitates, or particulates, powders, including freeze dried, rotary dried or spray dried powders, amorphous powders, tablets, capsules, syrup, suppositories, injections, emulsions, elixirs, suspensions or solutions. Formulations may optionally contain solvents, diluents, and other liquid vehicles, dispersion or suspension aids, surface active agents, pH modifiers, isotonic agents, thickening or emulsifying agents, stabilizers and preservatives, solid binders, lubricants and the like, as suited to the particular dosage form desired.

[00129] In some embodiments, the compositions of this disclosure are formulated for pharmaceutical administration to a subject. In some embodiments, Compound A is formulated as described in WO 2010/064722. Such pharmaceutical compositions of the present disclosure may be administered orally, parenterally, by inhalation spray, topically, rectally, nasally, buccally, vaginally or via an implanted reservoir. The term "parenteral" as used herein includes subcutaneous, intravenous, intramuscular, intra-articular, intra-synovial, intrasternal, intrathecal, intrahepatic, intralesional and intracranial injection or infusion techniques. In some embodiments, the compositions are administered orally, intravenously, or subcutaneously. The formulations of the disclosure may be designed to be short-acting, fast-releasing, or long-acting. Still further, the agents of the combination can be administered in a local rather than systemic means, such as administration (e.g., by injection) at a tumor site.

[00130] Liquid dosage forms for oral administration include, but are not limited to, pharmaceutically acceptable emulsions, microemulsions, solutions, suspensions, syrups and elixirs. In addition to the active compounds, the liquid dosage forms may contain inert diluents commonly used in the art such as, for example, water or other solvents, solubilizing agents and emulsifiers such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, cyclodextrins, dimethylformamide, oils (in particular, cottonseed, groundnut, corn, germ, olive, castor, and sesame oils), glycerol, tetrahydrofurfuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan, and mixtures thereof. Besides inert diluents, the oral compositions can also include adjuvants such as wetting agents, emulsifying and suspending agents, sweetening, flavoring, and perfuming agents.

[00131] Injectable preparations, for example, sterile injectable aqueous or oleaginous suspensions may be formulated according to the known art using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation may also be a sterile injectable solution, suspension or emulsion in a nontoxic parenterally acceptable diluent or solvent, for example, as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution, U.S.P. and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil can be employed including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid are used in the preparation of injectables. The injectable formulations can be sterilized, for example, by filtration through a bacterial-retaining filter, or by incorporating sterilizing agents in the form of sterile solid compositions which can be dissolved or dispersed in sterile water or other sterile injectable medium prior to use. Compositions formulated for parenteral administration may be injected by bolus injection or by timed push, or may be administered by continuous infusion.

[00132] Solid dosage forms for oral administration include capsules, tablets, pills, powders, and granules. In such solid dosage forms, at least one therapeutic agent of the combination is mixed with at least one inert, pharmaceutically acceptable excipient or carrier such as sodium citrate or dicalcium phosphate and/or a) fillers or extenders such as starches, lactose, sucrose, glucose, mannitol, and silicic acid, b) binders such as, for example, carboxymethylcellulose, alginates, gelatin, polyvinylpyrrolidinone, sucrose, and acacia, c)

humectants such as glycerol, d) disintegrating agents such as agar--agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates, and sodium carbonate, e) solution retarding agents such as paRaffin, f) absorption accelerators such as quaternary ammonium compounds, g) wetting agents such as, for example, cetyl alcohol and glycerol monostearate, h) absorbents such as kaolin and bentonite clay, and i) lubricants such as talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, and mixtures thereof. In the case of capsules, tablets and pills, the dosage form may also comprise buffering agents such as phosphates or carbonates.

[00133] Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugar as well as high molecular weight polyethylene glycols and the like. The solid dosage forms of tablets, dragees, capsules, pills, and granules can be prepared with coatings and shells such as enteric coatings and other coatings well known in the pharmaceutical formulating art. They may optionally contain opacifying agents and can also be of a composition that they release the active ingredient(s) only, or preferentially, in a certain part of the intestinal tract, optionally, in a delayed manner. Examples of embedding compositions that can be used include polymeric substances and waxes. Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugar as well as high molecular weight polyethylene glycols and the like.

[00134] One or more of the therapeutic agents of the combination can also be in micro-encapsulated form with one or more excipients as noted above. The solid dosage forms of tablets, dragees, capsules, pills, and granules can be prepared with coatings and shells such as enteric coatings, release controlling coatings and other coatings well known in the pharmaceutical formulating art. In such solid dosage forms the active compound may be admixed with at least one inert diluent such as sucrose, lactose or starch. Such dosage forms may also comprise, as is normal practice, additional substances other than inert diluents, e.g., tableting lubricants and other tableting aids such a magnesium stearate and microcrystalline cellulose. In the case of capsules, tablets and pills, the dosage forms may also comprise buffering agents. They may optionally contain opacifying agents and can also be of a composition that they release the active ingredient(s) only, or preferentially, in a certain part of

the intestinal tract, optionally, in a delayed manner. Examples of embedding compositions that can be used include polymeric substances and waxes.

