Abstract:
The present disclosure describes combination therapies useful for the treatment of cancer. In particular, the invention relates to a combination therapy which comprises a PD-1 axis binding antagonist and an ALK inhibitor for treating ALK-negative cancer.
COMBINATION OF A PD-1 AXIS BINDING ANTAGONIST AND AN ALK INHIBITOR FOR TREATING ALK-NEGATIVE CANCER

This application claims the benefit of U. S. Provisional Application No. 62/235,406 filed September 30, 2015, U. S. Provisional Application No. 62/385,430 filed September 9, 2016, and U.S. Provisional Application No. 62/393,609 filed September 12, 2016, the contents of which are hereby incorporated by reference in their entireties.

FIELD OF THE INVENTION

The present invention relates to combination therapies useful for the treatment of cancer. In particular, the invention relates to a combination therapy which comprises a PD-1 axis binding antagonist and an ALK inhibitor for treating cancer, including both ALK-positive and ALK-negative cancer.

BACKGROUND OF THE INVENTION

The mechanism of co-stimulation of T-cells has gained significant therapeutic interest in recent years for its potential to enhance cell-based immune response. Co-stimulatory molecules expressed on antigen-presenting cells (APCs) promote and induce T-cells to promote clonal expansion, cytokine secretion and effector function. In the absence of co-stimulation, T-cells can become refractory to antigen stimulation, do not mount an effective immune response, and further may result in exhaustion or tolerance to foreign antigens. Lenschow et al., Ann. Rev. Immunol. 14:233 (1996). Recently, it has been discovered that T cell dysfunction or anergy occurs concurrently with an induced and sustained expression of the inhibitory receptor, programmed death 1 polypeptide (PD-1). As a result, therapeutic targeting of PD-1 and other molecules which signal through interactions with PD-1, such as programmed death ligand 1 (PD-L1) and programmed death ligand 2 (PD-L2) are an area of intense interest. PD-L1 is overexpressed in many cancers and is often associated with poor prognosis (Okazaki T et al., Intern. Immun. 2007 19(7):813) (Thompson RH et al., Cancer Res 2006, 66(7):3381). Interestingly, the majority of tumor infiltrating T lymphocytes predominantly express PD-1, in contrast to T lymphocytes in normal
tissues and peripheral blood T lymphocytes indicating that up-regulation of PD-1 on tumor-reactive T cells can contribute to impaired antitumor immune responses (Ahmadzadeh et al, Blood 2009 1 14(8): 1537). This may be due to exploitation of PD-L1 signaling mediated by PD-L1 expressing tumor cells interacting with PD-1 expressing T cells to result in attenuation of T cell activation and evasion of immune surveillance (Sharpe et al., Nat Rev 2002) (Keir ME et al., 2008 Annu. Rev. Immunol. 26:677). Therefore, inhibition of the PD-L1/PD-1 interaction may enhance CD8+ T cell-mediated killing of tumors.

The inhibition of PD-1 axis signaling through its direct ligands (e.g., PD-L1, PD-L2) has been proposed as a means to enhance T cell immunity for the treatment of cancer (e.g., tumor immunity). Moreover, similar enhancements to T cell immunity have been observed by inhibiting the binding of PD-L1 to the binding partner B7-1. Furthermore, combining inhibition of PD-1 signaling with other pathways would further optimize therapeutic properties.

Anaplastic lymphoma kinase (ALK) is a member of the receptor tyrosine kinase superfamily, and at an amino acid sequence level is most closely related to members such as c-ros oncogene 1 (ROS1), leukocyte tyrosine kinase (LTK), the insulin receptor and c-Met (hepatic growth factor receptor) (Kostich M et al, Genome Biology 2002, 3, 1-12). ALK is largely expressed in the developing nervous system (Iwahara T et al, Oncogene 1997, 14, 439-449). Its relative abundance does tend to decrease in the adult animal, though its expression is maintained in certain regions of the brain, spinal cord and the eye (Vernersson et al., Gene Expression Patterns 2006, 6, 448-461).

ALK also has an important role in oncology (Webb TR et al, Expert Reviews in Anticancer Therapy 2009 9 331 -355). Point mutations in the full length ALK enzyme that lead to activation of the enzyme, and also increase in expression of the full length enzyme, have both been shown to lead to neuroblastoma (Ogawa S et al., Cancer Sci 2011 102:302-308). In addition, the fusion of ALK with other proteins due to genetic translocation events has also been shown to lead to activated kinase domain associated with cancer. A number of such ALK translocations leading to gene fusions are seen in lymphomas, the most prevalent being the nucleophosmin (NPM)-ALK fusion seen in anaplastic large cell lymphomas (ALCL). ALK fusion with EML4 leads to a chimeric protein (EML4-ALK) responsible for 2-7% of non-small cell lung carcinomas (NSCLC) (Soda M et al, Nature 2007 448 561 -567).
ALK inhibitors have been approved for the treatment of ALK-positive metastatic non-small cell lung cancer (NSCLC). However, since the EML4-ALK fusion kinase is aberrantly activated in only a small population of NSCLC carcinomas, there remains a need for optimal therapy for treating, stabilizing, preventing, and/or delaying development of various cancers in ALK-negative cancers. An optimal therapeutic treatment would provide combine blockade of PD-1 receptor/ligand interaction with an ALK inhibitor to bring further therapeutic benefit to patients suffering from cancers including both ALK-positive and ALK-negative cancers.

In addition to their direct activity on ALK-positive tumor cells, ALK inhibitors may affect the signaling and interaction of immune cells, including T cells and dendritic cells (DC). Without wishing to be bound by theory, it is believed that ALK inhibitors may exert immunomodulatory effects either directly or indirectly, e.g., by inhibition of wild-type ALK expressed on immune cells, or by stimulating or inhibiting the effects of downstream signaling pathways in immune cells. Alternately, it is possible that wild-type ALK activity is associated with immune suppressive mechanisms mediated by non-immune cells such as tumor cells and/or stromal cells. Thus, the combination of an ALK inhibitor and a PD-1 axis binding antagonist may provide beneficial effects even in ALK-negative cancers.

All references, publications, and patent applications disclosed herein are hereby incorporated by reference in their entirety.

**SUMMARY OF THE INVENTION**

The present invention describes a combination treatment comprising an ALK inhibitor and a PD-1 axis binding antagonist in cancer, including both ALK-positive and ALK-negative cancers.

Provided herein are methods for treating cancer or delaying progression of cancer, including both ALK-positive as well as ALK-negative cancers in an individual comprising administering to the individual an effective amount of a PD-1 axis binding antagonist and an ALK inhibitor. ALK-positive cancers include any cancer that tests positive for an activating ALK aberration such as an ALK fusion event or an oncogenic ALK mutation, and can also include cancers which have a c-Met amplification and/or ROS1 fusion and are otherwise known to have an alteration in c-Met or ROS1. Also provided herein are methods for treating or delaying progression of ALK-negative
cancers which also do not have c-Met amplification and/or ROS1 fusion and/or have no known alterations in either c-Met or ROS1. For example, in some embodiments the ALK-negative cancer will also have no known c-Met or ROS1 alterations, which have been predicted to exhibit sensitivity to an ALK inhibitor such as crizotinib alone. Zou HY et al. (2007, May 1) Cancer Res. 1;67(0):4408-17; Shaw AT et al. (2014, Nov 20). N.Engl.J.Med.; 371 (21): 1963-71.

Also provided herein is use of a PD-1 axis binding antagonist in the manufacture of a medicament for treating or delaying progression of cancer, including either an ALK-positive or an ALK-negative cancer, in an individual in combination with an ALK inhibitor. Also provided herein is use of an ALK inhibitor in the manufacture of a medicament for treating or delaying progression of an ALK-positive or ALK-negative cancer in an individual in combination with a PD-1 axis binding antagonist. Also provided herein is use of a PD-1 axis binding antagonist and an ALK inhibitor in the manufacture of medicaments for treating or delaying progression of an ALK-positive or ALK-negative cancer in an individual. Also provided herein is a process of manufacturing medicaments for treating or delaying progression of an ALK-positive or ALK-negative cancer in an individual, characterized by the use of a PD-1 axis binding antagonist and an ALK inhibitor. Also provided herein is a PD-1 axis binding antagonist for use in combination with an ALK inhibitor for treating or delaying progression of an ALK-negative cancer in the individual. Also provided herein is an ALK inhibitor for use in combination with a PD-1 axis binding antagonist for treating or delaying progression of an ALK-positive or ALK-negative cancer in the individual.

The ALK-positive cancer may be any cancer that tests positive for an activating ALK aberration such as an ALK fusion event or an oncogenic ALK mutation, including but not limited to EML4-ALK, KIF5B-ALK, TFG-ALK, KLC1-ALK, NPM-ALK, TMP3-ALK, TPM4-ALK, ATIC-ALK or CLTC-ALK. An individual who is diagnosed with an ALK-positive cancer will have a cancer which tests positive for any of the above-identified oncogenic mutations. The ALK-positive cancer may be a solid tumor. In some embodiments of the above treatment method, medicaments and uses of the invention, the cancer is a solid tumor and in some embodiments, the ALK-positive solid tumor is a melanoma, colon cancer, bladder cancer, breast cancer, clear cell kidney cancer, head/neck squamous cell carcinoma, rectal cancer, lung squamous cell carcinoma, thyroid cancer, bladder cancer, cervical cancer, uterine cancer, endometrial cancer, lung
adenocarcinoma, ovarian cancer, papillary kidney cancer, non-small-cell lung cancer (NSCLC), ovarian cancer, pancreatic cancer, prostate cancer, renal cell cancer, small-cell lung cancer (SCLC) and triple negative breast cancer. In some embodiments, the ALK-positive cancer may be at early stage or at late stage, and/or is an advanced or metastatic solid tumor malignancy. In some embodiments, the individual treated is a human. Moreover, ALK-positive cancers may include cancers which have a c-Met amplification and/or ROS1 fusion and are otherwise known to have an alteration in c-Met or ROS1.

By contrast, the ALK-negative cancer may be any cancer that does not test positive for an activating ALK aberration such as an ALK fusion event or an oncogenic ALK mutation, including but not limited to EML4-ALK, KIF5B-ALK, TFG-ALK, KLC1-ALK, NPM-ALK, TMP3-ALK, TPM4-ALK, ATIC-ALK or CLTC-ALK. An individual who is diagnosed with an ALK-negative cancer will have a cancer which tests negative for any of the above-identified oncogenic mutations, but may otherwise express wild-type ALK.

The ALK-negative cancer may be a solid tumor. In some embodiments of the above treatment method, medicaments and uses of the invention, the cancer is a solid tumor and in some embodiments, the ALK-negative solid tumor is a melanoma, colon cancer, bladder cancer, breast cancer, clear cell kidney cancer, head/neck squamous cell carcinoma, rectal cancer, lung squamous cell carcinoma, thyroid cancer, bladder cancer, cervical cancer, uterine cancer, endometrial cancer, lung adenocarcinoma, ovarian cancer, papillary kidney cancer, non-small-cell lung cancer (NSCLC), ovarian cancer, pancreatic cancer, prostate cancer, renal cell cancer, small-cell lung cancer (SCLC) and triple negative breast cancer. In some embodiments, the ALK-negative cancer may be at early stage or at late stage, and/or is an advanced or metastatic solid tumor malignancy.

In some embodiments, the individual treated is a human.

In some embodiments, the treatment results in sustained response in the individual after cessation of the treatment. In some embodiments, the treatment produces a complete response, a partial response, or stable disease in the individual.

Also provided herein are methods of enhancing immune function in an individual having an ALK-positive or ALK-negative cancer comprising administering an effective amount of a PD-1 axis binding antagonist and an ALK inhibitor.

Also provided herein is use of a PD-1 axis binding antagonist in the manufacture of a medicament for enhancing immune function in an individual having an ALK-positive
or ALK-negative cancer in combination with an ALK inhibitor. Also provided herein is
use of an ALK inhibitor in the manufacture of a medicament for enhancing immune
function in an individual having an ALK-positive or ALK-negative cancer in combination
with a PD-1 axis binding antagonist. Also provided herein is use of a PD-1 axis binding
antagonist and an ALK inhibitor in the manufacture of medicaments for enhancing
immune function in the individual having an ALK-positive or ALK-negative cancer. Also
provided herein is a manufacturing process of medicaments for enhancing immune
function in an individual, characterized by the use of a PD-1 axis binding antagonist and
an ALK inhibitor. Also provided herein is a PD-1 axis binding antagonist for use in
combination with an ALK inhibitor for enhancing immune function in the individual
having an ALK-positive or ALK-negative cancer. Also provided herein is an ALK
inhibitor for use in combination with a PD-1 axis binding antagonist for enhancing
immune function in the individual having an ALK-positive or ALK-negative cancer. In
some embodiments, the individual is a human.

In some embodiments, the PD-1 axis binding antagonist is a PD-1 binding
antagonist, a PD-L1 binding antagonist or a PD-L2 binding antagonist. In some
embodiments, the PD-1 binding antagonist inhibits binding of PD-1 to PD-L 1 and/or
binding of PD-1 to PD-L2. In some embodiments, the PD-1 binding antagonist is an
antibody (e.g., nivolumab, pembrolizumab, pidilizumab, mAb15 or mAb7 described
herein), an antigen binding fragments thereof, an immunoadhesin, a fusion protein, or
an oligopeptide. In some embodiments, the PD-1 binding antagonist is an
immunoadhesin comprising a PD-L2 extracellular domain fused to a Fc domain (e.g.,
AMP-224 described herein). In some embodiments, the PD-L1 binding antagonist
inhibits binding of PD-L1 to PD-1 and/or binding of PD-L 1 to B7-1. In some
embodiments, the PD-L 1 binding antagonist is an antibody (e.g., antibody
MDX-1105, MEDI4736 or MSB0010718C described herein), an antigen
binding fragments thereof, an immunoadhesin, a fusion protein, or an oligopeptide. In
some embodiments, the PD-L2 binding antagonist inhibits binding of PD-L2 to PD-1. In
some embodiments, the PD-L2 binding antagonist is an antibody, an antigen binding
fragments thereof, an immunoadhesin, a fusion protein, or an oligopeptide.

In some embodiments, the ALK inhibitor is a compound selected from the group
consisting of crizotinib (Pfizer; Xalkori®, PF-02341066), ceritinib (Novartis; Zykadia™,
LDK378), alectinib (Roche/Chugai; Alecensa®, RO542802, CH542802), PF-06463922
(Pfizer), NVP-TAE684 (Novartis), AP261 13 (Ariad), TSR-01 1 (Tesaro), X-396 (Xcovery), CEP-37440 (Cephalon/Teva) and RXDX-1 0 1 (Igynta; NMS-E628, Nerviano). Specific ALK inhibitors of the invention include the ALK inhibitors crizotinib or PF-06463922.

In some embodiments, the ALK inhibitor is a compound which has additional inhibitory activity against cMet, ROS1, RON, LTK, Trk (TrkA, TrkB, TrkC) and/or insulin receptor. cMet is a tyrosine kinase receptor involved in the stimulation of cell scattering, invasion, protection from apoptosis and angiogenesis. Comoglio PM, Giordano S, Trusolino L (June 2008). Nature Reviews Drug Discovery 7 (6): 504-1 6. ROS1 is also a receptor tyrosine kinase with genetic rearrangements that have been implicated in glioblastoma, non-small cell lung cancer (NSCLC), cholangiocarcinoma, ovarian cancer, gastric adenocarcinoma, colorectal cancer, inflammatory myofibroblastic tumor, angiosarcoma, and epithelioid hemangioendothelioma. Davies KD, Doebele RC. (201 3, Aug) Clin Cancer Res. 1:19(1 5):4040-5. RON-receptor activation leads to activation of common receptor tyrosine kinase downstream-signaling pathways, and to activation of MAPK, PI3K and beta-catenin in tumor models. Leonis MA, Thobe MN, Waltz, SE (2007, Aug). Future Oncol., 3(4): 441 -8. LTK or leukocyte receptor tyrosine kinase has been reported to be overexpressed in leukemia. Maru,Y, Hirai H, Takakaku, F. (1990, May) Oncogene Res. 5(3): 199-204. Tropomyosin receptor kinase (Trk), or TrkA, TrkB, TrkC are tyrosine kinases and are receptors which activated by specific neurotrophins and are involved in neuronal signal transduction. Huang EJ, Reichardt LF (2003, Jul) Annu. Rev. Biochem. 72: 609-642.

In some embodiments, the ALK inhibitor is administered sequentially (at separate times) or concurrently (during the same time) relative to the PD-1 axis binding antagonist. In some embodiments, the ALK inhibitor is administered either continuously or intermittently. In some embodiments, the ALK inhibitor is administered before administration of the PD-1 axis binding antagonist, simultaneously with administration of the PD-1 axis binding antagonist, or after administration of the PD-1 axis binding antagonist. In some embodiments, the ALK inhibitor and the PD-1 axis binding antagonist are administered with different dosing frequency. In some embodiments, selecting a dosage regimen (also referred to herein as an administration regimen) for a combination therapy of the invention 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 individual being treated. Preferably, a dosage regimen maximizes the amount of each therapeutic agent delivered to the patient consistent with an acceptable level of side effects. Accordingly, the dose amount and dosing frequency of each biotherapeutic and chemotherapeutic agent in the combination depends in part on the particular therapeutic agent, the severity of the cancer being treated, and patient characteristics.


Biotherapeutic agents in a combination therapy of the invention 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, bi-monthly, etc. A total weekly dose is generally at least about 0.05 Mg/kg, 0.2 Mg/kg, 0.5 Mg/kg, 1 Mg/kg, 10 Mg/kg, 100 Mg/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. (2003) Cancer Immunol. Immunother. 52:1 33-144.

Dosage units for a PD-1 axis binding antagonist (e.g., pembrolizumab, nivolumab) may be expressed as a flat dose, i.e., 100 mg, 200 mg, 300 mg, or as a patient-specific
dose, i.e., mg/kg (mg therapeutic agent/kg of body weight) or mg/m² (quantity in milligrams per square meter of body surface area).

In some embodiments that employ an antibody, antibody fragment or fusion fusion soluble receptor as the PD-1 axis binding antagonist in the combination therapy, the may comprise administering the antibody at a dose of about 0.5, 1, 2, 3, 5 or 10 mg/kg at intervals of about 7 days (± 2 days) or 14 days (± 2 days) or about 21 days (± 2 days) or about 30 days (± 2 days) throughout the course of treatment. Alternately, in some embodiments that employ an antibody, antibody fragment or fusion fusion soluble receptor as the PD-1 axis binding antagonist in the combination therapy, the dosing regimen will comprise administering the antibody a dose of from about 0.005 mg/kg to about 10 mg/kg, with intra-patient dose escalation. In other escalating dose embodiments, the interval between doses will be progressively shortened, e.g., about 30 days (± 2 days) between the first and second dose, about 14 days (± 2 days) between the second and third doses. In certain embodiments, the dosing interval will be about 14 days (± 2 days), for doses subsequent to the second dose. In certain embodiments, the dosing interval will be about 7 days (± 2 days), for doses subsequent to the second dose.

In certain embodiments, a subject will be administered an intravenous (IV) infusion of a medicament comprising any of the PD-1 axis binding antagonists described herein.