[00135] Dosage forms for topical or transdermal administration of one or more therapeutic agents of the combination include ointments, pastes, creams, lotions, gels, powders, solutions, sprays, inhalants or patches. The therapeutic agent is admixed under sterile conditions with a pharmaceutically acceptable carrier and any needed preservatives or buffers as may be required. Ophthalmic formulation, ear drops, and eye drops are also contemplated as being within the scope of this disclosure. Additionally, the present disclosure contemplates the use of transdermal patches, which have the added advantage of providing controlled delivery of one or more of the therapeutic agents of the combination to the body. Such dosage forms can be made by dissolving or dispensing the therapeutic agent in the proper medium. Absorption enhancers can also be used to increase the flux of the therapeutic agent across the skin. The rate can be controlled by either providing a rate controlling membrane or by dispersing the therapeutic agent in a polymer matrix or gel.

[00136] The combination described herein may be provided as a kit which comprises a first container and a second container and a package insert. The first container contains at least one dose of a pharmaceutical composition comprising an anti-PD-1 antagonist, the second container contains at least one dose of a pharmaceutical composition comprising a pan-RAF inhibitor, and the package insert, or label, which comprises instructions for treating a subject for a cancer using the pharmaceutical compositions. The first and second containers may be comprised of the same or different shape (e.g., vials, syringes and bottles) and/or material (e.g., plastic or glass). The kit may further comprise other materials that may be useful in administering the pharmaceutical compositions, such as diluents, filters, IV bags and lines, needles and syringes. In some embodiments of the kit, the anti-PD-1 antagonist is an anti-PD-1 antibody. In some embodiments of the kit, the anti-PD-1 antibody is nivolumab. In some embodiments of the kit, the anti-PD-1 antibody is pembrolizumab. In some embodiments of the kit, the pan-RAF inhibitor is Compound A or a pharmaceutically acceptable salt thereof.

[00137] These and other aspects of the disclosure, including the exemplary specific embodiments listed below, will be apparent from the teachings contained herein.

[00138] The present disclosure relates to methods for treating a subject suffering from a cancer by administering to the subject a pharmaceutical composition described herein, said method comprising: a) measuring at least one characteristic of at least one or more BRAF, NRAS and/or KRAS markers associated with gene mutation in a subject sample comprising tumor cells; b) identifying whether the at least one characteristic measured in step a) is informative for outcome upon treatment with the pharmaceutical composition; and c) determining to treat the subject with the pharmaceutical composition if the informative characteristic indicates that the tumor cells comprise at least one marker gene with a BRAF, NRAS and/or KRAS mutational status that indicates a favorable outcome to treatment with the pharmaceutical composition.

[00139] The present disclosure relates to methods for treating a subject suffering from a cancer by administering to the subject a pharmaceutical composition described herein, said method comprising: subjecting a nucleic acid sample from a cancer (tumor) sample from the subject to BRAF, NRAS, or KRAS mutational testing or PCR, wherein the presence of a mutation in either BRAF, NRAS, or KRAS gene indicates an increased likelihood of pharmacological effectiveness of the treatment.

[00140] The present disclosure relates to methods of treating a subject suffering from a cancer, said method comprising: i) obtaining a nucleic acid sample from a cancer sample from said subject; ii) subjecting the sample to BRAF, NRAS, or KRAS mutational testing or PCR and identifying the presence of at least one mutation in BRAF, NRAS, or KRAS gene; and iii) administering an effective amount of a combination described herein to the subject in whose sample the presence of at least one mutation in BRAF or KRAS gene is identified.

[00141] In some embodiments, a mutation in a marker can be identified by sequencing a nucleic acid, e.g., a DNA, RNA, cDNA or a protein correlated with the marker gene, e.g., a genotype marker gene, e.g., BRAF or NRAS. There are several sequencing methods known in the art to sequence nucleic acids. A nucleic acid primer can be designed to bind to a region comprising a potential mutation site or can be designed to complement the mutated sequence rather than the wild type sequence. Primer pairs can be designed to bracket a region comprising a potential mutation in a marker gene. A primer or primer pair can be used for sequencing one or both strands of DNA corresponding to the marker gene. A primer can be

used in conjunction with a probe, e.g., a nucleic acid probe, e.g., a hybridization probe, to amplify a region of interest prior to sequencing to boost sequence amounts for detection of a mutation in a marker gene. Examples of regions which can be sequenced include an entire gene, transcripts of the gene and a fragment of the gene or the transcript, e.g., one or more of exons or untranslated regions or a portion of a marker comprising a mutation site. Examples of mutations to target for primer selection and sequence or composition analysis can be found in public databases which collect mutation information, such as Database of Genotypes and Phenotypes (dbGaP) maintained by the National Center for Biotechnology Information (Bethesda, MD) and Catalogue of Somatic Mutations in Cancer (COSMIC) database maintained by the Wellcome Trust Sanger Institute (Cambridge, UK).

[00142] Sequencing methods are known to one skilled in the art. Examples of methods include the Sanger method, the SEQUENOM™ method and Next Generation Sequencing (NGS) methods. The Sanger method, comprising using electrophoresis, e.g., capillary electrophoresis to separate primer-elongated labeled DNA fragments, can be automated for high-throughput applications. The primer extension sequencing can be performed after PCR amplification of regions of interest. Software can assist with sequence base calling and with mutation identification. SEQUENOM™ MASSARRAY® sequencing analysis (San Diego, CA) is a mass-spectrometry method which compares actual mass to expected mass of particular fragments of interest to identify mutations. NGS technology (also called "massively parallel sequencing" and "second generation sequencing") in general provides for much higher throughput than previous methods and uses a variety of approaches (reviewed in Zhang et al. (2011) J. Genet. Genomics 38:95-109 and Shendure and Hanlee (2008) Nature Biotech. 26:1135-1145). NGS methods can identify low frequency mutations in a marker in a sample. Some NGS methods (see, e.g., GS-FLX Genome Sequencer (Roche Applied Science, Branford, CT), Genome analyzer (Illumina, Inc. San Diego, CA) SOLID™ analyzer (Applied Biosystems, Carlsbad, CA), Polonator G.007 (Dover Systems, Salem, NH), HELISCOPE™ (Helicos Biosciences Corp., Cambridge, MA)) use cyclic array sequencing, with or without clonal amplification of PCR products spatially separated in a flow cell and various schemes to detect the labeled modified nucleotide that is incorporated by the sequencing enzyme (e.g., polymerase or ligase). In one NGS method, primer pairs can be used in PCR reactions to amplify regions of interest. Amplified regions can be ligated into a concatenated product.

Clonal libraries are generated in the flow cell from the PCR or ligated products and further amplified ("bridge" or "cluster" PCR) for single-end sequencing as the polymerase adds a labeled, reversibly terminated base that is imaged in one of four channels, depending on the identity of the labeled base and then removed for the next cycle. Software can aid in the comparison to genomic sequences to identify mutations. Another NGS method is exome sequencing, which focuses on sequencing exons of all genes in the genome. As with other NGS methods, exons can be enriched by capture methods or amplification methods.

[00143] In some embodiments, DNA, e.g., genomic DNA corresponding to the wild type or mutated marker can be analyzed both by in situ and by in vitro formats in a biological sample using methods known in the art. DNA can be directly isolated from the sample or isolated after isolating another cellular component, e.g., RNA or protein. Kits are available for DNA isolation, e.g., QIAAMP® DNA Micro Kit (Qiagen, Valencia, CA). DNA also can be amplified using such kits.

[00144] In another embodiment, mRNA corresponding to the marker can be analyzed both by in situ and by in vitro formats in a biological sample using methods known in the art. Many expression detection methods use isolated RNA. For in vitro methods, any RNA isolation technique that does not select against the isolation of mRNA can be utilized for the purification of RNA from tumor cells (see, e.g., Ausubel et al., ed., Current Protocols in Molecular Biology, John Wiley & Sons, New York 1987-1999). Additionally, large numbers of tissue samples can readily be processed using techniques well known to those of skill in the art, such as, for example, the single-step RNA isolation process of Chomczynski (1989, U.S. Patent No. 4,843,155). RNA can be isolated using standard procedures (see e.g., Chomczynski and Sacchi (1987) Anal. Biochem.162:156-159), solutions (e.g., trizol, TRI REAGENT® (Molecular Research Center, Inc., Cincinnati, OH; see U.S. Patent No. 5,346,994) or kits (e.g., a QIAGEN® Group RNEASY® isolation kit (Valencia, CA) or LEUKOLOCK™ Total RNA Isolation System, Ambion division of Applied Biosystems, Austin, TX).

[00145] Additional steps may be employed to remove DNA from RNA samples. Cell lysis can be accomplished with a nonionic detergent, followed by microcentrifugation to remove the nuclei and hence the bulk of the cellular DNA. DNA subsequently can be isolated from the nuclei for DNA analysis. In one embodiment, RNA is extracted from cells of the

various types of interest using guanidinium thiocyanate lysis followed by CsCl centrifugation to separate the RNA from DNA (Chirgwin et al. (1979) Biochemistry 18:5294-99). Poly(A)+RNA is selected by selection with oligo-dT cellulose (see Sambrook et al. (1989) Molecular Cloning--A Laboratory Manual (2nd ed.), Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.). Alternatively, separation of RNA from DNA can be accomplished by organic extraction, for example, with hot phenol or phenol/chloroform/isoamyl alcohol. If desired, RNAse inhibitors may be added to the lysis buffer. Likewise, for certain cell types, it may be desirable to add a protein denaturation/digestion step to the protocol. For many applications, it is desirable to enrich mRNA with respect to other cellular RNAs, such as transfer RNA (tRNA) and ribosomal RNA (rRNA). Most mRNAs contain a poly(A) tail at their 3' end. This allows them to be enriched by affinity chromatography, for example, using oligo(dT) or poly(U) coupled to a solid support, such as cellulose or SEPHADEX.R™. medium (see Ausubel et al. (1994) Current Protocols In Molecular Biology, vol. 2, Current Protocols Publishing, New York). Once bound, poly(A)+mRNA is eluted from the affinity column using 2 mM EDTA/0.1% SDS.

[00146] A characteristic of a marker found in a sample, e.g., after obtaining a sample (e.g., a a tumor biopsy) from a test subject, can be assessed by any of a wide variety of well known methods for detecting or measuring the characteristic, e.g., of a marker or plurality of markers, e.g., of a nucleic acid (e.g., RNA, mRNA, genomic DNA, or cDNA) and/or translated protein. Non-limiting examples of such methods include immunological methods for detection of secreted, cell-surface, cytoplasmic, or nuclear proteins, protein purification methods, protein function or activity assays, nucleic acid hybridization methods, optionally including "mismatch cleavage" steps (Myers, et al. (1985) Science 230:1242) to digest mismatched, i.e. mutant or variant, regions and separation and identification of the mutant or variant from the resulting digested fragments, nucleic acid reverse transcription methods, and nucleic acid amplification methods and analysis of amplified products. These methods include gene array/chip technology, RT-PCR, TAQMAN® gene expression assays (Applied Biosystems, Foster City, CA), e.g., under GLP approved laboratory conditions, in situ hybridization, immunohistochemistry, immunoblotting, FISH (fluorescence in situ hybridization), FACS analyses, northern blot, southern blot, INFINIUM® DNA analysis Bead Chips (Illumina, Inc.,

San Diego, CA), quantitative PCR, bacterial artificial chromosome arrays, single nucleotide polymorphism (SNP) arrays (Affymetrix, Santa Clara, CA) or cytogenetic analyses.