In one embodiment of the invention, the PD-1 axis binding antagonist in the combination therapy is nivolumab pembrolizumab or avelumab (MSB001071 8C), which is administered intravenously or in a liquid dosage form at a dose selected from the group consisting of any one 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.

The optimal dose for a PD1 axis binding antagonist in combination with an ALK inhibitor may be identified by dose escalation of one or both of these agents. The ALK inhibitor may be administered orally (PO), either once daily (QD) or twice daily (BID), with or without food on a continuous schedule starting on Cycle 1 Day 1. A PD-1 axis binding antagonist such as avelumab may be administered as a 30-minute to 1-hr intravenous (IV) infusion every 2 weeks (Q2W), every 3 weeks (Q3W) or in case of dose reduction, every 4 weeks (Q4W), starting on Cycle 1 Day 1, except in the case of an ALK inhibitor
lead-in. On the day of ALK inhibitor administration, the ALK inhibitor may be given prior to or after administration of the PD-1 axis binding antagonist.

In another embodiment, an ALK inhibitor such as crizotinib can be administered at 250 mg BID, 200 mg BID, or 250 mg QD, and the PD-1 axis binding antagonist is administered at a starting dose of 2 mg/kg, or 5 mg/kg or 10 mg/kg, at a dosing interval of Q2W, Q3W or alternately Q4W. In one embodiment, the ALK inhibitor is administered at 250 mg BID for a 3-week lead-in period and then the PD-1 axis binding antagonist is administered at a starting dose of 2 mg/kg Q3W or 200 mg Q3W after the lead-in period. In another embodiment, the ALK inhibitor is administered at 250 mg BID and the PD-1 axis binding antagonist is administered at a starting dose of 2 mg/kg Q4W. In another embodiment, the ALK inhibitor is administered at 200 mg BID and PD-1 axis binding antagonist is administered at a starting dose of 2 mg/kg Q3W. In another embodiment, the ALK inhibitor is administered at 200 mg BID and the PD-1 axis binding antagonist is administered at a starting dose of 2 mg/kg Q4W. In another embodiment, the ALK inhibitor is administered at 250 mg QD and MK-3475 is administered at a starting dose of 2 mg/kg Q3W. In another embodiment, the ALK inhibitor is administered at 250 mg QD and the PD-1 axis binding antagonist is administered at a starting dose of 2 mg/kg Q4W.

In some embodiments, the patient is treated with a 3-week lead-in period of single-agent ALK inhibitor directly preceding the combination administration of the ALK inhibitor and PD-1 axis binding antagonist.

In some embodiments, a treatment cycle begins with the first day of combination treatment and last for 3 weeks. In such embodiments, the combination therapy is preferably administered for at least 18 weeks (6 cycles of treatment), more preferably at least 24 weeks (8 cycles of treatment), and even more preferably at least 2 weeks after the patient achieves a CR.

In some embodiments, the dose of ALK inhibitor is increased up to a maximum dose of 250 mg BID if the patient tolerates the combination treatment at a lower total dose of ALK inhibitor.

In some embodiments, the ALK inhibitor in the combination therapy is crizotinib, PF-06463922, ceritinib or alectinib. In some embodiments, an ALK inhibitor such as PF-06463922 is administered orally at a dose of 100 mg QD, 75 mg QD or 50 mg QD. In
some such embodiments, the ALK inhibitor is ceritinib, which is administered orally at a
dose of 750 mg QD. In other such embodiments, the ALK inhibitor is alectinib, which is
administered orally at a dose of 300 mg BID.

In another aspect, provided is a kit comprising a first container, a second
container and a package insert, wherein the first container comprises at least one dose
of a medicament of a PD-1 axis binding antagonist, and the second container
comprises at least one dose of an ALK inhibitor for treating or delaying progression of
an ALK-positive or ALK-negative cancer in an individual or enhancing immune function
in an individual having an ALK-positive or ALK-negative cancer. The kit may comprise a
PD-1 axis binding antagonist and a package insert comprising instructions for using the
PD-1 axis binding antagonist in combination with an ALK inhibitor to treat or delay
progression of an ALK-positive or ALK-negative cancer in an individual, or enhancing
immune function in an individual having an ALK-positive or ALK-negative cancer. The
kit may comprise an ALK inhibitor and a package insert comprising instructions for
using the ALK inhibitor in combination with a PD-1 axis binding antagonist to treat or
delay progression of an ALK-positive or ALK-negative cancer in an individual, or to
enhance immune function in an individual having an ALK-positive or ALK-negative
cancer. The kit may comprise a PD-1 axis binding antagonist and an ALK inhibitor, and
a package insert comprising instructions for using the PD-1 axis binding antagonist and
the ALK inhibitor to treat or delay progression of an ALK-positive or ALK-negative
cancer in an individual, or to enhance immune function in an individual having an ALK-
positive or ALK-negative cancer. The kit may further comprise instructions stating that
the medicaments are intended for use in treating an individual having a cancer that
tests negative for an activating ALK aberration such as an ALK fusion event or
oncogenic ALK mutation, such as EML4-ALK, KIF5B-ALK, TFG-ALK, KLC1-ALK, NPM-
ALK, TMP3-ALK, TPM4-ALK, ATIC-ALK or CLTC-ALK.

1. General Techniques

The techniques and procedures described or referenced herein are generally
well understood and commonly employed using conventional methodology by those
skilled in the art, such as, for example, the widely utilized methodologies described in
"ALK" means anaplastic lymphoma kinase receptor tyrosine kinase. All references to ALK herein will be understood to include references to both ALK and to oncogenic variants thereof, including ALK fusions (including without limitation EML4-ALK, KIF5B-ALK, TFG-ALK, KLC1-ALK and NPM-ALK) and selected oncogenic mutations of ALK.

"ALK inhibitor" means an inhibitor of anaplastic lymphoma kinase (ALK) and/or its oncogenic variants, i.e., ALK fusions and selected oncogenic mutations of ALK. Frequently, the inhibitor is a small molecule inhibitor of ALK. ALK inhibitors of the invention may also provide inhibition activity against c-Met, ROS1, RON, Trk, LTK and/or the insulin receptor.

"ALK-positive cancer" means any cancer which tests positive for an activating ALK aberration, such as an ALK fusion event or oncogenic ALK mutation, including but not limited to, EML4-ALK, KIF5B-ALK, TFG-ALK, KLC1-ALK, NPM-ALK, TMP3-ALK, TPM4-ALK, ATIC-ALK or CLTC-ALK. An individual who is diagnosed with or identified as having...
an ALK-positive cancer will have a cancer which tests positive for activating ALK aberrations.

Conversely, "ALK-negative cancer" means any cancer which tests negative for an activating ALK aberration, such as an ALK fusion event or oncogenic ALK mutation, including but not limited to, EML4-ALK, KIF5B-ALK, TFG-ALK, KLC1-ALK, NPM-ALK, TMP3-ALK, TPM4-ALK, ATIC-ALK or CLTC-ALK. An individual who is diagnosed with or identified as having an ALK-negative cancer will have a cancer which tests negative for activating ALK aberrations, but may otherwise express wild-type ALK. In some embodiments, wild-type ALK is expressed in the tumor microenvironment, or in any of the immune cells including but not limited to T cells, B cells, NK cells, dendritic cells, macrophages, myeloid-derived suppressor cells and/or in tumor and/or stromal cells.

In some embodiments, an individual who is diagnosed with or identified as having an ALK-negative cancer may furthermore express any one of or a combination of wild-type c-Met, ROS1, RON, LTK, Trk (TrkA, TrkB, TrkC), and/or insulin receptor in the tumor microenvironment, or in any of the immune cells including but limited to T cells, B cells, NK cells, dendritic cells, macrophages, myeloid-derived suppressor cells and/or in tumor and/or stromal cells.

A variety of methods for the detection of ALK aberrations have been described. (Shackelford RE et al., Genes Cancer, 2014, 5(1-2): 1-14). In some embodiments, status of the ALK gene is assessed using an FDA approved test. In some embodiments, the status of the ALK gene is detected using an assay that analyzes ALK or ALK fusion polynucleotides, such as the Vysis ALK Break Apart FISH Probe Kit (available from Abbott Molecular), or such as assays employing RT-PCR or Next Generation sequencing (NGS) technology. In other embodiments, the status of the ALK gene is inferred based on ALK expression, which is detected using a diagnostic anti-ALK antibody, or antigen binding fragment thereof, in an IHC assay on an FFPE or frozen tissue section of a tumor sample removed from a patient afflicted with cancer.

Specific ALK inhibitors useful as the ALK inhibitor in the treatment methods, medicaments and uses of the present invention, include crizotinib (Pfizer; Xalkori®, PF-02341 066), with the structure described in WHO Drug Information, Vol. 25, No. 1, page 54 (2011); ceritinib (Novartis; Zykdia™, LDK378), with the structure described in WHO
Drug Information, Vol. 28, No. 1, page 79 (2014); and alectinib (Roche/Chugai; Alecensa®, RO542802, CH542802), with the structure described in *WHO Drug Information*, Vol. 27, No. 3, page 70 (2013). In a preferred embodiment, the ALK inhibitor useful in the treatment methods, medicaments and uses of the present invention is crizotinib.

Additional examples of ALK inhibitors include, for example, PF-06463922 (Pfizer), NVP-TAE684 (Novartis), AP26113 (Ariad), TSR-011 (Tesaro), X-396 (Xcovery), CEP-37440 (Cephalon/Teva) and RXDX-101 (Ignyta; NMS-E628, Nerviano). (Wang et al., *Med. Chem. Commun.* 2014, 5:1266)

In an embodiment of the treatment methods, medicaments and uses of the present invention, the ALK inhibitor is the compound, 3-[(1R)-1-(2,6-dichloro-3-fluorophenyl)ethoxy]-5-(1-piperidin-4-ylpyrazol-4-yl)pyridin-2-amine, which is known as crizotinib or PF-02341066, having the following structure:

![Chemical Structure](image)

or a pharmaceutically acceptable salt thereof.

Crizotinib is an inhibitor of anaplastic lymphoma kinase (ALK) and its oncogenic variants (i.e., ALK fusion events and selected oncogenic ALK mutations), as well as the hepatocyte growth factor receptor (HGFR, c-Met), c-ros oncogene 1 (ROS1) and its oncogenic variants, Recepteur d’Origine Nantais (RON) receptor tyrosine kinases (RTKs), LTK, Trk (TrkA, TrkB, TrkC), and/or insulin receptor

Xalkori® (crizotinib) has been approved in the United States for the treatment of patients with metastatic non-small cell lung cancer (NSCLC) whose tumors are anaplastic lymphoma kinase (ALK)-positive as detected by an FDA-approved test, and has also been approved for the treatment of ALK-positive NSCLC in Europe, Japan and other jurisdictions.
Crizotinib, as well as pharmaceutically acceptable salts thereof, is described in International Publication Nos. WO 2006/021 884, WO 2006/021 881 and WO 2007/0661 85, and in U.S. Patent Nos. 7,858,643, 8,217,057 and 8,785,632. The use of crizotinib in treating abnormal cell growth, such as cancers, mediated by ALK or c-MET/HGFR is described in WO 2007/0661 7 and U.S. Patent No. 7,825,1 37. The use of crizotinib in treating ROS mediated cancers is described in WO201 3/01 7989. The contents of each of the foregoing patents and applications are incorporated herein by reference in their entirety. In another embodiment of the treatment methods, medicaments and uses of the present invention, the ALK inhibitor is the compound, (10fi)-7-amino-12-fluoro-2,10,16-trimethyl-15-oxo-1 0,15,16,1 7-tetrahydro-2H-8,4-(metheno)pyrazolo[4,3 -f][2,5,1 1]benzoxadiazacyclotetradecine-3-carbonitrile, which is known as PF-06463922, having the following structure:

![Chemical Structure](image-url)

or a pharmaceutically acceptable salt thereof.

PF-06463922 is a potent, macrocyclic inhibitor of both wild type and resistant mutant forms of ALK and c-ros oncogene 1 (ROS1) receptor tyrosine kinase. The preparation of PF-06463922 is disclosed in International Patent Publication No. WO201 3/1 32376 and in United States Patent No. 8,680,1 11. Solvates of PF-06463922 are disclosed in International Patent Publication No. WO 2014/207606. The contents of each of the foregoing patents and applications are incorporated herein by reference in their entirety.

References to crizotinib and PF-06463922 herein are understood to include references to the pharmaceutically acceptable salts and/or solvates thereof, unless otherwise indicated. The term "PD-1" refers to any form of PD-1 and variants thereof that retain at least part of the activity of PD-1. Alternative names for "PD-1" include CD279 and SLEB2. An exemplary human PD-1 is found as Uniprot Accession Number Q15116 (SEQ ID NO: 1). The term "PD-L1" refers to the ligand 1 binding partner and variants thereof. Alternative names for "PD-L1" include B7-H1, B7-4, CD274, and B7-H. An
exemplary human PD-L1 is found as Uniprot Accession Number Q9NZQ7 (SEQ ID NO: 2). The term "PD-L2" refers to the ligand 2 binding partner and variants thereof. Alternative names for "PD-L2" include B7-DC, Btdc, and CD273. An exemplary human PD-L2, is found as Uniprot Accession Number Q9BQ51 (SEQ ID NO: 3).

The term "PD-1 axis binding antagonist" is a molecule that inhibits the interaction of a PD-1 axis binding partner with either one or more of its binding partner, so as to remove T-cell dysfunction resulting from signaling on the PD-1 signaling axis - with a result being to restore or enhance T-cell function (e.g., proliferation, cytokine production, target cell killing). As used herein, a PD-1 axis binding antagonist includes a PD-1 binding antagonist, a PD-L1 binding antagonist and a PD-L2 binding antagonist.

The term "PD-1 binding antagonists" is a molecule that decreases, blocks, inhibits, abrogates or interferes with signal transduction resulting from the interaction of PD-1 with one or more of its binding partners, such as PD-L1, PD-L2. In some embodiments, the PD-1 binding antagonist is a molecule that inhibits the binding of PD-1 to its binding partners. In a specific aspect, the PD-1 binding antagonist inhibits the binding of PD-1 to PD-L1 and/or PD-L2. For example, PD-1 binding antagonists include anti-PD-1 antibodies, antigen binding fragments thereof, immunoadhesins, fusion proteins, oligopeptides and other molecules that decrease, block, inhibit, abrogate or interfere with signal transduction resulting from the interaction of PD-1 with PD-L1 and/or PD-L2. In one embodiment, a PD-1 binding antagonist reduces the negative co-stimulatory signal mediated by or through cell surface proteins expressed on T lymphocytes mediated signaling through PD-1 so as render a dysfunctional T-cell less dysfunctional (e.g., enhancing effector responses to antigen recognition). In some embodiments, the PD-1 binding antagonist is an anti-PD-1 antibody. In a specific aspect, a PD-1 binding antagonist is nivolumab (MDX-1106) described herein. In another specific aspect, a PD-1 binding antagonist is pembrolizumab (Keytruda®, Merck Inc., formerly known as MK-3475) described herein. In another specific aspect, a PD-1 binding antagonist is pidilizumab (CT-011, Curetech) described herein. In another specific aspect, a PD-1 binding antagonist is the antibody comprising a VH region produced by the expression vector with ATCC Accession No. PTA-121183 and having the VL region produced by the expression vector with ATCC Accession No. PTA-121182, also known as mAb7 or mAb15 (Rinat Neuroscience, Pfizer Inc) as described herein. In one embodiment, the PD-1 binding antagonist is an anti-PD-1 antibody comprising a heavy chain variable
region comprising the heavy chain variable region amino acid sequence from SEQ ID NO:4 and/or a light chain variable region comprising the light chain variable region amino acid sequence from SEQ ID NO:6. In a still further aspect, the PD-1 binding antagonist is an anti-PD-1 antibody comprising a heavy chain and/or a light chain sequence, wherein:

(a) the heavy chain sequence has at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to the mAb7 full length heavy chain sequence SEQ ID NO: 4 shown below:

QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYWINWVRQAPGQGLEWMGNIYPGSS
LTNYNEKFKVRMTYMDSTSTSTYMELSSQLSDELVYQAVYCARLSTGTFAYWGQGTTLV
TVSSASTKGPSVFLPFLPSVPSVSLGVTQTYCTNCRTKPSNTKVDKRQVEKGYGPPCPCPA
PEFLGGPSVFLFPPKDTLISRTPEVTCVVVDSDGSFNYAEGTVQNSDEPEVQFNWYVDGEVHNAK
TKPREEQFNYSTYRVSVLHQLDWNLNQEGKCKVSNKGLPSSIEKTISKAKGQPQP

(b) the light chain sequence has at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to the mAb7 full-length light chain sequence, SEQ ID NO: 6, shown below:

DIVMTQSPDSLAVSLGERATINCKSSQSLWDSGNQKNFLTWYQQKPQPPKLLIYWT
SYRESGVPRFSGSGSTDFLTITSSQAEVAVYCCNDYFYHPTHTFGGTKVEIKRG
TVAAPSFVIPPSDEQLKSGTASVVCLNNSPYPREAQKSHQWKVDNALQSGNSQESVTE
QDSKDESTYSLSTLSLTLSKADYEKHKVYACEVTHQGLSSPVTSFNRGEC (SEQ ID NO: 4); and/or

DIVMTQSPDSLAVSLGERATINCKSSQSLWDSGNQKNFLTWYQQKPQPPKLLIYWT
SYRESGVPRFSGSGSTDFLTITSSQAEVAVYCCNDYFYHPTHTFGGTKVEIKRG
TVAAPSFVIPPSDEQLKSGTASVVCLNNSPYPREAQKSHQWKVDNALQSGNSQESVTE
QDSKDESTYSLSTLSLTLSKADYEKHKVYACEVTHQGLSSPVTSFNRGEC (SEQ ID NO: 6).

The term "PD-L1 binding antagonists" is a molecule that decreases, blocks, inhibits, abrogates or interferes with signal transduction resulting from the interaction of PD-L1 with either one or more of its binding partners, such as PD-1, B7-1. In some embodiments, a PD-L1 binding antagonist is a molecule that inhibits the binding of PD-L1 to its binding partners. In a specific aspect, the PD-L1 binding antagonist inhibits binding of PD-L1 to PD-1 and/or B7-1. In some embodiments, the PD-L1 binding antagonists include anti-PD-L1 antibodies, antigen binding fragments thereof,
immunoadhesins, fusion proteins, oligopeptides and other molecules that decrease, block, inhibit, abrogate or interfere with signal transduction resulting from the interaction of PD-L1 with one or more of its binding partners, such as PD-1, B7-1. In one embodiment, a PD-L1 binding antagonist reduces the negative co-stimulatory signal mediated by or through cell surface proteins expressed on T lymphocytes mediated signaling through PD-L1 so as to render a dysfunctional T-cell less dysfunctional (e.g., enhancing effector responses to antigen recognition). In some embodiments, a PD-L1 binding antagonist is an anti-PD-L1 antibody. In a specific aspect, an anti-PD-L1 antibody is MPDL3280A (clone YW243.55.S70) described herein. In another specific aspect, an anti-PD-L1 antibody is MDX-1105 described herein. In yet another specific aspect, the anti-PD-L1 antibody is MEDI4736 as described herein. In yet another aspect, the anti-PD-L1 antibody is MSB0010718C as described herein.