[00147] Examples of techniques for detecting differences of at least one nucleotide between two nucleic acids include, but are not limited to, selective oligonucleotide hybridization, selective amplification, or selective primer extension. For example, oligonucleotide probes can be prepared in which the known polymorphic nucleotide is placed centrally (allele- or mutant-specific probes) and then hybridized to target DNA under conditions which permit hybridization only if a perfect match is found (Saiki et al. (1986) Nature 324:163); Saiki et al (1989) Proc. Natl Acad. Sci USA 86:6230; and Wallace et al. (1979) Nucl. Acids Res. 6:3543). Such allele specific oligonucleotide hybridization techniques can be used for the simultaneous detection of several nucleotide changes in different polymorphic or mutated regions of NRAS. For example, oligonucleotides having nucleotide sequences of specific allelic variants or mutants are attached to a solid support, e.g., a hybridizing membrane and this support, e.g., membrane, is then hybridized with labeled sample nucleic acid. Analysis of the hybridization signal thus can reveal the identity of the nucleotides of the sample nucleic acid.

EXAMPLES

Example 1. Tumor immunophenotype: NK-, T-, B- and Myeloid Cells in CT26

Experimental Procedure

[00148] Animals: Balb/c mice from JAX (approximately 20 grams body weight, female) were used. The animals were group-housed and provided food and water ad libitum prior to the experiment. Mice were maintained on a 12-hour light and dark cycle and maintained in the vivarium for at least 7 days after receipt prior to investigations.

[00149] Drugs used in this study: Compound A (12.5 mg/kg, Formulated in 0.05% Tween/0.5% Methocel at 1.25 mg/mL, dosed at 10 mL/kg, p.o.). Anti-PD-1 monoclonal antibody (200 ug/mouse, BioXcell, RMP1-14, dosed at 5 mL/kg, i.p.).

[00150] Cell Culture: CT26 cells (ATCC CRL-2638) were cultured in RPMI-1640 containing 10% FBS and pen/strep. Media was changed every 2-3 days and cells were split at ~80% confluence. A maximum of 20 passages was allowed prior to re-initiating the culture from the original cell stock

[00151] In vivo CT26 syngeneic model: Day-2 prior to tumor injection, the animal's fur was shaved from the rump up to the middle of back and extending to the flank of each side. At day 0, CT26 cells were suspended in RPMI medium and Matrigel (1:1) to final cell concentration of $2x10^6$/ml and injected 0.1ml ($0.2x10^6$ cells/mouse) subcutaneously in the flank. All procedures were performed under protocols approved by the Institutional Animal Care and Use Committee at TAKEDA CALIFORNIA INC. At Day 7 after inoculation, animals were sorted and randomized into 4 groups and treatment was initiated as illustrated in Table 1. Tumor size measured by caliper (width x length) and body weight were recorded twice a week until day 21. Tumor volumes were calculated using the formula TV = ($width^2$xlength) x 0.52 and tumor growth inhibition (TGI) was calculated using the formula (Vehicle – Treatment)/Vehicle x100 at each time point. Statistical significance was assessed by one-way ANOVA followed by Dunnett's post-test, comparing all groups versus vehicle (GraphPad Prism, GraphPad Software, Inc., La Jolla, CA, USA). Results are expressed as mean ± standard error of the mean (SEM). A probability level of $p<0.05$ was considered significant. At Day 14, 5 mice from each group were sacrificed and spleens and tumors were collected for FACS analysis.

Table 1

Group	Treatment	Dose	Frequency	Route	N (Tumor size)	N (FACS-Day14)
1	Vehicle	0.05% Tween80 /0.5% MC	qd D7-D20	p.o.	10	5
2	Compound A	12.5 mpk	qd D7-D20	p.o.	10	5
3	Anti-PD1	200 ug	D7,9,13,15,18	i.p.	10	5
4	Compound A +	12.5 mpk + 200 ug	qd D7-D20 + D7,9,13,15,18	p.o. + i.p.	10	5

	anti-PD1					

[00152] Flow cytometry was used to identify and enumerate different cell types in spleen and tumor. Spleens and tumors were removed and dissociated into single cell suspensions using the gentle MACS dissociator (Miltenyl Biotec). Spleens were processed in autoMACS buffer containing 0.5% BSA and tumors in RPMI-1640 media containing enzymes A, D and R provided in the MACS tumor dissociation kit. After red blood cell removal (eBioscience lysis buffer), cells were washed and suspended at 20x10e6 viable cells/ml. Single cell suspension was distributed into 96 well U bottom plate in PBS for viability staining (Zombie Aqua, Biolegend). Cells were then washed, blocked in Fc block (Becton Dickinson and Company) and subsequently stained using the following conjugated antibodies in 100ul final volume FACS buffer (PBS + 1% BSA). Conjugated antibodies used for staining of CD3, CD4, CD8, CD45, B220, PD-1, CD11b, γδ TCR, RORγt, FoxP3, NKp46, CD49b, Ly6G, Ly6C, F4/80 were purchased from eBioscience, BD Biosciences or Biolegend. After staining, cells were washed and fixed in either fixation buffer (eBioscience) for surface stains or fixation/permeabilization buffer (eBioscience) for additional intracellular staining (FoxP3, RORγt). Cells were acquired on a Canto II flow cytometer (BD Bioscience). The data was retrieved off the machine and analyzed with FlowJo software (Tree Star, Inc.) and GraphPad Prism (Version 5.04, GraphPad Software, Inc.). Each individual mouse value was entered for the parameters that were of interest. Then a one-way ANOVA with a post-dunnet's multiple comparison test comparing all groups versus vehicle was done to determine significance. Figures 1 and 2 show the results of the immunophenotyping. Results are expressed as mean ± standard error mean (SEM). A probability level of $p < 0.05$ was considered significant.

[00153] All references cited herein are incorporated by reference to the same extent as if each individual publication, database entry (e.g. Genbank sequences or GeneID entries), patent application, or patent, was specifically and individually indicated to be incorporated by reference. This statement of incorporation by reference is intended by Applicants, pursuant to 37 C.F.R. § 1.57(b)(1), to relate to each and every individual publication, database entry (e.g. Genbank sequences or GeneID entries), patent application, or patent, each of which is clearly identified in compliance with 37 C.F.R. § 1.57(b)(2), even if such citation is not immediately

adjacent to a dedicated statement of incorporation by reference. The inclusion of dedicated statements of incorporation by reference, if any, within the specification does not in any way weaken this general statement of incorporation by reference. Citation of the references herein is not intended as an admission that the reference is pertinent prior art, nor does it constitute any admission as to the contents or date of these publications or documents.

Example 2: A Phase Ib Clinical Study

[00154] A phase 1b study to evaluate the safety, tolerability, and pharmacodynamics of an investigational treatment of Compound A in combination with standard of care immune checkpoint inhibitors in patients with advanced melanoma is undertaken. Up to about 52 subjects are enrolled. Approximately 12 subjects are assigned in dose-escalation treatments, with up to 46 subjects in expansion cohorts.

[00155] The subjects are adults, either male or female, with histologically confirmed, unresectable stage III or IV melanoma, according to the AJCC staging system, and with a ECOG performance status of 0-1. Subjects are BRAF V600 mutation-positive or NRAS-mutation positive disease previously untreated with RAF, MEK or other inhibitors of the MAPK pathway. Subjects have adequate bone marrow reserve and renal and hepatic function. Subjects with active known or suspected autoimmune disease, or that are undergoing systemic treatment with either corticosteroids (>10mg prednisone or equivalents) or other immunosuppressive medications within 14 days of administration of a study drug administration, are excluded, as are subjects with prior treatment with an anti-PD-1, anti-PDL-1 or anti-PDL-2 antibodies.

Compound A is administered orally at 300 mg, 400 mg, or 600 mg once weekly. Intermediate doses can be tested. Nivolumab is administered IV at a dose of 3 mg/kg Q2W. Duration of treatment is up to 50 weeks and the period of evaluation is 12 months.

[00156] The primary objective in the dose escalation phase plus Part 1 limited cohort expansion is to determine the recommended Part 2 dose based on the initial safety profile of the combination treatments in each arm when administered to patients with advanced melanoma. The primary objective in Part 2 is to determine the initial antitumor activity of each combination arm.

[00157] The primary endpoint is the frequency of dose limiting toxicities (DLT)(part 1)

and overall response rate as measured by Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1(part 2). Secondary safety endpoints for this study are the frequency and severity of treatment emergent adverse events (TEAEs) including serious TEAEs, and TEAEs leading to treatment dioscontinuation. Secondary efficacy endpoints are duration of response (DOR), progression-free survival (PFS) and overall survival (OS). Serial tumor biopsies are used to assess immune cell infiltration in the tumor microenvironment at baseline, investigational single agent and post-combination treatment.

Claims:

1. A method of treating a subject suffering from a cancer, comprising administering to the subject:
   (i) a pan-RAF inhibitor or a pharmaceutically acceptable salt thereof; and
   (ii) a Programmed Death 1 protein (PD-1) antagonist; the amount of pan-RAF inhibitor and PD-1 antagonist is such that the combination thereof is therapeutically effective in the treatment of the cancer.

2. The method of claim 1, wherein the cancer is a KRAS, NRAS, or BRAF mutation positive cancer.

3. The method of claim 2, wherein the cancer is a KRAS mutation positive cancer.

4. The method of claim 3, wherein the cancer is a KRAS exon 2 mutation positive cancer.

5. The method of claim 3, wherein the cancer is a KRAS non-exon 2 mutation positive cancer.

6. The method of claim 2, wherein the cancer is a BRAF mutation positive cancer.

7. The method of claim 6, wherein the cancer is a BRAF V600 mutation positive cancer.

8. The method of claim 6, wherein the cancer is a non-V600 BRAF mutation positive cancer.

9. The method of claim 2, wherein the cancer is an NRAS mutation positive cancer.

10. The method of claim 9, wherein the cancer is NRAS non-exon 2 mutation positive cancer.

11. The method of claim 9, wherein the cancer is NRAS exon 2 mutation positive cancer.

12. The method of any one of claims 1-11, wherein the cancer is a solid tumor.

13. The method of any one of claims 1-11, wherein the cancer is a hematological malignancy.

14. The method of any one of claims 1-11, wherein the cancer is selected from skin cancer, ocular cancer, gastrointestinal cancer, thyroid cancer, breast cancer, ovarian cancer, central nervous system cancer, laryngeal cancer, cervical cancer, lymphatic system cancer, genitourinary tract cancer, bone cancer, biliary tract cancer, endometrial cancer, uterine cancer, liver cancer, lung cancer, prostate cancer, and colon cancer.