The term "PD-L2 binding antagonists" is a molecule that decreases, blocks, inhibits, abrogates or interferes with signal transduction resulting from the interaction of PD-L2 with either one or more of its binding partners, such as PD-1. In some embodiments, a PD-L2 binding antagonist is a molecule that inhibits the binding of PD-L2 to its binding partners. In a specific aspect, the PD-L2 binding antagonist inhibits binding of PD-L2 to PD-1. In some embodiments, the PD-L2 antagonists include anti-PD-L2 antibodies, antigen binding fragments thereof, immunoadhesins, fusion proteins, oligopeptides and other molecules that decrease, block, inhibit, abrogate or interfere with signal transduction resulting from the interaction of PD-L2 with either one or more of its binding partners, such as PD-1. In one embodiment, a PD-L2 binding antagonist reduces the negative co-stimulatory signal mediated by or through cell surface proteins expressed on T lymphocytes mediated signaling through PD-L2 so as to render a dysfunctional T-cell less dysfunctional (e.g., enhancing effector responses to antigen recognition). In some embodiments, a PD-L2 binding antagonist is an immunoadhesin.

Table 1 below provides a list of the amino acid sequences of exemplary PD-1 axis binding antagonists for use in the treatment method, medicaments and uses of the present invention. CDRs are underlined for mAb7 and mAb15.
| SEQ ID NO: 4 | mAb7 or mAbl5 full-length heavy chain | OVOLVQSGAEVKPGASVKVSCKASGYTFTSYWINWVROAPG
QGLEWMGNNYPGSULTNYNEKFKNRVTMTRDTSTSTVYMELSS
LRSEDTAVYYCARLSTGTFAYWGOGLTVTVSSASTKGPSVFPLA
PCRSRTSESTAAAGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL
QSSGLYSLSSVTVPSSSLGTKTYYTCNVHDHPSNTKVDKRVESK
YGPPCPAPCPAEFLGGPSVFLFPPKDTLMSRTPEVTCVVVDVS
QEDPEVQFENWYVGVEVHNAKTKPREEQFNSTYRVVSVLTVLH
QDWLNGKEYKCVKSNKLPLSSIEKTISAKGQPREPQVYTLPPS
QEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPVL
DSDGSFFLYSRLTVDKSRWQEGNVFSCVMHEALHNHYTQKSL
SLSLGK |
| SEQ ID NO: 5 | mAb7 or mAb15 full-length heavy chain without the C-terminal lysine | OVOLVQSGAEVKPGASVKVSCKASGYTFTSYWINWVROAPG
QGLEWMGNNYPGSULTNYNEKFKNRVTMTRDTSTSTVYMELSS
LRSEDTAVYYCARLSTGTFAYWGOGLTVTVSSASTKGPSVFPLA
PCRSRTSESTAAAGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL
QSSGLYSLSSVTVPSSSLGTKTYYTCNVHDHPSNTKVDKRVESK
YGPPCPAPCPAEFLGGPSVFLFPPKDTLMSRTPEVTCVVVDVS
QEDPEVQFENWYVGVEVHNAKTKPREEQFNSTYRVVSVLTVLH
QDWLNGKEYKCVKSNKLPLSSIEKTISAKGQPREPQVYTLPPS
QEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPVL
DSDGSFFLYSRLTVDKSRWQEGNVFSCVMHEALHNHYTQKSL
SLSLGK |
| SEQ ID NO: 6 | mAb7 full-length light chain | DIVMTQSPDSLAVSLGERATINCKSSOSLWDSGNOKNFLTWYQ
KPGOPPKLIIYWTsyRESGVpDRFSGSGSTDFLTISSLOAEDEV
AVYYCONDYFYPHTGGGTKEIKRGTVAAPSIFFPSSDEOLKS
GTASVVCALLNFFPREAKVQWKDNLQSGNSQESVTEQDSDK
STYSLSSTLKSDYEHKVVACEVTHQGLSSPVTKSFNRGEC |
| SEQ ID NO: 7 | mAB7 light chain | OVOLVQSGAEVKPGASVKVSCKASGYTFTSYWINWVROAPG
QGLEWMGNNYPGSULTNYNEKFKNRVTMTRDTSTSTVYMELSS
LRSEDTAVYYCARLSTGTFAYWGOGLTVTVSS |
| SEQ ID NO: 8 | OVOLVOSGAEVKKPGASVKVSCKASGYTFTSYWINWVROAPG | QGLEWMMGNIWPGLTNFFJRVTMTDRTSTTTVYMELESS |
| mAB15 heavy chain | LRSEDTAVYYCARLLLTGTFAYWGOGLTVTVS |
| SEQ ID NO: 9 | DIVMTQPSDLSLGERATINCKSSQSLWDGSNQKNFTWYQ | QKPGQPPPILYYWTYSYRESGVPRDFSNGSGTDFLTITSSLOQAE |
| mAB15 light chain | DVAVYYQNDYFYPHTFGGGTKVE |
| SEQ ID NO: 10 | QVQLVESGGGWQPGRSLRLDCKAGITFSNSGMSKHVRQAPGKG | LEWVAVrWYDGSKRYYADSVKGRFTISRDNSKNTLFLQMNSLRL |
| Nivolumab, MDX1106, full length heavy chain | AEDTAVYYCATNDDYWQGTLTVSSASTKPSVFPLAPCSRST |
| | SESAALGCLVDYFPEPVTWSNSGALTSGVHTFPAVLQSSGLY |
| | SLSSVVTNPSSSLGTYYCTNDHDKPSNTKVRGESYGGPCPCCPA |
| | PEFLGGPSVFLFPPKPDITLMISRTPEVTCWVDVQEDPEVQFNW |
| | YYDGVEVHNAATKPREEQFNSTYRWVSLSLTVDLHQDWLNGKEYK |
| | CKVSNKGLPSSIEKTISAKAQRENPQVYTLPPS |
| | EEMTKQVSLTCLVKGFPYPSDIAVEWESNGQPEKNYKTTPVLSDGFSFLYSLRTVDKSRWQEGNVFSCSVMEALHNHYTQKSLSLSLGK |
| SEQ ID NO: 11 | EIVLTQPSATLSLSPGERATLSRASQSVYSLAWYQPGQAPRL | LITYASRNATGIPARFSGSGSDTDFLTISSLEPEDFAVYYCQSS |
| Nivolumab, MDX1106, full length light chain | NWPRTFQGKVEIRTVAAPSVFIFPSPDEQLSGTAHSVCLNNF |
| | YPREAVQWKVDNALQSGNSQESVTEQDSTYSLSTLTLKAD |
| | YEKHKVVACEVTHQGLSSPVTSFNRGEC |
| SEQ ID NO: | 12 | Pembrolizumab, MK3475, full length heavy chain | QVQLVQSGVEVKPGASVKVSCKASGYTFTNYMYWVRQA PGQGLEWMMGNNPSNGGTFNEKFKNRTLTTSSTTAYM ELKSLQFFDVTAVYCARRDYRFDGDGKYWGGGTGVTTVSSA STKGPVSFAPCSRTSESTAAALGCLVKDYFEPVTWNS GALTSGVHTFPALVQLQSLSSVVTVPSSSLGTKTYTCNV DHKPSNTKVDKRESKYGPCCPAPAEFGPLGSVEFLFPK PKDMLSMRTPETCVVDSQEDPEVQFNWYVDDGEVEVNA KTKPREEQFNSTYRRVSVLTHQDDLWNGKEYKCKVSNKGL PSSIIEKTISAKGQPREPQYTLPPSQEEMTKQVSLTCVK GFYPSDIAVEWESNGQPENNYKTPVLDSDGSFFLYSRTLTV DKSRRWQEGNVFSCSVMHEALTHQYKSLSLGK |
| SEQ ID NO: | 13 | Pembrolizumab, MK3475, full length light chain | EIVLTQSPATLSLSPGERATLSCRASKGVSTSGYSYLHWYQQ KPGQAPRLLLALASYLESGVPARFSGSGSSTGTDFTLTISSLEPE DFAVYYCQHSDRLPLTFGGGTKEIKRTAAPSVFIFPSDE QLKSgtasvcllnnFypreakvqvkvdnaLQSGNSQESVT EQDSKDMTSLSSTLSKADYEKHKVACEVTHQLSSPVTV KSFNRGEC |
| SEQ ID NO: | 14 | AMP224, without signal sequence | LFTVTVPKELYIEEHSNVTECNFTGHSVNLGAIATSLQKVEN DTSPHRERATLLEEQLPGKASHHPVQVRDEQGYYCIIIYGV A WDKYKLTLKVASYRKNTHILKVPDEVEELTCQATGYPLAEV SWPNVSPANTHSRTPEGLYQVTSRLKPPGRNFSCVFWNT HVRELTLASIDLQSMERPHTHPEMKSCDKTHTCCPAPELLG GPSVFLLFPKPDKTLMSRTPETCVWDVSHEDPEVKNWYVDG VEVHNAKTPREEQYNSTYRWSVLTHQDDLWNGKEYKCKVSNKALPAPIEKTISAKGQPREPQYTLPPSRDELTKQV SLTCVKGFFYPSDIAVEWESNGQPENNYKTPVLDSDGSFFLYSKLTVDKSRWQQGNYVCSVMHEALTHQYKSLSLGK |
| SEQ ID NO: 15 | YW243.55.S70 heavy chain | EVQLVESGGGLVQPGGSLRLSCAASGFTFSDSW1IHVRQAPGK
GLEWVAWISPYGGSTYYADSVKGRFTISADTSKNTAYLQMNSLR
AEDTAVYYCARRHWPGGFDYWQGTLVT/VS |
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| SEQ ID NO: 16 | YW243.55.S70 light chain | DIQMTQSPSSLSASVGDRVTITCRASQDVSTAVAWYQQKPGKAP
KLLIYSAFLYSVPSRFSGSGTDFTLTISSLQPEDFATYYCQQ
YLYH PATFGQGTKVEIKR |
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| SEQ ID NO: 17 | MSB0010718C heavy chain  | EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYMWVRQAPGKGL
EWVSIYPSGASFTYADKGRFTISRDSKNTLTYLQMNSLRAEDTA
VYCYCARLGT/VTTVT/DYWQ GTLVTSS |
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| SEQ ID NO: 18 | MSB0010718C light chain  | Q3ALTQPASVSGSPGQSITISCTGTSSDVGGYNSWYQQHPGK
APKLMYIDSNRPVSNSNGSRSOGKSGNTASLTISGLQAEDADYY
CSSYTSSSTRVFTGTGT/VTVL |
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The term "dysfunction" in the context of immune dysfunction, refers to a state of reduced immune responsiveness to antigenic stimulation. The term includes the common elements of both exhaustion and/or anergy in which antigen recognition may occur, but the ensuing immune response is ineffective to control infection or tumor growth.

The term "dysfunctional", as used herein, also includes refractory or unresponsive to antigen recognition, specifically, impaired capacity to translate antigen
recognition into downstream T-cell effector functions, such as proliferation, cytokine production (e.g., IL-2) and/or target cell killing.

The term "anergy" refers to the state of unresponsiveness to antigen stimulation resulting from incomplete or insufficient signals delivered through the T-cell receptor (e.g. increase in intracellular Ca$^{2+}$ in the absence of ras-activation). T cell anergy can also result upon stimulation with antigen in the absence of co-stimulation, resulting in the cell becoming refractory to subsequent activation by the antigen even in the context of costimulation. The unresponsive state can often be overridden by the presence of Interleukin-2. Anergic T-cells do not undergo clonal expansion and/or acquire effector functions.

The term "exhaustion" refers to T cell exhaustion as a state of T cell dysfunction that arises from sustained TCR signaling that occurs during many chronic infections and cancer. It is distinguished from anergy in that it arises not through incomplete or deficient signaling, but from sustained signaling. It is defined by poor effector function, sustained expression of inhibitory receptors and a transcriptional state distinct from that of functional effector or memory T cells. Exhaustion prevents optimal control of infection and tumors. Exhaustion can result from both extrinsic negative regulatory pathways (e.g., immunoregulatory cytokines) as well as cell intrinsic negative regulatory (costimulatory) pathways (PD-1, B7-H3, B7-H4, etc.).

"Enhancing T-cell function" means to induce, cause or stimulate a T-cell to have a sustained or amplified biological function, or renew or reactivate exhausted or inactive T-cells. Examples of enhancing T-cell function include: increased secretion of γ-interferon from CD8$^+$ T-cells, increased proliferation, increased antigen responsiveness (e.g., viral, pathogen, or tumor clearance) relative to such levels before the intervention. In one embodiment, the level of enhancement is as least 50%, alternatively 60%, 70%, 80%, 90%, 100%, 120%, 150%, 200%. The manner of measuring this enhancement is known to one of ordinary skill in the art.

A "T cell dysfunctional disorder" is a disorder or condition of T-cells characterized by decreased responsiveness to antigenic stimulation. In a particular embodiment, a T-cell dysfunctional disorder is a disorder that is specifically associated with inappropriate increased signaling through PD-1. In another embodiment, a T-cell dysfunctional disorder is one in which T-cells are anergic or have decreased ability to secrete cytokines, proliferate, or execute cytolytic activity. In a specific aspect, the decreased
responsiveness results in ineffective control of a pathogen or tumor expressing an immunogen. Examples of T have upcell dysfunctional disorders characterized by T-cell dysfunction include unresolved acute infection, chronic infection and tumor immunity.

"Tumor immunity" refers to the process in which tumors evade immune recognition and clearance. Thus, as a therapeutic concept, tumor immunity is "treated" when such evasion is attenuated, and the tumors are recognized and attacked by the immune system. Examples of tumor recognition include tumor binding, tumor shrinkage and tumor clearance.

"Immunogenicity" refers to the ability of a particular substance to provoke an immune response. Tumors are immunogenic and enhancing tumor immunogenicity aids in the clearance of the tumor cells by the immune response. Examples of enhancing tumor immunogenicity include treatment with anti-PDL antibodies and an ALK inhibitor.

"Sustained response" refers to the sustained effect on reducing tumor growth after cessation of a treatment. For example, the tumor size may remain to be the same or smaller as compared to the size at the beginning of the administration phase. In some embodiments, the sustained response has a duration at least the same as the treatment duration, at least 1.5X, 2.0X, 2.5X, or 3.0X length of the treatment duration.

The term "antibody" includes monoclonal antibodies (including full length antibodies which have an immunoglobulin Fc region), antibody compositions with polypitopic specificity, multispecific antibodies (e.g. bispecific antibodies, diabodies, and single-chain molecules, as well as antibody fragments (e.g., Fab, F(ab')2, and Fv). The term "immunoglobulin" (Ig) is used interchangeably with "antibody" herein.

The basic 4-chain antibody unit is a heterotetrameric glycoprotein composed of two identical light (L) chains and two identical heavy (H) chains. An IgM antibody consists of 5 of the basic heterotetramer units along with an additional polypeptide called a J chain, and contains 10 antigen binding sites, while IgA antibodies comprise from 2-5 of the basic 4-chain units which can polymerize to form polyvalent assemblages in combination with the J chain. In the case of IgGs, the 4-chain unit is generally about 150,000 daltons. Each L chain is linked to an H chain by one covalent disulfide bond, while the two H chains are linked to each other by one or more disulfide bonds depending on the H chain isotype. Each H and L chain also has regularly spaced intrachain disulfide bridges. Each H chain has at the N-terminus, a variable domain (VH) followed by three constant domains (CH) for each of the a and γ chains and four CH
domains for \( \mu \) and \( \varepsilon \) isotypes. Each L chain has at the N-terminus, a variable domain (VL) followed by a constant domain at its other end. The VL is aligned with the \( \text{VH} \) and the CL is aligned with the first constant domain of the heavy chain (CH 1). Particular amino acid residues are believed to form an interface between the light chain and heavy chain variable domains. The pairing of a VH and VL together forms a single antigen-binding site. For the structure and properties of the different classes of antibodies, see e.g., Basic and Clinical Immunology, 8th Edition, Daniel P. Sties, Abba Terr and Tristram G. Parsolw (eds), Appleton & Lange, Norwalk, CT, 1994, page 71 and Chapter 6. The L chain from any vertebrate species can be assigned to one of two clearly distinct types, called kappa and lambda, based on the amino acid sequences of their constant domains. Depending on the amino acid sequence of the constant domain of their heavy chains (CH), immunoglobulins can be assigned to different classes or isotypes. There are five classes of immunoglobulins: IgA, IgD, IgE, IgG and IgM, having heavy chains designated \( \alpha \), \( \delta \), \( \varepsilon \), \( \gamma \) and \( \mu \), respectively. The \( \gamma \) and \( \alpha \) classes are further divided into subclasses on the basis of relatively minor differences in the CH sequence and function, e.g., humans express the following subclasses: IgG1, IgG2A, IgG2B, IgG3, IgG4, IgA1 and IgA2.

The "variable region" or "variable domain" of an antibody refers to the amino-terminal domains of the heavy or light chain of the antibody. The variable domains of the heavy chain and light chain may be referred to as "VH" and "VL", respectively. These domains are generally the most variable parts of the antibody (relative to other antibodies of the same class) and contain the antigen binding sites.

The term "variable" refers to the fact that certain segments of the variable domains differ extensively in sequence among antibodies. The V domain mediates antigen binding and defines the specificity of a particular antibody for its particular antigen. However, the variability is not evenly distributed across the entire span of the variable domains. Instead, it is concentrated in three segments called hypervariable regions (HVRs) both in the light-chain and the heavy chain variable domains. The more highly conserved portions of variable domains are called the framework regions (FR).

The variable domains of native heavy and light chains each comprise four FR regions, largely adopting a beta-sheet configuration, connected by three HVRs, which form loops connecting, and in some cases forming part of, the beta-sheet structure. The HVRs in each chain are held together in close proximity by the FR regions and, with the
HVRs from the other chain, contribute to the formation of the antigen binding site of antibodies (see Kabat et al., Sequences of Immunological Interest, Fifth Edition, National Institute of Health, Bethesda, MD (1991)). The constant domains are not involved directly in the binding of antibody to an antigen, but exhibit various effector functions, such as participation of the antibody in antibody-dependent cellular toxicity.

The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally occurring mutations and/or post-translation modifications (e.g., isomerizations, amidations) that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic site. In contrast to polyclonal antibody preparations which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. In addition to their specificity, the monoclonal antibodies are advantageous in that they are synthesized by the hybridoma culture, uncontaminated by other immunoglobulins. The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by a variety of techniques, including, for example, the hybridoma method (e.g., Kohler and Milstein. Nature, 256:495-97 (1975); Hongo et al, Hybridoma, 14 (3): 253-260 (1995), Harlow et al., Antibodies: A Laboratory Manual, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988); Hammerling et al, in: Monoclonal Antibodies and T-Cell Hybridomas 563-681 (Elsevier, N.Y., 1981)), recombinant DNA methods (see, e.g., U.S. Patent No. 4,816,567), phage-display technologies (see, e.g., Clackson et al, Nature, 352: 624-628 (1991); Marks et al, J. Mol. Biol. 222: 581 -597 (1992); Sidhu et al, J. Mol. Biol 338(2): 299-310 (2004); Lee et al, J. Mol. Biol. 340(5): 1073-1093 (2004); Fellouse, Proc. Natl. Acad. Sci. USA 101 (34): 12467-12472 (2004); and Lee et al, J. Immunol. Methods 284(1 -2): 119-132 (2004), and technologies for producing human or human-like antibodies in animals that have parts or all of the human immunoglobulin loci or genes encoding human immunoglobulin sequences (see, e.g., WO 1998/24893; WO1996/34096; WO 1996/33735; WO 1991/10741; Jakobovits et al, Proc. Natl. Acad. Sci. USA 90: 255 1

The term "naked antibody" refers to an antibody that is not conjugated to a cytotoxic moiety or radiolabel.