15. The method of claim 14, wherein the cancer is non-small cell lung cancer.

16. The method of claim 14, wherein the cancer is colorectal cancer.

17. The method of claim 14, wherein the cancer is skin.

18. The method of claim 17, wherein the skin cancer is melanoma.

19. The method of any one of claims 1-18, wherein the pan-RAF inhibitor is selected from (R)-2-(1-(6-amino-5-chloropyrimidine-4-carboxamide)ethyl)-N-(5-chloro-4-(trifluoromethyl)pyridin-2-yl)thiazole-5-carboxamide, N-{7-cyano-6-[4-fluoro-3-({[3-(trifluoromethyl)phenyl]acetyl}amino)phenoxy]-1,3-benzothiazol-2-yl}cyclopropanecarboxamide or a pharmaceutically acceptable salt thereof.

20. The method of claim 19, wherein the pan-RAF inhibitor is (R)-2-(1-(6-amino-5-chloropyrimidine-4-carboxamide)ethyl)-N-(5-chloro-4-(trifluoromethyl)pyridin-2-yl)thiazole-5-carboxamide or a pharmaceutically acceptable salt thereof.

21. The method of claim 19, wherein the pan-RAF inhibitor is N-{7-cyano-6-[4-fluoro-3-({[3-(trifluoromethyl)phenyl]acetyl}amino)phenoxy]-1,3-benzothiazol-2-yl}cyclopropanecarboxamide or a pharmaceutically acceptable salt thereof.

22. The method of any one of claims 1-21, wherein the PD-1 antagonist is:
    (i) a monoclonal antibody, or an antigen binding fragment thereof, which specifically binds to human PD-1 and blocks the binding of human PD-L1 to human PD-1; or
    (ii) a monoclonal antibody, or an antigen binding fragment thereof, which specifically binds to human PD-L1 and blocks the binding of human PD-L1 to human PD-1.

23. The method of claim 20, wherein the PD-1 antagonist is selected from pembrolizumab, nivolumab, atezolizumab, durvalumab, and avelumab.

24. A pharmaceutical composition comprising:
    (i) a pan-RAF inhibitor or a pharmaceutically acceptable salt thereof; and
    (ii) a PD-1 antagonist; the amount of said pan-RAF kinase inhibitor and PD-1 antagonist is such that the combination thereof is effective in the treatment of a cancer.

25. A kit which comprises a first container, a second container and a package insert, wherein the first container comprises at least one dose of a pharmaceutical composition comprising an antagonist of a Programmed Death 1 protein (PD-1), the second container comprises at least one dose of a pharmaceutical composition comprising a pan-RAF inhibitor or a pharmaceutically acceptable salt thereof, and the package insert comprises instructions for treating a subject for a cancer using the pharmaceutical compositions.

26. The kit of claim 25, wherein the PD-1 antagonist is selected pembrolizumab or nivolumab.

27. The kit of claim 26, wherein the pan-RAF inhibitor is (R)-2-(1-(6-amino-5-chloropyrimidine-4-carboxamide)ethyl)-N-(5-chloro-4-(trifluoromethyl)pyridin-2-yl)thiazole-5-carboxamide or a pharmaceutically acceptable salt thereof.

Figure 1

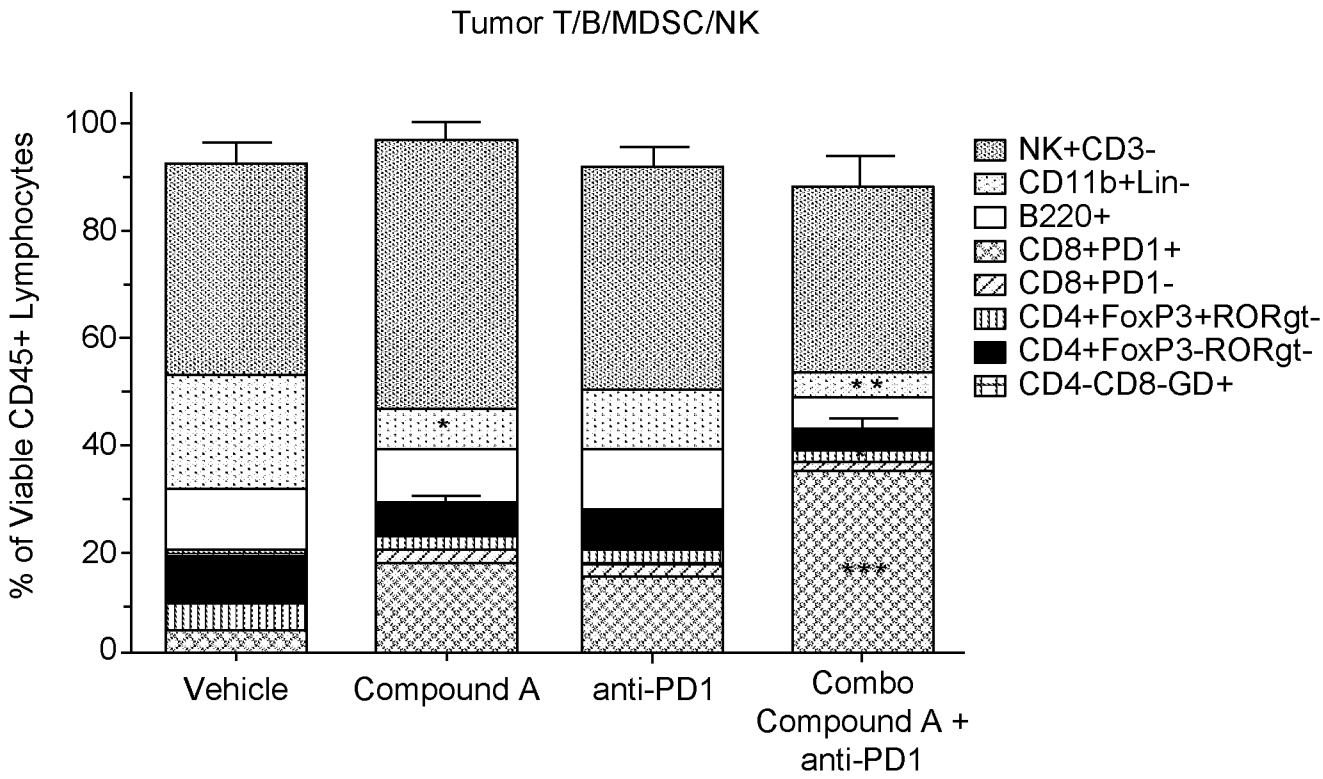

Tumor T/B/MDSC/NK
% of Viable CD45+ Lymphocytes
100
80
60
40
20
0
Vehicle
Compound A
anti-PD1
Combo Compound A + anti-PD1
NK+CD3-
CD11b+Lin-
B220+
CD8+PD1+
CD8+PD1-
CD4+FoxP3+RORgt-
CD4+FoxP3-RORgt-
CD4-CD8-GD+
*
**
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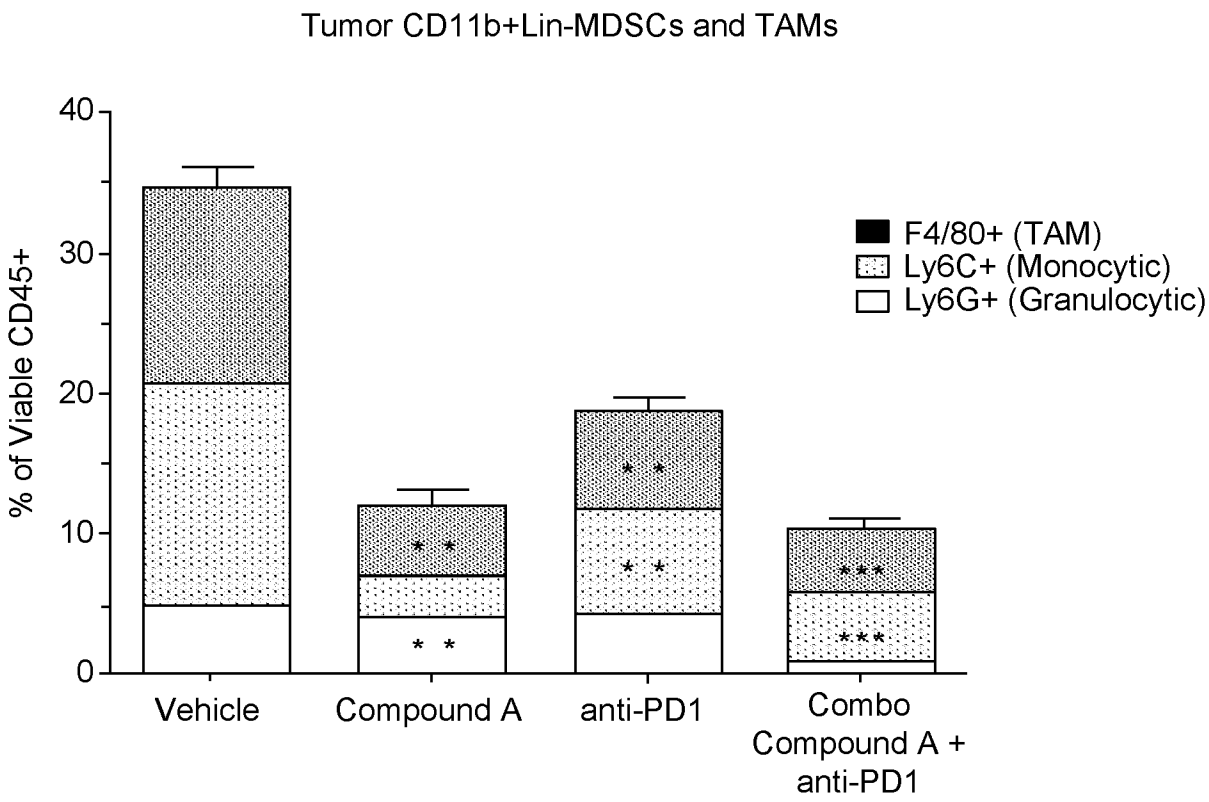
Figure 2


Tumor CD11b+Lin-MDSCs and TAMs
% of Viable CD45+
40
30
20
10
0
F4/80+ (TAM)
Ly6C+ (Monocytic)
Ly6G+ (Granulocytic)
Vehicle
Compound A
anti-PD1
Combo
Compound A +
anti-PD1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2017/023539

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. ☐ forming part of the international application as filed:

      ☐ in the form of an Annex C/ST.25 text file.