The terms "full-length antibody," "intact antibody" or "whole antibody" are used interchangeably to refer to an antibody in its substantially intact form, as opposed to an antibody fragment. Specifically whole antibodies include those with heavy and light chains including an Fc region. The constant domains may be native sequence constant domains (e.g., human native sequence constant domains) or amino acid sequence variants thereof. In some cases, the intact antibody may have one or more effector functions.

An "antibody fragment" comprises a portion of an intact antibody, preferably the antigen binding and/or the variable region of the intact antibody. Examples of antibody fragments include Fab, Fab', F(ab')2 and Fv fragments; diabodies; linear antibodies (see U.S. Patent 5,641,870, Example 2; Zapata et al, Protein Eng. 8(10): 1057-1062 [1995]); single-chain antibody molecules and multispecific antibodies formed from antibody fragments. Papain digestion of antibodies produced two identical antigen-binding fragments, called "Fab" fragments, and a residual "Fc" fragment, a designation reflecting the ability to crystallize readily. The Fab fragment consists of an entire L chain along with the variable region domain of the H chain (VH), and the first constant domain of one heavy chain (Cn1). Each Fab fragment is monovalent with respect to antigen binding, i.e., it has a single antigen-binding site. Pepsin treatment of an antibody yields a single large F(ab')2 fragment which roughly corresponds to two disulfide linked Fab fragments having different antigen-binding activity and is still capable of cross-linking antigen. Fab' fragments differ from Fab fragments by having a few additional residues at the carboxy terminus of the CH1 domain including one or more cysteines from the antibody hinge region. Fab'-SH is the designation herein for Fab' in which the cysteine residue(s) of the constant domains bear a free thiol group. F(ab')2 antibody fragments
originally were produced as pairs of Fab' fragments which have hinge cysteines between them. Other chemical couplings of antibody fragments are also known.

The Fc fragment comprises the carboxy-terminal portions of both H chains held together by disulfides. The effector functions of antibodies are determined by sequences in the Fc region, the region which is also recognized by Fc receptors (FcR) found on certain types of cells.

"Fv" is the minimum antibody fragment which contains a complete antigen-recognition and -binding site. This fragment consists of a dimer of one heavy- and one light-chain variable region domain in tight, non-covalent association. From the folding of these two domains emanate six hypervariable loops (3 loops each from the H and L chain) that contribute the amino acid residues for antigen binding and confer antigen binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three HVRs specific for an antigen) has the ability to recognize and bind antigen, although at a lower affinity than the entire binding site.

"Single-chain Fv" also abbreviated as "sFv " or "scFv " are antibody fragments that comprise the VH and VL antibody domains connected into a single polypeptide chain. Preferably, the sFv polypeptide further comprises a polypeptide linker between the VH and VL domains which enables the sFv to form the desired structure for antigen binding. For a review of the sFv, see Pluckthun in The Pharmacology of Monoclonal Antibodies, vol. 1 13, Rosenberg and Moore eds., Springer-Verlag, New York, pp. 269-315 (1994).

"Functional fragments" of the antibodies of the invention comprise a portion of an intact antibody, generally including the antigen binding or variable region of the intact antibody or the Fc region of an antibody which retains or has modified FcR binding capability. Examples of antibody fragments include linear antibody, single-chain antibody molecules and multispecific antibodies formed from antibody fragments.

The term "diabodies" refers to small antibody fragments prepared by constructing sFv fragments (see preceding paragraph) with short linkers (about 5-10 residues) between the VH and VL domains such that inter-chain but not intra-chain pairing of the V domains is achieved, thereby resulting in a bivalent fragment, i.e., a fragment having two antigen-binding sites. Bispecific diabodies are heterodimers of two "crossover" sFv fragments in which the VH and VL domains of the two antibodies are present on different polypeptide chains. Diabodies are described in greater detail in, for

The monoclonal antibodies herein specifically include "chimeric" antibodies (immunoglobulins) in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is(are) identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity (U.S. Patent No. 4,816,567; Morrison et al., Proc. Natl. Acad. Sci. USA, 81:6851-6855 (1984)). Chimeric antibodies of interest herein include PRIMATIZED® antibodies wherein the antigen-binding region of the antibody is derived from an antibody produced by, e.g., immunizing macaque monkeys with an antigen of interest. As used herein, "humanized antibody" is used a subset of "chimeric antibodies."

"Humanized" forms of non-human (e.g., murine) antibodies are chimeric antibodies that contain minimal sequence derived from non-human immunoglobulin. In one embodiment, a humanized antibody is a human immunoglobulin (recipient antibody) in which residues from an HVR (hereinafter defined) of the recipient are replaced by residues from an HVR of a non-human species (donor antibody) such as mouse, rat, rabbit or non-human primate having the desired specificity, affinity, and/or capacity. In some instances, framework ("FR") residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, humanized antibodies may comprise residues that are not found in the recipient antibody or in the donor antibody. These modifications may be made to further refine antibody performance, such as binding affinity. In general, a 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 sequence, and all or substantially all of the FR regions are those of a human immunoglobulin sequence, although the FR regions may include one or more individual FR residue substitutions that improve antibody performance, such as binding affinity, isomerization, immunogenicity, etc. The number of these amino acid substitutions in the FR are typically no more than 6 in the H chain, and in the L chain,
no more than 3. The humanized antibody optionally will also comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin.


A "human antibody" is an antibody that possesses an amino-acid sequence corresponding to that of an antibody produced by a human and/or has been made using any of the techniques for making human antibodies as disclosed herein. This definition of a human antibody specifically excludes a humanized antibody comprising non-human antigen-binding residues. Human antibodies can be produced using various techniques known in the art, including phage-display libraries. Hoogenboom and Winter, J. Mol. Biol., 227:381 (1991); Marks et al., J. Mol. Biol., 222:581 (1991). Also available for the preparation of human monoclonal antibodies are methods described in Cole et al., Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, p. 77 (1985); Boerner et al., J. Immunol., 147(l):86-95 (1991). See also van Dijk and van de Winkel, Curr. Opin. Pharmacol, 5:368-74 (2001). Human antibodies can be prepared by administering the antigen to a transgenic animal that has been modified to produce such antibodies in response to antigenic challenge, but whose endogenous loci have been disabled, e.g., immunized xenomice (see, e.g., U.S. Pat. Nos. 6,075, 181 and 6,150,584 regarding XENOMOUSE™ technology). See also, for example, Li et al, Proc. Natl. Acad. Sci. USA, 103:3557-3562 (2006) regarding human antibodies generated via a human B-cell hybridoma technology.

The term "hypervariable region," "HVR," or "HV" when used herein refers to the regions of an antibody variable domain which are hypervariable in sequence and/or form structurally defined loops. Generally, antibodies comprise six HVRs; three in the VH (H1, H2, H3), and three in the VL (L1, L2, L3). In native antibodies, H3 and L3 display the most diversity of the six HVRs, and H3 in particular is believed to play a unique role in conferring fine specificity to antibodies. See, e.g., Xu et al, Immunity 3:37-45 (2000); Johnson and Wu, in Methods in Molecular Biology 248:1-25 (Lo, ed., Human Press, Totowa, NJ, 2003). Indeed, naturally occurring camelid antibodies consisting of a heavy chain only are functional and stable in the absence of light chain.

A number of HVR delineations are in use and are encompassed herein. The Kabat Complementarity Determining Regions (CDRs) are based on sequence variability and are the most commonly used (Kabat et al. Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD. (1991)). Chothia refers instead to the location of the structural loops (Chothia and Lesk, J. Mol. Biol. 196:901-917 (1987)). The AbM HVRs represent a compromise between the Kabat HVRs and Chothia structural loops, and are used by Oxford Molecular's AbM antibody modeling software. The "contact" HVRs are based on an analysis of the available complex crystal structures. The residues from each of these HVRs are noted below.

<table>
<thead>
<tr>
<th>Loop</th>
<th>Kabat</th>
<th>AbM</th>
<th>Chothia</th>
<th>Contact</th>
</tr>
</thead>
<tbody>
<tr>
<td>L1</td>
<td>L24-L34</td>
<td>L24-L34</td>
<td>L26-L32</td>
<td>L30-L36</td>
</tr>
<tr>
<td>L2</td>
<td>L50-L56</td>
<td>L50-L56</td>
<td>L50-L52</td>
<td>L46-L55</td>
</tr>
<tr>
<td>L3</td>
<td>L89-L97</td>
<td>L89-L97</td>
<td>L91-L96</td>
<td>L89-L96</td>
</tr>
<tr>
<td>H1</td>
<td>H31-H35B</td>
<td>H26-H35B</td>
<td>H26-H32</td>
<td>H30-H35B (Kabat numbering)</td>
</tr>
<tr>
<td>H1</td>
<td>H31-H35</td>
<td>H26-H35</td>
<td>H26-H32</td>
<td>H30-H35 (Chothia numbering)</td>
</tr>
<tr>
<td>H2</td>
<td>H50-H65</td>
<td>H50-H58</td>
<td>H53-H55</td>
<td>H47-H58</td>
</tr>
<tr>
<td>H3</td>
<td>H95-H102</td>
<td>H95-H102</td>
<td>H96-H101</td>
<td>H93-H101</td>
</tr>
</tbody>
</table>

HVRs may comprise "extended HVRs" as follows: 24-36 or 24-34 (L1), 46-56 or 50-56 (L2) and 89-97 or 89-96 (L3) in the VL and 26-35 (H1), 50-65 or 49-65 (H2) and 93-102, 94-102, or 95-102 (H3) in the VH. The variable domain residues are numbered according to Kabat et al., supra, for each of these definitions.

The expression "variable-domain residue-numbering as in Kabat" or "amino-acid-position numbering as in Kabat," and variations thereof, refers to the numbering system used for heavy-chain variable domains or light-chain variable domains of the compilation of antibodies in Kabat et al., supra. Using this numbering system, the actual linear amino acid sequence may contain fewer or additional amino acids corresponding to a shortening of, or insertion into, a FR or HVR of the variable domain. For example, a heavy-chain variable domain may include a single amino acid insert (residue 52a according to Kabat) after residue 52 of H2 and inserted residues (e.g. residues 82a, 82b, and 82c, etc. according to Kabat) after heavy-chain FR residue 82. The Kabat
numbering of residues may be determined for a given antibody by alignment at regions of homology of the sequence of the antibody with a "standard" Kabat numbered sequence.

"Framework" or "FR" residues are those variable-domain residues other than the HVR residues as herein defined.

A "human consensus framework or "acceptor human framework " is a framework that represents the most commonly occurring amino acid residues in a selection of human immunoglobulin VL or VH framework sequences. Generally, the selection of human immunoglobulin VL or VH sequences is from a subgroup of variable domain sequences.

Generally, the subgroup of sequences is a subgroup as in Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD (1991). Examples include for the VL, the subgroup may be subgroup kappa i, kappa ii, kappa iii or kappa IV as in Kabat et al, supra. Additionally, for the VH, the subgroup may be subgroup i, subgroup ii, or subgroup III as in Kabat et al., supra. Alternatively, a human consensus framework can be derived from the above in which particular residues, such as when a human framework residue is selected based on its homology to the donor framework by aligning the donor framework sequence with a collection of various human framework sequences. An acceptor human framework "derived from" a human immunoglobulin framework or a human consensus framework may comprise the same amino acid sequence thereof, or it may contain pre-existing amino acid sequence changes. In some embodiments, the number of pre-existing amino acid changes are 10 or less, 9 or less, 8 or less, 7 or less, 6 or less, 5 or less, 4 or less, 3 or less, or 2 or less.

An "amino-acid modification" at a specified position, e.g. of the Fc region, refers to the substitution or deletion of the specified residue, or the insertion of at least one amino acid residue adjacent the specified residue. Insertion "adjacent" to a specified residue means insertion within one to two residues thereof. The insertion may be N-terminal or C-terminal to the specified residue. The preferred amino acid modification herein is a substitution.

An "affinity-matured" antibody is one with one or more alterations in one or more HVRs thereof that result in an improvement in the affinity of the antibody for antigen, compared to a parent antibody that does not possess those alteration(s). In one

As use herein, the term "specifically binds to" or is "specific for" refers to measurable and reproducible interactions such as binding between a target and an antibody, which is determinative of the presence of the target in the presence of a heterogeneous population of molecules including biological molecules. For example, an antibody that specifically binds to a target (which can be an epitope) is an antibody that binds this target with greater affinity, avidity, more readily, and/or with greater duration than it binds to other targets. In one embodiment, the extent of binding of an antibody to an unrelated target is less than about 10% of the binding of the antibody to the target as measured, e.g., by a radioimmunoassay (RIA). In certain embodiments, an antibody that specifically binds to a target has a dissociation constant (Kd) of < 1 µM, < 100 nM, < 10 nM, < 1 nM, or < 0.1 nM. In certain embodiments, an antibody specifically binds to an epitope on a protein that is conserved among the protein from different species. In another embodiment, specific binding can include, but does not require exclusive binding.

As used herein, the term "immunoadhesin" designates antibody-like molecules which combine the binding specificity of a heterologous protein (an "adhesin") with the effector functions of immunoglobulin constant domains. Structurally, the immunoadhesins comprise a fusion of an amino acid sequence with the desired binding specificity which is other than the antigen recognition and binding site of an antibody (i.e., is "heterologous"), and an immunoglobulin constant domain sequence. The adhesin part of an immunoadhesin molecule typically is a contiguous amino acid sequence comprising at least the binding site of a receptor or a ligand. The immunoglobulin constant domain sequence in the immunoadhesin may be obtained from any immunoglobulin, such as IgG-1, IgG-2 (including IgG2A and IgG2B), IgG-3, or IgG-4 subtypes, IgA (including IgA-1 and IgA-2), IgE, IgD or IgM. The Ig fusions
preferably include the substitution of a domain of a polypeptide or antibody described herein in the place of at least one variable region within an Ig molecule. In a particularly preferred embodiment, the immunoglobulin fusion includes the hinge, CH2 and CH3, or the hinge, CH1, CH2 and CH3 regions of an IgGl molecule. For the production of immunoglobulin fusions see also US Patent No. 5,428, 130 issued June 27, 1995. For example, useful immunoadhesins as second medicaments useful for combination therapy herein include polypeptides that comprise the extracellular or PD-1 binding portions of PD-L1 or PD-L2 or the extracellular or PD-L1 or PD-L2 binding portions of PD-1, fused to a constant domain of an immunoglobulin sequence, such as a PD-L1 ECD - Fc, a PD-L2 ECD - Fc, and a PD-1 ECD - Fc, respectively. Immunoadhesin combinations of Ig Fc and ECD of cell surface receptors are sometimes termed soluble receptors.

A "fusion protein" and a "fusion polypeptide" refer to a polypeptide having two portions covalently linked together, where each of the portions is a polypeptide having a different property. The property may be a biological property, such as activity in vitro or in vivo. The property may also be simple chemical or physical property, such as binding to a target molecule, catalysis of a reaction, etc. The two portions may be linked directly by a single peptide bond or through a peptide linker but are in reading frame with each other.

A "PD-1 oligopeptide " "PD-L1 oligopeptide " or "PD-L2 oligopeptide" is an oligopeptide that binds, preferably specifically, to a PD-1, PD-L1 or PD-L2 negative costimulatory polypeptide, respectively, including a receptor, ligand or signaling component, respectively, as described herein. Such oligopeptides may be chemically synthesized using known oligopeptide synthesis methodology or may be prepared and purified using recombinant technology. Such oligopeptides are usually at least about 5 amino acids in length, alternatively at least about 6, 7, 8, 9, 10, 1 1 , 12, 13, 14, 15, 16, 17, 18, 19, 20, 2 1 , 22, 23, 24, 25, 26, 27, 28, 29, 30, 3 1 , 32, 33, 34, 35, 36, 37, 38, 39, 40, 4 1 , 42, 43, 44, 45, 46, 47, 48, 49, 50, 5 1 , 52, 53, 54, 55, 56, 57, 58, 59, 60, 6 1 , 62, 63, 64, 65, 66, 67, 68, 69, 70, 7 1 , 72, 73, 74, 75, 76, 77, 78, 79, 80, 8 1 , 82, 83, 84, 85, 86, 87, 88, 89, 9 0 , 9 1 , 92, 93, 94, 95, 96, 97, 98, 99, or 100 amino acids in length or more. Such oligopeptides may be identified using well known techniques. In this regard, it is noted that techniques for screening oligopeptide libraries for oligopeptides that are capable of specifically binding to a polypeptide target are well known in the art

A "blocking" antibody or an "antagonist" antibody is one that inhibits or reduces a biological activity of the antigen it binds. In some embodiments, blocking antibodies or antagonist antibodies substantially or completely inhibit the biological activity of the antigen. The anti-PD-L1 antibodies of the invention block the signaling through PD-1 so as to restore a functional response by T-cells (e.g., proliferation, cytokine production, target cell killing) from a dysfunctional state to antigen stimulation.

An "agonist" or activating antibody is one that enhances or initiates signaling by the antigen to which it binds. In some embodiments, agonist antibodies cause or activate signaling without the presence of the natural ligand.

The term "Fc region" herein is used to define a C-terminal region of an immunoglobulin heavy chain, including native-sequence Fc regions and variant Fc regions. Although the boundaries of the Fc region of an immunoglobulin heavy chain might vary, the human IgG heavy-chain Fc region is usually defined to stretch from an amino acid residue at position Cys226, or from Pro230, to the carboxyl-terminus thereof. The C-terminal lysine (residue 447 according to the EU numbering system) of the Fc region may be removed, for example, during production or purification of the antibody, or by recombinantly engineering the nucleic acid encoding a heavy chain of the antibody. Accordingly, a composition of intact antibodies may comprise antibody populations with all K447 residues removed, antibody populations with no K447 residues removed, and antibody populations having a mixture of antibodies with and without the K447 residue. Suitable native-sequence Fc regions for use in the antibodies of the invention include human IgG1, IgG2 (IgG2A, IgG2B), IgG3 and IgG4.
"Fc receptor" or "FcR" describes a receptor that binds to the Fc region of an antibody. The preferred FcR is a native sequence human FcR. Moreover, a preferred FcR is one which binds an IgG antibody (a gamma receptor) and includes receptors of the FcyRI, FcyRII, and FcyRIII subclasses, including allelic variants and alternatively spliced forms of these receptors, FcyRII receptors include FcyRIIA (an "activating receptor") and FcyRIIB (an "inhibiting receptor"), which have similar amino acid sequences that differ primarily in the cytoplasmic domains thereof. Activating receptor FcyRIIA contains an immunoreceptor tyrosine-based activation motif (ITAM) in its cytoplasmic domain. Inhibiting receptor FcyRIIB contains an immunoreceptor tyrosine-based inhibition motif (ITIM) in its cytoplasmic domain, (see M. Daeron, Annu. Rev. Immunol. J. 5:203-234 (1997). FcRs are reviewed in Ravetch and inet, Annu. Rev. Immunol. 9:457-92 (1991); Capel et al, Immunomethods 4:25-34 (1994); and de Haas et al., J. Lab. Clin. Med. 126:330-41 (1995). Other FcRs, including those to be identified in the future, are encompassed by the term "FcR" herein.

The term "Fc receptor" or "FcR" also includes the neonatal receptor, FcRn, which is responsible for the transfer of maternal IgGs to the fetus. Guyer et al, J. Immunol. 117:587 (1976) and Kim et al, J. Immunol. 24:249 (1994). Methods of measuring binding to FcRn are known (see, e.g., Ghetie and Ward, Immunol. Today 18(12):592-8 (1997); Ghetie et al, Nature Biotechnology 15(7):637-40 (1997); Hinton et al, J. Biol. Chem. 279(8):6213-6 (2004); WO 2004/92219 (Hinton et al). Binding to FcRn in vivo and serum half-life of human FcRn high-affinity binding polypeptides can be assayed, e.g., in transgenic mice or transfected human cell lines expressing human FcRn, or in primates to which the polypeptides having a variant Fc region are administered. WO 2004/42072 (Presta) describes antibody variants which improved or diminished binding to FcRs. See also, e.g, Shields et al, J. Biol. Chem. 9(2): 6591 -6604 (2001).

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

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

"Carriers" as used herein include pharmaceutically acceptable carriers, excipients, or stabilizers that are nontoxic to the cell or mammal being exposed thereto at the dosages and concentrations employed. Often the physiologically acceptable carrier is an aqueous pH buffered solution. Examples of physiologically acceptable carriers include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid; low molecular weight (less than about 10 residues) polypeptide; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, arginine or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-forming counterions such as sodium; and/or nonionic surfactants such as TWEEN™, polyethylene glycol (PEG), and PLURONICS™.

A "package insert" refers to instructions customarily included in commercial packages of medicaments that contain information about the indications customarily included in commercial packages of medicaments that contain information about the indications, usage, dosage, administration, contraindications, other medicaments to be combined with the packaged product, and/or warnings concerning the use of such medicaments, etc.

As used herein, the term "treatment" refers to clinical intervention designed to alter the natural course of the individual or cell being treated during the course of clinical pathology. Desirable effects of treatment include decreasing the rate of disease
progression, ameliorating or palliating the disease state, and remission or improved prognosis. For example, an individual is successfully "treated" if one or more symptoms associated with cancer are mitigated or eliminated, including, but are not limited to, reducing the proliferation of (or destroying) cancerous cells, decreasing symptoms resulting from the disease, increasing the quality of life of those suffering from the disease, decreasing the dose of other medications required to treat the disease, delaying the progression of the disease, and/or prolonging survival of individuals. As used herein, "delaying progression of a disease" means to defer, hinder, slow, retard, stabilize, and/or postpone development of the disease (such as cancer). This delay can be of varying lengths of time, depending on the history of the disease and/or individual being treated. As is evident to one skilled in the art, a sufficient or significant delay can, in effect, encompass prevention, in that the individual does not develop the disease. For example, a late stage cancer, such as development of metastasis, may be delayed.

An "effective amount" is at least the minimum concentration required to effect a measurable improvement or prevention of a particular disorder. An effective amount herein may vary according to factors such as the disease state, age, sex, and weight of the patient, and the ability of the antibody to elicit a desired response in the individual. An effective amount is also one in which any toxic or detrimental effects of the treatment are outweighed by the therapeutically beneficial effects. For prophylactic use, beneficial or desired results include results such as eliminating or reducing the risk, lessening the severity, or delaying the onset of the disease, including biochemical, histological and/or behavioral symptoms of the disease, its complications and intermediate pathological phenotypes presenting during development of the disease. For therapeutic use, beneficial or desired results include clinical results such as decreasing one or more symptoms resulting from the disease, increasing the quality of life of those suffering from the disease, decreasing the dose of other medications required to treat the disease, enhancing effect of another medication such as via targeting, delaying the progression of the disease, and/or prolonging survival. In the case of cancer or tumor, an effective amount of the drug may have the effect in reducing the number of cancer cells; reducing the tumor size; inhibiting (i.e., slow to some extent or desirably stop) cancer cell infiltration into peripheral organs; inhibit (i.e., slow to some extent and desirably stop) tumor metastasis; inhibiting to some extent tumor growth; and/or relieving to some extent one or more of the symptoms associated
with the disorder. An effective amount can be administered in one or more administrations. For purposes of this invention, an effective amount of drug, compound, or pharmaceutical composition is an amount sufficient to accomplish prophylactic or therapeutic treatment either directly or indirectly. As is understood in the clinical context, an effective amount of a drug, compound, or pharmaceutical composition may or may not be achieved in conjunction with another drug, compound, or pharmaceutical composition. Thus, an "effective amount" may be considered in the context of administering one or more therapeutic agents, and a single agent may be considered to be given in an effective amount if, in conjunction with one or more other agents, a desirable result may be or is achieved.

As used herein, "in combination with" or "in conjunction with" refers to administration of one treatment modality in addition to another treatment modality. As such, "in combination with" or "in conjunction with" refers to administration of one treatment modality before, during, or after administration of the other treatment modality to the individual.

As used herein, "complete response" or "CR" refers to disappearance of all target lesions; "partial response" or "PR" refers to at least a 30% decrease in the sum of the longest diameters (SLD) of target lesions, taking as reference the baseline SLD; and "stable disease" or "SD" refers to neither sufficient shrinkage of target lesions to qualify for PR, nor sufficient increase to qualify for PD, taking as reference the smallest SLD since the treatment started.

As used herein, "progressive disease" or "PD" refers to at least a 20% increase in the SLD of target lesions, taking as reference the smallest SLD recorded since the treatment started or the presence of one or more new lesions.

As used herein, "progression free survival" (PFS) refers to the length of time during and after treatment during which the disease being treated (e.g., cancer) does not get worse. Progression-free survival may include 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.

As used herein, "overall response rate" (ORR) refers to the sum of complete response (CR) rate and partial response (PR) rate.

As used herein, "overall survival" refers to the percentage of individuals in a group who are likely to be alive after a particular duration of time.
A "chemotherapeutic agent" is a chemical compound useful in the treatment of cancer. Examples of chemotherapeutic agents include alkylating agents such as thiotepa and cyclophosphamide (CYTOXAN®); alkyl sulfonates such as busulfan, improsulfan, and piposulfan; aziridines such as. benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylmelamines including altretamine, triethylenemelamine, trietylenephosphoramide and trimethylolomelamine; acetogenins (especially bullatacin and bullatanone); delta-9-tetrahydrocannabinol (dronabinol, MARINOL®); beta-lapachone; lapachol; colchicines; betulinic acid; a camptothecin (including the synthetic analogue topotecan (HYCAMTIN®), CPT-11 (irinotecan, CAMPTOSAR®), acetylcamptothecin, scopoletin, and 9-aminocamptothecin); bryostatin; pemetrexed; callystatin; CC-1065 (including its adozelesin, carzelesin and bizelesin synthetic analogues); podophyllotoxin; podophyllinic acid; teniposide; cryptophycins (particularly cryptophycin 1 and cryptophycin 8); dolastatin; duocarmycin (including the synthetic analogues, KW-2189 and CB 1-TM1); eleutherobin; pancratistatin; TLK-286; CDP323, an oral alpha-4 integrin inhibitor; a sarcodictyin; spongistatin; nitrogen mustards such as chlorambucil, chlornaphazine, chlornophamide, estramustine, ifosfamide, mechlorethamine, mechloretamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, and ranimustine; antibiotics such as the enediyne antibiotics (e.g., calicheamicin, especially calicheamicin gamma I I and calicheamicin omegal I (see, e.g., Nicolaou et al, Angew. Chem Intl. Ed. Engl., 33 : 183-186 (1994)); dynemicin, including dynemicin A; an esperamicin; as well as neocarzinostatin chromophore and related chromoprotein enediyne antibiotic chromophores), aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, carabichin, carminomycin, carzinophilin, chromomycinis, dactinomycin, daunorubicin, detorubicin, 6 diazo-5 oxo-L-norleucine, doxorubicin (including ADRIAMYCIN®, morpholino doxorubicin, cyanomorpholino doxorubicin, 2 pyrrolino doxorubicin, doxorubicin HC1 liposome injection (DOXIL®) and deoxydoxorubicin), epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins such as mitomycin C, mycophenolic acid, nogalamycin, olivomycins, peplomycin, potfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites such as methotrexate, gemcitabine (GEMZAR®), tegafur
(UFTORAL®), capecitabine (XELODA®), an epothilone, and 5-fluorouracil (5-FU); folic acid analogues such as denopterin, methotrexate, pteropterin, trimetrexate; pyrimidine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, thioguanine; folic acid analogues such as ancitabine, azacitidine, 6-azaauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine, and imatinib (a 2-phenylaminopyrimidine derivative), as well as other c- it inhibitors; anti-adrenals such as aminogluthethimide, mitotane, trilostane; folic acid replenisher such as folinic acid; aceglatone; aldophosphamide glycoside; aminolevulinic acid; eniluracil; amsacrine; bestrabucil; bisantrene; edatraxate; defoafamine; demecolcine; diaziquone; elfornithine; triaziquone; 2,2',2"-trichlorotriethylamine; trichotheccenes (especially T-2 toxin, verrucarin A, roditin A and anguidine); urethan; vindesine (ELDIS1 NE®, FILDESIN®); dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); thiota; taxoids, e.g., paclitaxel (TAXOL®), albumin-engineered nanoparticle formulation of paclitaxel (ABRAXANE™), and doxetaxel (TAXOTERE®); chlorambucil; 6-thioguanine; mercaptopurine; methotrexate; platinum analogs such as cisplatin and carboplatin; vinblastine (VELBAN®); platinum; etoposide (VP-1 6); ifosfamide; mitoxantrone; vincristine (ONCOVIN®); oxaliplatin; leucovovin; vinorelbin (NAVELBINE®); novantrone; edatrexate; daunomycin; aminopterin; ibandronate; topoisomerase inhibitor RFS 2000; difluoromethylomithine (DMFO); retinoids such as retinoic acid; pharmaceutically acceptable salts, acids or derivatives of any of the above; as well as combinations of two or more of the above such as CHOP, an abbreviation for a combined therapy of cyclophosphamide, doxorubicin, vincristine, and prednisolone, and FOLFOX, an abbreviation for a treatment regimen with oxaliplatin (ELOXATIN™) combined with 5-FU and leucovovin.

Additional examples of chemotherapeutic agents include anti-hormonal agents that act to regulate, reduce, block, or inhibit the effects of hormones that can promote the growth of cancer, and are often in the form of systemic, or whole-body treatment. They may be hormones themselves. Examples include anti-estrogens and selective
estrogen receptor modulators (SERMs), including, for example, tamoxifen (including NOLVADEX® tamoxifen), raloxifene (EVISTA®), droloxifene, 4-hydroxytamoxifen, trioxifene, keoxifene, LY 1 1 701 8, onapristone, and toremifene (FARESTON®); anti-progesterones; estrogen receptor down-regulators (ERDs); estrogen receptor antagonists such as fulvestrant (FASLODEX®); agents that function to suppress or shut down the ovaries, for example, leutinizing hormone-releasing hormone (LHRH) agonists such as leuprolide acetate (LUPRON® and ELIGARD®), goserelin acetate, buserelin acetate and triptерелин; anti-androgens such as flutamide, nilutamide and bicalutamide; and aromatase inhibitors that inhibit the enzyme aromatase, which regulates estrogen production in the adrenal glands, such as, for example, 4(5)-imidazoles, aminogluthethimide, megestrol acetate (MEGASE®), exemestane (AROMAS IN®), formestane, fadrozole, vorozole (RJVISOR®), letrozole (FEMARA®), and anastrozole (ARIMIDEX®). In addition, such definition of chemotherapeutic agents includes bisphosphonates such as clodronate (for example, BONEFOS® or OSTAC®), etidronate (DIDROCAL®), NE-58095, zoledronic acid/zoledronate (ZOMETA®), alendronate (FOSAMAX®), pamidronate (AREDIA®), tiludronate (SKELID®), or risedronate (ACTONEL®); as well as troxacitabine (a 1,3-dioxolane nucleoside cytosine analog); anti-sense oligonucleotides, particularly those that inhibit expression of genes in signaling pathways implicated in abherant cell proliferation, such as, for example, PKC-alpha, Raf, H-Ras, and epidermal growth factor receptor (EGF-R); vaccines such as THERATOPE® vaccine and gene therapy vaccines, for example, ALLOVECTIN® vaccine, LEUVECTIN® vaccine, and VAXID® vaccine; topoisomerase 1 inhibitor (e.g., LURTOTECAN®); an anti-estrogen such as fulvestrant; a Kit inhibitor such as imatinib or EXEL-0862 (a tyrosine kinase inhibitor); EGFR inhibitor such as erlotinib or cetuximab; an anti-VEGF inhibitor such as bevacizumab; arinotecan; rmRH (e.g., ABARELIX®); lapatinib and lapatinib ditosylate (an ErbB-2 and EGFR dual tyrosine kinase small-molecule inhibitor also known as GW57201 6); 17AAG (geldanamycin derivative that is a heat shock protein (Hsp) 90 poison), and pharmaceutically acceptable salts, acids or derivatives of any of the above.

As used herein, the term "cytokine" refers generically to proteins released by one cell population that act on another cell as intercellular mediators or have an autocrine effect on the cells producing the proteins. Examples of such cytokines include lymphokines, monokines; interleukins ("ILs") such as IL- 1 , IL- 1a , IL- 2 , IL- 3 , IL- 4 , IL- 5 ,
As used herein, the term "chemokine" refers to soluble factors (e.g., cytokines) that have the ability to selectively induce chemotaxis and activation of leukocytes. They also trigger processes of angiogenesis, inflammation, wound healing, and tumorigenesis. Example chemokines include IL-8, a human homolog of murine keratinocyte chemoattractant (KC).

A pharmaceutically acceptable salt may involve the inclusion of another molecule such as an acetate ion, a succinate ion or other counter ion. The counter ion may be any organic or inorganic moiety that stabilizes the charge on the parent compound. Furthermore, a pharmaceutically acceptable salt may have more than one charged atom in its structure. Instances where multiple charged atoms are part of the pharmaceutically acceptable salt can have multiple counter ions. Hence, a pharmaceutically acceptable salt can have one or more charged atoms and/or one or more counter ion.

If the compound of the invention is a base, the desired pharmaceutically acceptable salt may be prepared by any suitable method available in the art, for example, treatment of the free base with an inorganic acid, such as hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, methanesulfonic acid, phosphoric acid and the like, or with an organic acid, such as acetic acid, maleic acid, succinic acid, mandelic acid, fumaric acid, malonic acid, pyruvic acid, oxalic acid, glycolic acid, salicylic acid, a pyranosidyl acid, such as glucuronic acid or galacturonic acid, an alpha hydroxy acid, such as citric acid or tartaric acid, an amino acid, such as aspartic acid or glutamic acid, an aromatic acid, such as benzoic acid or cinnamic acid, a sulfonic acid, such as p-toluenesulfonic acid or ethanesulfonic acid, or the like.
If the compound of the invention is an acid, the desired pharmaceutically acceptable salt may be prepared by any suitable method, for example, treatment of the free acid with an inorganic or organic base, such as an amine (primary, secondary or tertiary), an alkali metal hydroxide or alkaline earth metal hydroxide, or the like.

Illustrative examples of suitable salts include, but are not limited to, organic salts derived from amino acids, such as glycine and arginine, ammonia, primary, secondary, and tertiary amines, and cyclic amines, such as piperidine, morpholine and piperazine, and inorganic salts derived from sodium, calcium, potassium, magnesium, manganese, iron, copper, zinc, aluminum and lithium.

Prodrugs are also contemplated for use in the methods, medicaments and uses of the present invention. The term "prodrug", as employed herein, denotes a compound that is a drug precursor which, upon administration to a subject, undergoes chemical conversion by metabolic or chemical processes to yield the active pharmaceutical ingredient or a salt thereof. A discussion of prodrugs is provided in T. Higuchi and V. Stella, Pro-drugs as Novel Delivery Systems (1987) 14 of the A.C.S. Symposium Series, and in Bioreversible Carriers in Drug Design, (1987) Edward B. Roche, ed., American Pharmaceutical Association and Pergamon Press, both of which are incorporated herein by reference thereto.

The phrase "pharmaceutically acceptable" indicates that the substance or composition must be compatible chemically and/or toxicologically, with the other ingredients comprising a formulation, and/or the mammal being treated therewith.

A "solvate" refers to an association or complex of one or more solvent molecules and a compound of the invention. Examples of solvents that form solvates include, but are not limited to, water, isopropanol, ethanol, methanol, DMSO, ethyl acetate, acetic acid and ethanolamine. The term "hydrate" refers to the complex where the solvent molecule is water.

It is understood that aspects and variations of the invention described herein include "consisting of" and/or "consisting essentially of" aspects and variations.

III. Methods

In one aspect, provided herein is a method for treating or delaying progression of an ALK-positive or ALK-negative cancer in an individual comprising administering to the individual an effective amount of a PD-1 axis binding antagonist and an ALK inhibitor. In
some embodiments, the treatment results in sustained response in the individual after
cessation of the treatment.

The methods of this invention may find use in treating conditions where
enhanced immunogenicity is desired such as increasing tumor immunogenicity for the
treatment of cancer. A variety of cancers may be treated, or their progression may be
delayed, in individuals who do not test positive for an activating ALK aberration such as
an ALK fusion event or an oncogenic ALK mutation.

In some embodiments, the individual has melanoma. In some embodiments, the
individual has colorectal cancer. In other embodiments, the individual has melanoma,
colon cancer, bladder cancer, breast cancer, clear cell kidney cancer, head/neck
squamous cell carcinoma, rectal cancer, lung squamous cell carcinoma, thyroid cancer,
bladder cancer, cervical cancer, uterine cancer, endometrial cancer, lung
adenocarcinoma, ovarian cancer, papillary kidney cancer, non-small-cell lung cancer
(NSCLC), ovarian cancer, pancreatic cancer, prostate cancer, renal cell cancer, small-
cell lung cancer (SCLC) and triple negative breast cancer.

In some embodiments, the individual is a mammal, such as domesticated
animals (e.g., cows, sheep, cats, dogs, and horses), primates (e.g., humans and non-
human primates such as monkeys), rabbits, and rodents (e.g., mice and rats). In some
embodiments, the individual is a human.

In another aspect, provided herein is a method of enhancing immune function in
an individual having an ALK-positive or ALK-negative cancer comprising administering
an effective amount of a PD-1 axis binding antagonist and an ALK inhibitor.

In some embodiments, the cancer has elevated levels of T-cell infiltration.

In some embodiments, the combination therapy of the invention comprises
administration of a PD-1 axis binding antagonist and an ALK inhibitor. The PD-1 axis
binding antagonist and the ALK inhibitor may be administered in any suitable manner
known in the art. For example, the PD-1 axis binding antagonist and the ALK inhibitor
may be administered sequentially (at different times) or concurrently (at the same time).