      ☐ on paper or in the form of an image file.

   b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. ☐ furnished subsequent to the international filing date for the purposes of international search only:

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      ☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. ☒ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

   Although a sequence listing has been filed or furnished, it was not used for the purposes of this search.

   Since only one version or copy of a sequence listing has been filed or furnished, the statements under item 2 are not required.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2017/023539

A. CLASSIFICATION OF SUBJECT MATTER

A61K 31/395 (2006.01) ***A61K 31/428 (2006.01)*** ***A61K 31/4745 (2006.01)***

According to International Patent Classification (IPC)

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

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

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

EPODOC, WPIAP, CAPlus/MEDLINE/EMBASE/BIOSIS: pan-RAF kinase inhibitor; PD-1 or PD-L1 antagonist; pembrolizumab; nivolumab, atezolizumab, durvalumab; and equivalent terms.
REGISTRY: CAS no. 1228591-30-7; CAS no. 1096708-71-2; CAS no. 1163719-56-9; CAS no. 878739-06-1; CAS no. 1454682-72-4; CAS no. 284461-73-0

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2015/126903 A1 (HEALTH RESEARCH, INC.) 27 August 2015 (whole document, in particular, paragraphs [0034], [0039], [0041], [0049], [0061] & [0064]-[0066])	1-18 & 22-26
Y	BRAUNER E. ET AL., Combining BRAF inhibitor and anti PD-L1 antibody dramatically improves tumor regression and anti tumor immunity in an immunocompetent murine model of anaplastic thyroid cancer. *Oncotarget*, 2 March 2016, Vol. 7, No. 13, pages 17194-17211 [Retrieved on 2017-05-26] <DOI: 10.18632/ONCOTARGET.7839> (whole document)	1-27
Y	COOPER Z.A. ET AL., Response to BRAF Inhibition in Melanoma Is Enhanced When Combined with Immune Checkpoint Blockade. *Cancer Immunology Research*, 29 April 2014, Vol. 2, No. 7, pages 643-654 [Retrieved on 2017-05-26] <DOI: 10.1158/2326-6066.CIR-13-0215> (whole document)	1-27

☒ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

*Special categories of cited documents:

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
26/05/2017 *(day/month/year)*	09/06/2017 *(day/month/year)*

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2017/023539

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	HU-LIESKOVAN S. ET AL., Combining Targeted Therapy With Immunotherapy in BRAF-Mutant Melanoma: Promise and Challenges. *Journal of clinical oncology*, 20 July 2014, Vol. 32, No. 21, pages 2248-2254 [Retrieved on 2017-05-26] <DOI: 10.1200/JCO.2013.52.1377> (whole document)	1-27
Y	WO 2014/193898 A1 (MERCK SHARP & DOHME CORP. & GLAXOSMITHKLINE LLC) 4 December 2014 (whole document, in particular, claims 1-24)	1-27
Y	WO 2015/148828 A1 (MILLENNIUM PHARMACEUTICALS, INC.) 1 October 2015 (paragraphs [0003]-[0006], [0008], [0023], [0038]-[00141], [00255]-[00276], [00287], [00312]-[00317]; Examples 13 & 14)	1-27
Y	NAKAMURA A. ET AL., Antitumor Activity of the Selective Pan-RAF Inhibitor TAK-632 in BRAF Inhibitor-Resistant Melanoma. *Cancer Research*, 11 October 2013, Vol. 73, No. 23, pages OF1-OF13 [Retrieved on 2017-05-26] <DOI: 10.1158/0008-5472.CAN-13-1825> (whole document)	1-27
Y	US 2015/0126533 A1 (ARITA T. ET AL.) 7 May 2015 (paragraphs [0004]-[0007], [0019], [0022], [0023], [0045] & [0063]; Table 3)	1-27
Y	WO 2015/095819 A2 (BIOMED VALLEY DISCOVERIES, INC.) 25 June 2015 (paragraphs [0013]-[0016], [0082]-[0087], [0101]-[0103], [0114], [0115], [0151] & [0259]-[0264]; Table 1; Example 8; Figures 47, 48, 53, 54; claims 61, 71, 80 & 90)	1-27
A	WO 2009/006389 A2 (SUNESIS PHARMACEUTICALS, INC.) 8 January 2009 (whole document)	-
A	WO 2010/064722 A1 (TAKEDA PHARMACEUTICAL COMPANY LIMITED) 10 June 2010 (whole document)	-
A	LLIEVA K.M. ET AL., Effects of BRAF mutations and BRAF inhibition on immune responses to melanoma. *Molecular Cancer Therapeutics*, 10 November 2014, Vol. 13, No. 12, pages 2769–2783 [Retrieved on 2017-05-26] <DOI: 10.1158/1535-7163.MCT-14-0290> (whole document)	-
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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/US2017/023539

Note: This Annex lists known patent family members relating to the patent documents cited in this International Search Report. This Authority is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		US 2010/216810 A1	26/08/2010