In some embodiments, the ALK inhibitor is administered continuously. In some
embodiments, the ALK inhibitor is administered intermittently. In some embodiments,
the ALK inhibitor is administered before administration of the PD-1 axis binding
antagonist. In some embodiments, the ALK inhibitor is administered simultaneously with
administration of the PD-1 PD-1 axis binding antagonist. In some embodiments, the ALK inhibitor is administered after administration of the PD-1 PD-1 axis binding antagonist.

In some embodiments, provided is a method for treating or delaying progression of an ALK-positive or ALK-negative cancer in an individual comprising administering to the individual an effective amount of a PD-1 axis binding antagonist and an ALK inhibitor, further comprising administering an additional therapy. The additional therapy may be radiation therapy, surgery (e.g., lumpectomy and a mastectomy), chemotherapy, gene therapy, DNA therapy, viral therapy, RA therapy, immunotherapy, bone marrow transplantation, nanotherapy, monoclonal antibody therapy, or a combination of the foregoing. The additional therapy may be in the form of adjuvant or neoadjuvant therapy. In some embodiments, the additional therapy is the administration of small molecule enzymatic inhibitor or anti-metastatic agent. In some embodiments, the additional therapy is the administration of side-effect limiting agents (e.g., agents intended to lessen the occurrence and/or severity of side effects of treatment, such as anti-nausea agents, etc.). In some embodiments, the additional therapy is radiation therapy. In some embodiments, the additional therapy is surgery. In some embodiments, the additional therapy is a combination of radiation therapy and surgery. In some embodiments, the additional therapy is gamma irradiation. In some embodiments, the additional therapy is therapy targeting PI3K/A T/mTOR pathway, HSP90 inhibitor, tubulin inhibitor, apoptosis inhibitor, and/or chemopreventative agent. The additional therapy may be one or more of the chemotherapeutic agents described hereabove.

The PD-1 axis binding antagonist and the ALK inhibitor may be administered by the same route of administration or by different routes of administration. In some embodiments, the PD-1 axis binding antagonist is administered intravenously, intramuscularly, subcutaneously, topically, orally, transdermal\(^\wedge\), intraperitoneally, intraportally, by implantation, by inhalation, intrathecal\(^\wedge\), intraventricularly, or intranasally. In some embodiments, the ALK inhibitor is administered intravenously, intramuscularly, subcutaneously, topically, orally, transdermal\(^\wedge\), intraperitoneally, intraoritall\(^\wedge\), by implantation, by inhalation, intrathecal\(^\wedge\), intraventricularly, or intranasally. An effective amount of the PD-1 axis binding antagonist and the ALK inhibitor may be administered for prevention or treatment of disease. The appropriate dosage of the PD-1 axis binding antagonist and/or the ALK inhibitor may be determined
based on the type of disease to be treated, the type of the PD-1 axis binding antagonist and the ALK inhibitor, the severity and course of the disease, the clinical condition of the individual, the individual's clinical history and response to the treatment, and the discretion of the attending physician.

Any of the PD-1 axis binding antagonists and the ALK inhibitors known in the art or described below may be used in the methods.

IV. PD-1 Axis Binding Antagonists

Provided herein is a method for treating or delaying progression of an ALK-positive or ALK-negative cancer in an individual comprising administering to the individual an effective amount of a PD-1 axis binding antagonist and an ALK inhibitor. For example, a PD-1 axis binding antagonist includes a PD-1 binding antagonist, a PD-L1 binding antagonist and a PD-L2 binding antagonist.

In some embodiments, the PD-1 binding antagonist is a molecule that inhibits the binding of PD-1 to its ligand binding partners. In a specific aspect the PD-1 ligand binding partners are PD-L1 and/or PD-L2. In another embodiment, a PD-L1 binding antagonist is a molecule that inhibits the binding of PD-L1 to its binding partners. In a specific aspect, PD-L1 binding partners are PD-1 and/or B7-1. In another embodiment, the PD-L2 binding antagonist is a molecule that inhibits the binding of PD-L2 to its binding partners. In a specific aspect, a PD-L2 binding partner is PD-1. The antagonist may be an antibody, an antigen binding fragment thereof, an immunoadhesin, a fusion protein, or an oligopeptide.

In some embodiment, the PD-1 binding antagonist is an anti-PD-1 antibody (e.g., a human antibody, a humanized antibody, or a chimeric antibody). In some embodiments, the anti-PD-1 antibody is selected from the group consisting of nivolumab (MDX-1 106), pembrolizumab (Merck 3475), pidilizumab (CT-01 1), mAb1 5, and mAb7. In some embodiments, the PD-1 binding antagonist is an immunoadhesin (e.g., an immunoadhesin comprising an extracellular or PD-1 binding portion of PD-L1 or PD-L2 fused to a constant region (e.g., an Fc region of an immunoglobulin sequence). In some embodiments, the PD-1 binding antagonist is AMP-224 (amino acid sequence as shown in SEQ ID NO: 14). In some embodiments, the PD-1 binding antagonist is nivolumab (heavy and light chain variable sequences as shown in SEQ ID NOS: 8 and 9 respectively; CAS Registry Number: 946414-94-4), also known as MDX-1106, MDX-1 106-04, ONO-4538 or BMS-936558, an anti-PD-1 antibody described in
WO2006/121168. In other embodiments, the PD-1 binding antagonist is pembrolizumab (heavy and light chain variable sequences as shown in SEQ ID Nos: 11 and 12, respectively), also known as MK-3475 or Merck-3745, SCH-900475, an anti-PD-1 antibody described in WO2009/143355. In still other embodiments, the PD-1 binding antagonist is pidilizumab, also known as CT-011, hBAT or hBAT-1, an anti-PD-1 antibody described in WO2009/101611. In yet other embodiments, the PD-1 binding antagonist is AMP-224, also known as B7-DCIg, a PD-L2-Fc fusion soluble receptor described in WO2010/027827 and WO2011/066342.

In some embodiments, the PD-1 axis binding antagonist is an anti-PD-L1 antagonist. In some embodiments, the PD-L1 binding antagonist is anti-PD-L1 antibody. In some embodiments, the anti-PD-L1 antibody is capable of inhibiting binding between PD-L1 and PD-1 and/or between PD-L1 and B7-1. In some embodiments, the anti-PD-L1 antibody is a monoclonal antibody. In some embodiments, the anti-PD-L1 antibody is an antibody fragment selected from the group consisting of Fab, Fab'-SH, Fv, scFv, and (Fab')2 fragments. In some embodiments, the anti-PD-L1 antibody is a humanized antibody. In some embodiments, the anti-PD-L1 antibody is a human antibody. In some embodiments, the anti-PD-L1 binding antagonist is selected from the group consisting of MPDL3280A (YW243.55.S70 or atezolizumab), MDX-105, MEDI4736 and MSB0010718C. In some embodiments, the PD-L1 binding antagonist is MPDL3280 (from clone YW243.55.S70, heavy and light chain variable region sequences shown in SEQ ID Nos. 14 and 15, respectively), an anti-PD-L1 antibody described in WO2010/077634 Al. In other embodiments, the PD-L1 binding antagonist is MDX-105, also known as BMS-936559, an anti-PD-L1 antibody described in WO2007/005874. In yet other embodiments, the PD-L1 binding antagonist is MEDI4736, an anti-PD-L1 described in WO2011/066389. In other embodiments, the PD-L1 binding antagonist is MSB0010718C (heavy and light chain variable sequences as shown in SEQ ID NO: 17 and 18, respectively) is an anti-PD-L1 described in WO13079174.

The anti-PD-L1 antibodies useful in this invention, including compositions containing such antibodies, such as YW243.55.S70 as described in WO2010/077634, may be used in combination with a ALK inhibitor to treat an ALK-positive or ALK-negative cancer.
In a still further specific aspect, the antibody described herein (such as an anti-PD-1 antibody, an anti-PD-L1 antibody, or an anti-PD-L2 antibody) further comprises a human or murine constant region. In a still further aspect, the human constant region is selected from the group consisting of IgG1, IgG2, IgG3, IgG4. In a still further specific aspect, the human constant region is IgG1. In a still further aspect, the murine constant region is selected from the group consisting of IgG1, IgG2A, IgG2B, IgG3. In a still further aspect, the murine constant region is IgG2A. In a still further specific aspect, the antibody has reduced or minimal effector function. In a still further specific aspect, the minimal effector function results from production in prokaryotic cells. In a still further specific aspect the minimal effector function results from an "effector-less Fc mutation" or aglycosylation. In a still further aspect, the effector-less Fc mutation is an N297A or D265A N297A substitution in the constant region.

In a still further aspect, provided herein are nucleic acids encoding any of the antibodies described herein. In some embodiments, the nucleic acid further comprises a vector suitable for expression of the nucleic acid encoding any of the previously described anti-PD-L1, anti-PD-1, or anti-PD-L2 antibodies. In a still further specific aspect, the vector further comprises a host cell suitable for expression of the nucleic acid. In a still further specific aspect, the host cell is a eukaryotic cell or a prokaryotic cell. In a still further specific aspect, the eukaryotic cell is a mammalian cell, such as Chinese Hamster Ovary (CHO).

The antibody or antigen binding fragment thereof, may be made using methods known in the art, for example, by a process comprising culturing a host cell containing nucleic acid encoding any of the previously described anti-PD-L1, anti-PD-1, or anti-PD-L2 antibodies or antigen-binding fragment in a form suitable for expression, under conditions suitable to produce such antibody or fragment, and recovering the antibody or fragment.

In a still further embodiment, the invention provides for a composition comprising an anti-PD-L1, an anti-PD-1, or an anti-PD-L2 antibody or antigen binding fragment thereof as provided herein and at least one pharmaceutically acceptable carrier. In some embodiments, the anti-PD-L1, anti-PD-1, or anti-PD-L2 antibody or antigen binding fragment thereof administered to the individual is a composition comprising one or more pharmaceutically acceptable carrier. Any of the pharmaceutically acceptable carrier described herein or known in the art may be used.
V. ALK inhibitors

The invention provides methods for treating cancer or delaying progression of an ALK-positive or ALK-negative cancer in an individual comprising administering an effective amount of a PD-1 axis binding antagonist and an ALK inhibitor. Any known ALK inhibitors are intended, as described above, the content of which are incorporated herein by reference. The ALK inhibitor administered may be in a pharmaceutical composition or formulation. In some embodiments, the pharmaceutical composition or formulation comprises one or more ALK inhibitors described herein and a pharmaceutically acceptable carrier or excipient.

In some embodiments, the ALK inhibitor is a competitive inhibitor of ALK.

VI. Kits

In another aspect, provided is a kit comprising a PD-1 axis binding antagonist and/or an ALK inhibitor for treating or delaying progression of a cancer in an individual or for enhancing immune function of an individual having cancer. In some embodiments, the kit comprises a PD-1 axis binding antagonist and a package insert comprising instructions for using the PD-1 axis binding antagonist in combination with an ALK inhibitor to treat or delay progression of an ALK-positive or ALK-negative cancer in an individual or to enhance immune function of an individual having cancer. In some embodiments, the kit comprises an ALK inhibitor and a package insert comprising instructions for using the ALK inhibitor in combination with a PD-1 axis binding antagonist to treat or delay progression of an ALK-positive or ALK-negative cancer in an individual or to enhance immune function of an individual having cancer. In some embodiments, the kit comprises a PD-1 axis binding antagonist and an ALK inhibitor, and a package insert comprising instructions for using the PD-1 axis binding antagonist and the ALK inhibitor to treat or delay progression of an ALK-positive or ALK-negative cancer in an individual or to enhance immune function of an individual having cancer. Any of the PD-1 axis binding antagonists and/or ALK inhibitors described herein may be included in the kits.

In some embodiments, the kit comprises a container containing one or more of the PD-1 axis binding antagonists and ALK inhibitors described herein. Suitable containers include, for example, bottles, vials (e.g., dual chamber vials), syringes (such as single or dual chamber syringes) and test tubes. The container may be formed from a variety of materials such as glass or plastic. In some embodiments, the kit may
comprise a label (e.g., on or associated with the container) or a package insert. The label or the package insert may indicate that the compound contained therein may be useful or intended for treating or delaying progression of an ALK-positive or ALK-negative cancer in an individual or for enhancing immune function of an individual having cancer. The kit may further comprise other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, and syringes.

EXAMPLES

The invention can be further understood by reference to the following examples, which are provided by way of illustration and are not meant to be limiting.

Example 1: ALK inhibitor impact on T cell proliferation and viability

To assess the relative impact of ALK inhibitors, PF-06463922 and PF-02341066 (crizotinib, Xalkori®, Pfizer Inc) on CD3/CD28-stimulated effector cells, activation assays were performed which compared the ALK inhibitors to tyrosine kinase inhibitors, sunitinib and axitinib (Sutent®, and Inlyta®, Pfizer Inc). In published studies, administration of sunitinib and axitinib resulted in dose-dependent inhibition of both cell proliferation and cell viability but with axitinib showing only minor toxicity effects in peripheral blood mononuclear cells (PMBCs). Stehle F. et al. J Biol Chem. 2013 Jun 7;288(23): 16334-4.

Briefly, human cells were isolated from healthy donors’ PBMCs by using EasySep™ Human T Cell Enrichment Kit (Stemcell Technologies) according to manufacturer’s instruction. ALK inhibitors PF-06463922 and crizotinib were titrated such that the final culture concentration was 0.1% DMSO in a total volume of 150 µl per well. Cells were cultured in RPMI 1640 medium supplemented with 10% FCS and 100 I.U./mL penicillin/streptomycin in humidified incubator at 37 °C with 5% CO2. Post isolation, cells were directly stimulated with Dynabeads® Human T-Activator CD3 or CD3/CD28 (Life Technologies) in the presence of each compound at indicated concentration ranges, .001, .01, 0.1, 1, 10 µg/ml concentration, that cover clinically effective concentration for three days: (PF-06463922 at 100 nM, crizotinib at 80 nM, sunitinib at 12-20 nM, axitinib at 0.2-0.6 nM or DMSO vehicle). Culture supernatants were collected on Day 2 or 3 for cytokine analysis. Plates were then harvested on DNA specific filter papers (Perkin Elmer) using Harvester96 (Tomtec Life Sciences). The
Radiolabeled filters were covered with beta scintillation liquid (Perkin Elmer) and read in Microbeta® counter plates (Perkin Elmer). $^3$H Thymidine was pulsed on Day 2 for T cell proliferation assay. The impact by sunitinib and axitinib on T cell proliferation and viability were repeated as previously published (Figures 1A and 1B). Moreover, treatment of activated T cells with ALK inhibitors PF-06463922 and crizotinib did not have adverse effects on T cell proliferation, with dosage levels that exceeded sunitinib and more closely approximated axitinib (Figure 1A).

Table 3 below provides the thymidine incorporation (CPM) values for each of the respective inhibitors.

<table>
<thead>
<tr>
<th>log[Agonist], μM</th>
<th>Sunitinib</th>
<th>Axitinib</th>
<th>PF-06463922</th>
<th>Crizotinib</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>Mean</td>
<td>Mean</td>
<td>Mean</td>
<td>Mean</td>
</tr>
<tr>
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<td>493570.3</td>
<td>437978</td>
<td>399866.7</td>
<td>432360.3</td>
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<td>±5214.787</td>
<td>±10135.82</td>
<td>±29488.63</td>
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<tr>
<td>-3</td>
<td>469071</td>
<td>394950</td>
<td>401448.3</td>
<td>421221.3</td>
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<td>±48070.7</td>
<td>±19054.05</td>
<td>±37205.07</td>
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<td>-2</td>
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<td>408592.3</td>
<td>411422.3</td>
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<td>±13911.18</td>
<td>±11690</td>
<td>±13377.95</td>
<td>±42508.08</td>
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<tr>
<td>-1</td>
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<td>416579.3</td>
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<td>±39073.88</td>
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<td>±23523.21</td>
<td>±47103.88</td>
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<td>±105.9733</td>
<td>±9004.015</td>
<td>±37421.4</td>
<td>±34171.33</td>
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<td>258875.3</td>
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<td>±25269.1</td>
<td>±18298.53</td>
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<td>2</td>
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<td>94120.66</td>
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<td>±25</td>
<td>±31076.09</td>
<td>±2728.719</td>
<td>±104.9704</td>
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</tbody>
</table>

Cell viability was measured by Cell-Titer Glo Luminescent Cell Viability assay® (Promega, Madison). Figure 1B indicates that PF-06463922 and crizotinib did not result in dose-dependent decreases in T cell viability, with dosage levels that again exceeded sunitinib and more closely approximated axitinib. By analogy, PF-06463922
and crizotinib appears suitable for implementation in dosing regimens which incorporate immunotherapy. Table 4 below provides the Luciferase RLU values reflecting T cell viability for each of the respective inhibitors.

Table 4: Luciferase RLU

<table>
<thead>
<tr>
<th>log[Agonist], µM</th>
<th>Sunitinib</th>
<th>Axitinib</th>
<th>PF-06463922</th>
<th>crizotinib</th>
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<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Mean</td>
<td>Mean</td>
<td>Mean</td>
</tr>
<tr>
<td>0</td>
<td>98.24227</td>
<td>92.62644</td>
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<td>94.22272</td>
</tr>
<tr>
<td>±2.168515</td>
<td>±0.522128</td>
<td>±0.756657</td>
<td>±1.813741</td>
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</tr>
<tr>
<td>-3</td>
<td>96.57127</td>
<td>97.36216</td>
<td>98.16747</td>
<td>89.07632</td>
</tr>
<tr>
<td>±1.710999</td>
<td>±1.185823</td>
<td>±0.660638</td>
<td>±2.842621</td>
<td></td>
</tr>
<tr>
<td>-2</td>
<td>98.03569</td>
<td>95.54089</td>
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<td>±2.63407</td>
<td>±3.149818</td>
<td>±0.620189</td>
<td>±1.564283</td>
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<td>-1</td>
<td>91.57024</td>
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</tr>
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<td>±2.195313</td>
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<td>±2.287887</td>
<td>±1.826977</td>
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<td>13.95176</td>
<td>89.78527</td>
<td>89.118</td>
<td>91.71838</td>
</tr>
<tr>
<td>±7.493037</td>
<td>±1.428145</td>
<td>±1.283329</td>
<td>±2.35425</td>
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<tr>
<td>2</td>
<td>0.850193</td>
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<td>88.6231</td>
<td>64.23526</td>
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<td>±0.259612</td>
<td>±0.877947</td>
<td>±1.572783</td>
<td>±3.748341</td>
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<tr>
<td>3</td>
<td>1.826584</td>
<td>78.81053</td>
<td>40.36231</td>
<td>1.47364</td>
</tr>
<tr>
<td>±0.58776</td>
<td>±3.223431</td>
<td>±9.73577</td>
<td>±0.16934</td>
<td></td>
</tr>
</tbody>
</table>

Example 2: ALK inhibition enhanced anti-tumor activity of anti-PD-L1 antibodies in ALK-negative colorectal and melanoma tumors in vivo

To evaluate the anti-tumor efficacy of combination immunotherapy against established CT26 and B16 tumors, anti-PD-L1 dosing regimens were selected based on prior studies that showed efficacy in the CT26 and B16 models (data not shown). Both CT26 colon carcinoma and B16 melanoma cell lines have published mutanome and transcriptome data, with no known ALK fusion events or oncogenic mutations. Castle et al, BMC Genomics, 15:190 (13 Mar 2014); Castle et al, CAN 11:3722 (11 Jan 2012).
As shown in Figures 2A-2C, administration of an ALK inhibitor, an anti-PD-L1 antibody, or isotype alone did not consistently inhibit CT26 tumor growth when these single agent treatments were started in tumors of 100 - 150 mm³ in size. On day 21 post tumor inoculation, administration of each of an anti-PD-L1 antibody and an ALK inhibitor (PF-06463922) individually, resulted in 41.3% and 28.3% of tumor growth inhibition (TGI) respectively relative to the isotype control (p < 0.001). By contrast, as shown in Figures 2D and 2E, when animals were concurrently administrated an ALK inhibitor and an anti-PD-L1 antibody, a dramatic efficacy of 72% TGI, (2/10 animals were tumor free) relative to isotype controls (p < 0.0001) ensued. The percentage of TGI was defined as 1 - (Tumor Volume Treated / Tumor Volume isotype control) x 100.

Similarly, an anti-tumor effect of the ALK inhibitor/anti-PD-L1 combination was observed in the B16 skin melanoma cancer model as shown in Figures 3A through 3D. As shown in Figure 3D, at the end of study (day 22 post tumor implant), 2 out of 10 animals had a complete response (tumor free), as compared to no animals having a complete response in those groups administered with a single agent isotype, ALK inhibitor or anti-mPD-L1 antibody.

Materials and Methods

Mice. Six- to eight-week old female Balb/c mice were purchased from The Jackson Laboratories (Farmington, CT). Mice were maintained and all animal experiments were conducted according to the protocols approved by the Institutional Animal Care and Use Committee of Rinat, South San Francisco and Worldwide Research and Development (WRD), La Jolla, Pfizer Inc.

Cell lines. The CT26 colon carcinoma cell line was purchased from ATCC® CRL-2638™. B16-F10 (B16) melanoma cell lines were purchased from ATCC® CRL-6475™. Cells were cultured in DMEM medium supplemented with 10% fetal bovine serum, and 100 I.U./mL penicillin/streptomycin at 37°C in an atmosphere of 5% CO2 in air, and IMPACT-tested for pathogens at Research Animal Diagnostic Laboratory (RADIL) (Columbia, MO). Pathogen-free cells growing in an exponential growth phase were harvested and used for tumor inoculation.

Antibodies for immunotherapy. Rat anti-mouse PD-L1 mAb (clone MIH5) was purchased from eBioscience. Rat IgG2a isotype control were purchased from BioXcell (clone 2A3)(West Lebanon, NH).
In vivo tumor efficacy studies. Balb/c mice were inoculated subcutaneously at the right lower flank with 0.1 ml of 1x10^6 CT26 murine colorectal cells in PBS. For the second study, C57BL/6 mice were inoculated subcutaneously in the right lower flank with 0.1 ml of 1x10^6 B16 skin melanoma cells. When tumor bearing mice achieved a mean tumor volume of 100 to 150 mm^3, mice were randomly assigned to 1 of 4 treatment groups, n=10. Group 1: received 10 mg/kg of an isotype control antibody (ratIgG2a, clone2A3) intraperitoneal^a every 3 days for a total of 3 doses; Group 2: received 10 mg/kg anti-PD-L1 antibody (MIH5) (ratIgG2a, purchased from eBioscience) intraperitoneally every 3 days for a total of 3 doses; Group 3: received 3 mg/kg of PF-06463922 an ALK inhibitor, orally, once daily for 12 days; Group 4: received 10 mg/kg each of an anti-PD-L1 antibody MIH5, LOTE20708-102, intraperitoneally every 3 days for a total of 3 doses plus PF-06463922 an ALK inhibitor orally, once daily for 12 days. Mice were monitored for tumor growth and body weight changes.

In the CT26 carcinoma model, blockade of PD-L1 with anti-PD-L1 antibody MIH5 or treatment with ALK inhibitor PF-06463922 was partially effective compared with isotype antibody treated group as a single agent therapy at preventing tumor growth (Figures 2B and 2C). Combination treatment with anti-PD-L1 antibody and ALK inhibitor significantly inhibited tumor growth and was significantly more effective than anti-PD-L1 antibody or ALK inhibitor treatment alone (Figures 2D, 2E). Furthermore, co-treatment at an early stage of tumor growth resulted not only in significant reduction of tumor volume but also demonstrated a sustained response. Early intervention resulted in about a 20% complete response (tumor free) and 50% partial response that were maintained for at least 21 days. These results indicate that ALK inhibition enhanced the anti-tumor activity of PD-L1 blockade and therefore worked synergistically with anti-PD-L1 antibodies to inhibit tumor growth (Figure 2E).

Table 5: Average tumor volume (mm^3)

<table>
<thead>
<tr>
<th>Tumor vol (mm^3)</th>
<th>Rat IgG2a</th>
<th>Anti-mPDL1</th>
<th>PF-06463922</th>
<th>Anti-mPDL1 + PF-06463922</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>Mean</td>
<td>Mean</td>
<td>Mean</td>
<td>Mean</td>
</tr>
</tbody>
</table>
Moreover, early intervention in the second B16 melanoma study, resulted in a 20% complete response (2/10 animals tumor free) maintained for 21 days that was not seen in any of the single agent groups as shown in Figure 3D.

### Example 3: ALK inhibition of crizotinib but not alectinib enhanced anti-tumor activity of anti-PD-L1 antibody in ALK-negative colorectal tumors in vivo

To evaluate the anti-tumor efficacy of ALK inhibitors crizotinib and alectinib in combination with immunotherapeutics against established CT26 and MC38 colon carcinoma cell lines, anti-PD-L1 dosing regimens were selected as in Example 2. CT26 colon carcinoma cell line has published mutanome and transcriptome data, with no known ALK fusion events or oncogenic mutations. Castle et al, BMC Genomics. 15:190 (13 Mar 2014); Castle et al, CAN 11:3722 (11 Jan 2012). In-house mRNA data analysis on murine syngeneic tumor cell lines and their in vivo derived tissues revealed that MC38, CT26, and B16F10 are negative in ALK and ROS1 mRNA level, while positive in cMET mRNA expression when compared to a house keeping gene.

As shown in Figures 4A-4B, in the CT26 Syngeneic studies, no anti-tumor effects were observed from treatment with anti-mPD-L1 alone or with any dose of crizotinib alone compared to the isotype control antibody treated group. By Day 25, anti-mPD-L1 in combination with 5, 20, or 40 mg/kg crizotinib resulted in tumor growth inhibition (TGI) of 65.4% (P value = 0.0003) with 40% complete tumor regression (4 out 10 animals tumor free), 74.3% (P value < 0.0001) with 40% complete tumor regression (4 out 10 animals tumor free), or 61.3% (P value = 0.0004) with 30% complete tumor regression (3 out 10 animals tumor free), respectively (Figure 4A, 4B). Percentage of TGI was defined as \((\frac{\text{Tumor volumeTreated}}{\text{Tumor volumeisotype control}}) \times 100\%\).  

<table>
<thead>
<tr>
<th>Day</th>
<th>Tumor Volume</th>
<th>Tumor Volume</th>
<th>Tumor Volume</th>
<th>Tumor Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 5 ±12.06385</td>
<td>Day 9 ±34.61</td>
<td>Day 14 ±95.021</td>
<td>Day 17 ±160.731</td>
</tr>
<tr>
<td></td>
<td>183.04 ±12.52759</td>
<td>263.34 ±46.57493</td>
<td>545.33 ±96.03331</td>
<td>962.02 ±180.1961</td>
</tr>
<tr>
<td></td>
<td>182.29 ±12.6828</td>
<td>253.14 ±34.7816</td>
<td>548.44 ±96.76349</td>
<td>570.0889 ±21.5698</td>
</tr>
<tr>
<td></td>
<td>181.85 ±14.20208</td>
<td>185.5556 ±254.881</td>
<td>323.4 ±292.6458</td>
<td>662.9556 ±294.3596</td>
</tr>
<tr>
<td></td>
<td>179.65 ±285.9975</td>
<td>249.5714 ±95.76349</td>
<td>236.76 ±245.9813</td>
<td>622.9556 ±249.3596</td>
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</table>
Similarly, an enhanced anti-tumor effect of the ALk inhibitor crizotinib/anti-PD-L1 combination was observed in the MC38 colon carcinoma model. As shown in Figure 4C, by Day 24, anti-mPD-L1 in combination with crizotinib resulted in tumor growth inhibition (TGI) of 56.6% (P value = 0.0017) compared to the crizotinib alone and 57.4% (P value = 0.0011) compared to the anti-mPDL1 treated group.

As shown in Figure 4D, ALK-inhibitor alectinib in combination with anti-mPDL1 treatment did not show significant improvement over either single agent alone. The lack of efficacy may result from any number of factors, including but not limited to alectinib's selectivity for ALK and other kinases.

Materials and Methods

Mice. Six- to eight-week old female Balb/c or C57BL/6 mice were purchased from The Jackson Laboratories. Mice were maintained and all animal experiments were conducted according to the protocols approved by the Institutional Animal Care and Use Committee of Rinat, South San Francisco and Worldwide Research and Development (WRD), La Jolla, Pfizer Inc.

Materials. CT26 cell lines, anti-PD-L1 antibody and Rat IgG isotypes (IgG2a and IgG2b) were purchased from BioXCell (clones 2A3 and LTF2, respectively) and prepared as described in Example 2 above.

MC38 cell lines. MC38 colon carcinoma cell line was kindly provided by Dr. Antoni Ribas at UCLA, California. Cells were cultured in DMEM medium supplemented with 10% fetal bovine serum, and 100 I.U./mL penicillin/streptomycin at 37°C in an atmosphere of 5% CO2 in air, and IMPACT-tested for pathogens at Research Animal Diagnostic Laboratory (RADIL) (Columbia, MO). Pathogen-free cells growing in an exponential growth phase were harvested and used for tumor inoculation.

In vivo tumor efficacy studies. Balb/c mice were inoculated subcutaneously at the right lower flank with 0.1 ml of 0.1 x 10^6 CT26 murine colorectal cells in PBS. For the MC38 study, C57BL/6 mice were inoculated subcutaneously in the right lower flank with 0.2 x 10^6 MC38 colon carcinoma cells. When tumor bearing mice achieved a mean tumor volume of 50 to 150 mm^3, mice were randomly assigned to 1 of 4 treatment groups, n=10. Group 1 received 10 mg/kg of an isotype control antibody intraperitoneal^ every 3 days for a total of 3 doses; Group 2 received 10 mg/kg anti-PD-L1 antibody (MIH5)
intraperitoneally every 3 days for a total of 3 doses; Group 3-5 received one of three doses of ALK inhibitor (in CT26, Group 3-5 received 5, 20, and 40 mg/kg of crizotinib or 60 mg/kg alectinib, while MC38 Group 3 received 40 mg/kg crizotinib) each orally, once daily for 12 days; Group 6 received 10 mg/kg each of an anti-PD-L1 antibody MIH5, intraperitoneal^ every 3 days for a total of 3 doses plus ALK inhibitor orally, once daily for 12 days (for CT26 cell line, 5, 20, and 40 mg/kg crizotinib or 60 mg/kg alectinib, and for MC38 cell line, 40 mg/kg crizotinib). Mice were monitored for tumor growth and body weight changes.

In the CT26 carcinoma model, blockade of PD-L1 with anti-PD-L1 antibody MIH5 or treatment with ALK inhibitors were not effective compared with isotype antibody treated group as a single agent therapy at inhibiting preventing tumor growth (Figures 2C, 4A, and 4B). In both CT26 and MC38 models, combination treatment with anti-PD-L1 antibody and crizotinib synergistically inhibited tumor growth and was significantly more effective than anti-PD-L1 antibody or crizotinib alone (Figures 4A, 4B and 4C). Furthermore, co-treatment at an early stage of tumor growth resulted not only in significant reduction of tumor volume but also demonstrated a sustained response. Early intervention resulted in about a 30-40% complete response (tumor free). These results indicate that ALK inhibition with crizotinib enhanced the anti-tumor activity of PD-L1 blockade and therefore worked synergistically with anti-PD-L1 antibodies to inhibit tumor growth (Figures 4A, 4B and 4C).

Table 6. Tumor Measurement for Crizotinib Dose Escalation and Anti-mPDL1 Combination in CT26 Model Over Time

<table>
<thead>
<tr>
<th>Days</th>
<th>Rat IgG2a</th>
<th>Anti-mPDL</th>
<th>Crizotinib (5mg/kg)</th>
<th>Crizotinib (20mg/kg)</th>
<th>Crizotinib (40mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>89.6 ± 9.3</td>
<td>90.2 ± 8.3</td>
<td>90.3 ± 8.1</td>
<td>90.3 ± 8.0</td>
<td>94.4 ± 7.9</td>
</tr>
<tr>
<td>10</td>
<td>189.4 ± 12.7</td>
<td>146.5 ± 19.1</td>
<td>140.9 ± 18.0</td>
<td>156.1 ± 21.9</td>
<td>127.0 ± 9.8</td>
</tr>
<tr>
<td>14</td>
<td>253.2 ± 14.0</td>
<td>204.5 ± 32.3</td>
<td>246.6 ± 54.7</td>
<td>234.2 ± 49.0</td>
<td>218.5 ± 31.5</td>
</tr>
</tbody>
</table>
### Table 6. Tumor Measurement for Crizotinib Dose Escalation and Anti-mPDL1 Combination in CT26 Model Over Time

<table>
<thead>
<tr>
<th>Days</th>
<th>Tumor Volume (mm³) (Mean ± SEM)</th>
<th>Anti-mPDL (5mg/kg)</th>
<th>Crizotinib (20mg/kg)</th>
<th>Crizotinib (40mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rat IgG2a</td>
<td>344.3 ± 407.1 ±</td>
<td>299.3 ± 319.6 ±</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td></td>
<td>106.7 ± 122.2 ±</td>
<td>67.1 ±</td>
<td></td>
</tr>
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<td>22</td>
<td></td>
<td>698.1 ± 587.4 ±</td>
<td>749.8 ± 631.3 ±</td>
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<tr>
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<td>121.3 ± 205.7 ±</td>
<td>220.0 ± 158.5 ±</td>
<td>101.1 ±</td>
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<tr>
<td>25</td>
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<td>979.4 ± 868.0 ±</td>
<td>1161.1 ± 934.9 ±</td>
<td>1074.5 ±</td>
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<tr>
<td></td>
<td></td>
<td>198.5 ± 333.5 ±</td>
<td>415.4 ± 223.8 ±</td>
<td>136.2 ±</td>
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</table>

<table>
<thead>
<tr>
<th>Days</th>
<th>Anti-mPDL1 + Crizotinib (5mg/kg)</th>
<th>P value</th>
<th>Anti-mPDL1 + Crizotinib (20mg/kg)</th>
<th>P value</th>
<th>Anti-mPDL1 + Crizotinib (40mg/kg)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>90.2 ± 7.5 ns</td>
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<td>89.7 ± 7.3 ns</td>
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</tr>
<tr>
<td>10</td>
<td>110.7 ± 20.6 ns</td>
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<td>110.9 ± 18.1 ns</td>
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<td>103.5 ± 8.8 ns</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>164.9 ± 32.2 ns</td>
<td></td>
<td>183.7 ± 32.6 ns</td>
<td></td>
<td>166.7 ± 24.6 ns</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>163.4 ± 44.1 ns</td>
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<td>148.8 ± 36.0 ns</td>
<td></td>
<td>200.4 ± 43.6 ns</td>
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<tr>
<td>22</td>
<td>346.6 ± ns</td>
<td></td>
<td>264.6 ± 84.2 ns</td>
<td></td>
<td>402.3 ± 107.4 ns</td>
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</tr>
<tr>
<td>25</td>
<td>402.0 ± 0.0003 ns</td>
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<td>239.9 ± 104.1 &lt; 0.0001</td>
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<td>416.2 ± 148.3 4</td>
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</table>

Tumor volume (mm³) is shown as Mean ± SEM. P value is the comparison of anti-mPD-L1 + Crizotinib vs. Crizotinib treated group. P < 0.05 is considered statistically significant, and "ns" denotes non-significant difference.
Table 7. Tumor Measurements for Crizotinib and Anti-mPDL1 Combination in MC38 Model Over Time

<table>
<thead>
<tr>
<th>Days</th>
<th>Rat IgG2b (Mean ± SEM)</th>
<th>Anti-mPDL1 (Mean ± SEM)</th>
<th>Crizotinib (Mean ± SEM)</th>
<th>Anti-mPDL1 + Crizotinib (Mean ± SEM)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>77.9 ± 5.4</td>
<td>78.2 ± 5.6</td>
<td>78.1 ± 6.0</td>
<td>78.1 ± 6.3</td>
<td>ns</td>
</tr>
<tr>
<td>14</td>
<td>175.3 ± 12.8</td>
<td>166.5 ± 11.5</td>
<td>169.7 ± 16.3</td>
<td>111.2 ± 9.6</td>
<td>ns</td>
</tr>
<tr>
<td>19</td>
<td>439.5 ± 50.5</td>
<td>322.3 ± 28.7</td>
<td>291.7 ± 26.2</td>
<td>214.5 ± 20.6</td>
<td>ns</td>
</tr>
<tr>
<td>24</td>
<td>905.8 ± 89.9</td>
<td>590.8 ± 69.3</td>
<td>580.5 ± 67.0</td>
<td>251.8 ± 31.4</td>
<td>0.0017</td>
</tr>
<tr>
<td>27</td>
<td>1555 ± 166.4</td>
<td>830.4 ± 83.1</td>
<td>836.5 ± 137.8</td>
<td>401.7 ± 50.6</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Tumor volume (mm³) is shown as Mean ± SEM. P value is the comparison of Anti-mPDL1 + Crizotinib vs. Crizotinib treated group. P < 0.05 is considered statistically significant, and "ns" denotes non-significant difference.

By contrast, intervention with alectinib in the CT26 carcinoma study did not result in a significant improvement any of the single agent groups (Figure 4D).
Table 8. Tumor Measurements for Alectinib and Anti-mPDL1 Combination in CT26 Model Over Time

<table>
<thead>
<tr>
<th>Days</th>
<th>Rat IgG2a</th>
<th>Anti-mPDL1</th>
<th>Alectinib</th>
<th>Anti-mPDL1 + Alectinib</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>119.7 ±</td>
<td>119.1 ±</td>
<td>119.1 ±</td>
<td>119.2 ±</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>11.6</td>
<td>12.4</td>
<td>12.5</td>
<td>12.8</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>268.2 ±</td>
<td>253.5 ±</td>
<td>293.4 ±</td>
<td>230.0 ±</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>36.6</td>
<td>59.8</td>
<td>54.2</td>
<td>40.3</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>530.2 ±</td>
<td>438.4 ±</td>
<td>546.8 ±</td>
<td>337.7 ±</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>103.5</td>
<td>152.8</td>
<td>98.0</td>
<td>81.5</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>1076.4 ±</td>
<td>738.9 ±</td>
<td>1114.2 ±</td>
<td>816.7 ±</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>247.5</td>
<td>210.4</td>
<td>217.0</td>
<td>247.7</td>
<td></td>
</tr>
<tr>
<td>27</td>
<td>1759.9 ±</td>
<td>1285.9 ±</td>
<td>1892.5 ±</td>
<td>1202.4 ±</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>357.7</td>
<td>414.6</td>
<td>283.1</td>
<td>295.0</td>
<td></td>
</tr>
</tbody>
</table>

Tumor volume (mm\(^3\)) is shown as Mean ± SEM. P value is the comparison of Anti-mPDL1 + Alectinib vs. Alectinib treated group. P < 0.05 is considered statistically significant, and "ns" denotes non-significant difference.

Example 4: CD8+ T cells is required for enhanced anti-tumor efficacy resulting from crizotinib in combination with anti-PD-L1 antibody treatment. To evaluate the effect of crizotinib in combination with anti-PD-L1 on immune cells, *in vivo* depletion of CD4\(^+\), CD8\(^+\), or CD4\(^+\) concurrently with CD8\(^+\) T cells were performed prior to combination treatment in CT26 syngeneic model.

As shown in Figure 5, compared to isotype-depletion antibody treatment, *in vivo* depletion of CD4\(^+\) T cells did not affect the anti-tumor efficacy of the ALK/PD-L1 combination treated group. However *in vivo* depletion of CD8\(^+\) T cells alone, or the concurrent depletion of both CD4\(^+\) and CD8\(^+\) T cells, led to loss of anti-tumor efficacy by the ALK/PD-L1 combination treatment group. By Day 21, as compared to the isotype depletion antibody-treated group (Group I), the anti-CD8\(^+\) depletion antibody group...
(Group 3) or the anti-CD4⁺ and anti-CD8⁺ concurrent depletion antibodies group (Group 4) resulted in tumor growth inhibition (TGI) of -92.95% (P value <0.0001) and -91.5% (P value <0.0001) respectively. Percentage of TGI was defined as (1 - Tumor Volume depletion / Tumor Volume isotype control) x 100%.

Materials and Methods

Mice. Six- to eight-week old female Balb/c mice were purchased from The Jackson Laboratories. Mice were maintained and all animal experiments were conducted according to the protocols approved by the Institutional Animal Care and Use Committee of Rinat, South San Francisco and Worldwide Research and Development (WRD), La Jolla, Pfizer Inc.

Materials. CT26 cell lines, Rat IgG2b (clone LTF-2), anti-mouse CD4 (Rat IgG2b, GK1.5) and anti-mouse CD8 (Rat IgG2b, 2.43) were purchased from Bioexcel (West Lebanon, NH) and prepared as described in Example 2 above. As described above, rat anti-mouse PD-L1 mAb (clone MIH5) was purchased from eBioscience. In vivo tumor efficacy studies. Balb/c mice were inoculated subcutaneously at the right lower flank with 0.1 ml of 0.1x10⁶ CT26 murine colorectal cells in PBS. When tumor bearing mice achieved a mean tumor volume of 50 to 150 mm³, mice were randomly assigned to 1 of 4 treatment groups, n= 10. Depletion antibodies were given intraperitoneal^ every 3 days and started one day prior to any drug treatment (crizotinib or PD-L1 or combo). Group 1 received 10 mg/kg of an isotype control depletion antibody (IgG2b); Group 2 received 10 mg/kg anti-CD4 depletion antibody (Rat IgG2b, GK1.5); Group 3 received 10 mg/kg anti-CD8 depletion antibody (Rat IgG2b, 2.43) and Group 4 was administered combination of anti-CD4 and anti-CD8 depletion antibodies. Crizotinib was dosed orally at 20 mg/kg, QD for 14 days. Murine surrogate anti-PD-L1 antibody (MIH5) was given intraperitoneal^ every 3 days for a total of 3 doses and started on concurrently with Crizotinib. Mice were monitored for tumor growth and body weight changes.

In the CT26 carcinoma model, in vivo depletion of CD4⁺ T cells does not affect the anti-tumor efficacy under combination treatment when compared to isotype depletion antibody treated group. In vivo depletion of CD8⁺ T cells alone, or concurrent depletion of CD4⁺ and CD8⁺ T cells, leads to a significant loss of anti-tumor efficacy for the
combination treatment group. By Day 21, compared to isotype depletion antibody treated group, anti-CD8+ depletion antibody or anti-CD4+ and anti-CD8+ depletion antibodies concurrently treated group results in tumor growth inhibition (TGI) of -92.95% (P value <0.0001) and -91.5% (P value <0.0001) respectively. These results indicate that the synergetic anti-tumor efficacy from ALK inhibition with crizotinib and PD-L1 blockade is indeed mediated through the regulation of immune cells.

Table 9. Tumor Measurements for in vivo CD4+ and CD8+ T cell depletion with Crizotinib and Anti-mPDL1 Combination treatment in CT26 Model Over Time

<table>
<thead>
<tr>
<th>Day(s)</th>
<th>Rat IgG2b depletion</th>
<th>CD4+ Depletion</th>
<th>CD8+ Depletion</th>
<th>CD4+ plus CD8+ Depletion</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>6666.44 ± 44.55</td>
<td>6655.99 ± 44.44</td>
<td>6655.48 ± 44.33</td>
<td>65.4 ± 4.4</td>
<td>ns</td>
</tr>
<tr>
<td>14</td>
<td>1333 ± 66.55</td>
<td>1068 ± 1177.22</td>
<td>2328 ± 2255.00</td>
<td>378.0 ± 20.5</td>
<td>ns</td>
</tr>
<tr>
<td>17</td>
<td>11447 ± 119966.00</td>
<td>8604 ± 6606.33</td>
<td>8604 ± 73.3</td>
<td>&lt; 0.0001</td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>261 ± 990.66</td>
<td>624 ± 11388.22</td>
<td>120.6 ± 34423.44</td>
<td>1626.5 ± 115.8</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>

Tumor volume (mm³) is shown as Mean ± SEM. P value is the comparison of CD8+ depletion vs. Rat IgG2b treated group. P < 0.05 is considered statistically significant, and "ns" denotes non-significant difference.

Example 5: ALK inhibition with crizotinib enhanced anti-tumor activity of avelumab in ALK-negative colorectal tumors in vivo.

To evaluate the anti-tumor efficacy of combination immunotherapy against established MC38 tumors, avelumab dosing regimens were selected as 20 mg/kg per mouse intraperitoneal (i.p.) for total 3 doses and 3-4 days apart.

This study was conducted in MC38 colon carcinoma model, where the animals were treated with crizotinib at 40 mg/kg, QD for 12 days combined with fixed avelumab dose. Avelumab is a fully human IgG1 antibody that recognizes both human and mouse PD-L1. Administration of avelumab alone resulted in 49.7% TGI (P value <0.0001), crizotinib alone resulted in 48.5% TGI (P value <0.0001), and the combination of avelumab with crizotinib led to 65% TGI (P value <0.0001) compared with the isotype
control antibody treated group (Table 10 below). No statistically significant effect on body weight change was observed in animals under all treatment conditions.

Materials and Methods

5 Mice. Six- to eight-week old female C57BL/6 mice were purchased from The Jackson Laboratories (Farmington, CT). Mice were maintained and all animal experiments were conducted according to the protocols approved by the Institutional Animal Care and Use Committee of Rinat, South San Francisco and Worldwide Research and Development (WRD), La Jolla, Pfizer Inc.

10 Cell lines. MC38 colon carcinoma cell line was kindly provided by Dr. Antoni Ribas at UCLA, California. Cells were cultured in DMEM medium supplemented with 10% fetal bovine serum, and 100 I.U./mL penicillin/streptomycin at 37°C in an atmosphere of 5% CO2 in air, and IMPACT-tested for pathogens at Research Animal Diagnostic Laboratory (RADIL) (Columbia, MO). Pathogen-free cells growing in an exponential growth phase were harvested and used for tumor inoculation.

Antibodies for immunotherapy. Avelumab mAb (human IgG1) was provided by Merck Serono at 20 mg/mL concentration, Lot:508203. Human IgG1 was prepared in-house (Lot No. NB1 23249p 1 92CA1 1 0124). Antibody was prepared at concentration of 1mg/mL in phosphate buffered saline (PBS) (Invitrogen, Carlsbad, CA), and dosed 0.2 mL per mouse intraperitoneal^ (i.p.) for 3 doses 3-4 days apart.

In vivo tumor efficacy studies. C57BL/6 mice were used for subcutaneous tumor inoculation with $2 \times 10^5$ MC38 cells in 0.1 mL of PBS. When tumors reached an average of 100 mm$^3$, mice were randomized into groups of 10 animals per group, and treatment was started on the same day. Tumor size was measured in two dimensions using a digital caliper, and the volume was expressed in mm$^3$ using the formula: $V = 0.5 \times L \times W^2$ where L and W are the long and short diameters of the tumor, respectively. Body weight was recorded weekly. Percentage of TGI was defined as $1 - \frac{(Tumor\ volume_{\ treated}}{Tumor\ volume_{\ isotype\ control}) \times 100$. Treatment Group 1 received 20 mg/kg of an isotype control antibody (humanIgG1) intraperitoneal^ every 4 days for a total of 3 doses; Group 2 received 20 mg/kg avelumab intraperitoneal^ every 4 days for a total of 3 doses; Group 3 received 40 mg/kg of crizotinib, orally, once daily for 12 days; Group 4 received 20 mg/kg avelumab intraperitoneal^ every 4 days for a total of 3 doses plus 40 mg/kg
crizotinib orally, once daily for 12 days. Mice were monitored for tumor growth and body weight changes.

As shown in Figures 6A and 6B, a significant anti-tumor effect of the crizotinib/avelumab combination was observed in the MC38 colon carcinoma model.

Table 10. Tumor Measurements for MC38 Model Over Time

<table>
<thead>
<tr>
<th>Days</th>
<th>Huummaann IgG1 (Mean ± SEM)</th>
<th>Aaveluummaabb (Mean ± SEM)</th>
<th>Crizotinib (Mean ± SEM)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>7788.22 ± 55.11</td>
<td>7788.33 ± 55.44</td>
<td>7788.11 ± 66.00</td>
<td>ns</td>
</tr>
<tr>
<td>14</td>
<td>119999.66 ± 114455.56</td>
<td>116699.77 ± 11.1</td>
<td>ns</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>465.4 ± 307.1 ± 291.7 ± 210.8</td>
<td>11.1</td>
<td>&lt; 0.0001</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>3355.77 ± 2255.55 ± 2266.22</td>
<td>3300.77 ± 114699.77 ± 5.56</td>
<td>1.0005</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>110066.00 ± 6622.00 ± 6677.00</td>
<td>556633 ± 2255.55 ± 3300.77</td>
<td>0.0001</td>
<td></td>
</tr>
</tbody>
</table>

Tumor volume (mm³) is shown as Mean ± SEM. P value is the comparison of Avelumab + Crizotinib vs. isotype control antibody (human IgG1) treated group. P < 0.05 is considered statistically significant, and "ns" denotes non-significant difference.

Example 6: Studies to evaluate efficacy and safety of the ALK inhibitors, crizotinib and lorlatinib (PF06463922), in combination with PD-L1 inhibitor, avelumab.

The ALK inhibitors, crizotinib and lorlatinib, and PD-L1 inhibitor, avelumab, in combination can be studied in an open-label dose finding study to evaluate safety, efficacy, pharmacokinetics and pharmacodynamics in patients with either ALK-positive or ALK-negative locally advanced or metastatic NSCLC. The study can begin with a screening period of up to and including 28 days prior to the first dose of study drugs to assess eligibility. The treatment period begins on the first day of the first cycle (14 days), see combination schedule in Tables A and B below, and lasts for 2 cycles. Treatment with ALK inhibitors, crizotinib or lorlatinib, with avelumab may for example continue until the patient experiences unacceptable toxicity that precludes further treatment and/or disease progression.

The study can consist of dose-finding and dose-expansion phase.
1. Dose-finding phase

Both ALK-positive and ALK-negative groups will be evaluated to identify the maximum tolerated dose (MTD) and recommended Phase 2 dose (RP2D). Determination of the MTD will be performed using modified toxicity probability index (mTPI) with dose de-escalation from the approved prescribed dose of crizotinib (250 mg BID), 100 mg QD of lorlatinib and the RP2D of avelumab (10 mg/kg Q2W). In the ALK-positive group (Group B), a dose expansion will evaluate an additional 12 patients at the MTD/RP2D to further assess safety, pharmacokinetics, pharmacodynamics and antitumor activity of the combination.

Table A. Crizotinib plus Avelumab Dose Levels (Group A) in patients with ALK-negative NSCLC

<table>
<thead>
<tr>
<th>Crizotinib</th>
<th>Avelumab</th>
<th>Avelumab</th>
</tr>
</thead>
<tbody>
<tr>
<td>250 mg BID PO</td>
<td>DL-1A*</td>
<td>DL 0 (Start)</td>
</tr>
<tr>
<td>200 mg BID PO</td>
<td>DL-1A-1C</td>
<td>DL-1C**</td>
</tr>
<tr>
<td>250 mg QD PO</td>
<td>DL-1A-2C</td>
<td>DL-2C**</td>
</tr>
</tbody>
</table>

* A denotes dose reduction attributed to avelumab (A=avelumab)

** 1C denotes dose reduction attributed to crizotinib (C=crizotinib), -2C denotes dose reduction attributed to crizotinib (C=crizotinib).

Table B. Lorlatinib plus Avelumab Dose Levels (Group B) in patients with ALK-positive NSCLC

<table>
<thead>
<tr>
<th>Lorlatinib</th>
<th>Avelumab</th>
<th>Avelumab</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 mg QD PO</td>
<td>DL-1A*</td>
<td>DL-0 (Start)</td>
</tr>
<tr>
<td>75 mg QD PO</td>
<td>DL-1A*-1P**</td>
<td>DL-1P**</td>
</tr>
<tr>
<td>50 mg QD PO</td>
<td>DL-1A*-2P**</td>
<td>DL-2P**</td>
</tr>
</tbody>
</table>

* A denotes dose reduction attributed to avelumab (A=avelumab)

** 1P, -2P denotes dose reduction attributed to lorlatinib, PF-06643922 (P=PF-06643922)

The MTD estimate is the highest dose tested of crizotinib and avelumab or lorlatinib and avelumab associated with the occurrence of DLTs within the first 2 cycles of treatment in <33% of patients. The RP2D is the dose of crizotinib and avelumab or lorlatinib and avelumab in combination chosen for further clinical development. If the MTD proves to
be clinically feasible for long-term administration in a reasonable number of patients, this dose may become the RP2D. Further experience in Phase 1b may result in a RP2D dose lower than the MTD.

2. Expansion phase

In Group B, a dose expansion will evaluate an additional 12 patients at the MTD/RP2D to further assess the safety, pharmacokinetics, pharmacodynamics and antitumor activity of the combination of lorlatinib with avelumab in ALK-positive patients with NSCLC.

Phase 2 (Group A only)

After the MTD is identified and the RP2D is determined in Group A, the 12 patients treated at the RP2D will be considered Stage 1 of Simon's Optimal Two-Stage design. If 3 or more patients of the 12 in Phase 1b have a confirmed response per RECIST 1.1, then in Phase 2 an additional 33 patients will be enrolled and treated. If there are fewer than 3 patients who have a confirmed objective response in the first 12 patients treated at the RP2D, then Phase 2 will not be opened for enrollment. Patients with ALK-negative NSCLC who are enrolled and subsequently determined by retrospective central testing to be positive for ALK gene rearrangement, ROS1 gene translocation, c-Met gene amplification, or c-Met exon 14 deletion may be replaced.

Dose-expansion

In the ALK-positive group (Group B, above), a dose expansion will evaluate an additional 12 patients at the MTD/RP2D to further assess the safety, pharmacokinetics, pharmacodynamics and antitumor activity of the combination.

In the ALK-negative group (Group A, above), after the MTD is identified and the RP2D is determined, the 12 patients treated at the RP2D will be considered Stage 1 of Simon's Optimal Two-Stage design. If 3 or more patients of the 12 in Phase 1b have a confirmed response per RECIST 1.1, then in Phase 2 an additional 33 patients will be enrolled and treated. If there are fewer than 3 patients who have a confirmed objective response in the first 12 patients treated at the RP2D, then Phase 2 will not be opened for enrollment. Patients with ALK-negative NSCLC who are enrolled and subsequently determined by retrospective central testing to be positive for ALK gene rearrangement, ROS1 gene translocation, c-Met gene amplification, or c-Met exon 14 deletion may be replaced. Patients with NSCLC containing epidermal growth factor receptor (EGFR)
mutations are permitted into the ALk-negative group if they have exhausted appropriate targeted therapy for these mutations. The data cut-off for the primary clinical study report can occur once all patients in the expansion phase have completed treatment or have discontinued earlier.

Example 7: Study to evaluate efficacy and safety of ALK-inhibitor, crizotinib in combination with PD-L1 inhibitor, avelumab, in patients with ALK-negative NSCLC

Group A patients were enrolled on the basis of locally approved testing for ALK-negative NSCLC tumor biopsy, subject to confirmation with retrospective central testing. Four patients from Group A had data available for Investigator Assessment: 3 patients with Overall Response as Progressive Disease and 1 patient with unconfirmed Stable Disease. These data are preliminary and have not yet been verified against source documents. The amount of time between first dose and follow-up scan at Investigator Assessment was approximately 8 weeks, per protocol. The clinical study from which this preliminary data was generated specifies RECIST v1.1 for evaluation of response.

All references cited herein are incorporated by reference to the same extent as if each individual publication, database entry (e.g. Genbank sequences or GenelD 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 GenelD 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.
We claim:

1. A method for treating or delaying progression of a cancer in an individual comprising administering to the individual an effective amount of a PD-1 axis binding antagonist and an ALK inhibitor.

2. The method of claim 1, wherein the cancer is an ALK-positive cancer.

3. The method of claim 1, wherein the cancer is an ALK-negative cancer.

4. The method of claim 1, wherein the PD-1 axis binding antagonist is selected from the group consisting of a PD-1 binding antagonist, a PD-L1 binding antagonist and a PD-L2 binding antagonist.

5. The method of Claim 4, wherein the PD-1 axis binding antagonist is a PD-1 binding antagonist.

6. The method of Claim 5, wherein the PD-1 binding antagonist inhibits the binding of PD-1 to its ligand binding partners.

7. The method of Claim 6, wherein the PD-1 binding antagonist inhibits the binding of PD-1 to PD-L1.

8. The method of Claim 6, wherein the PD-1 binding antagonist inhibits the binding of PD-1 to PD-L2.

9. The method of Claim 6, wherein the PD-1 binding antagonist inhibits the binding of PD-1 to both PD-L1 and PD-L2.

10. The method of claim 6, wherein the PD-1 binding antagonist is AMP-224.

11. The method of Claim 1, wherein the PD-1 axis binding antagonist is an antibody or antibody fragment thereof.

12. The method of Claim 10, wherein the PD-1 axis binding antagonist is an anti-PD-1 antibody.

13. The method of Claim 12, wherein the anti-PD-1 antibody is selected from the group consisting of nivolumab (MDX-1106), pembrolizumab (MK-3475), pidilizumab (CT-011) or an antibody comprising a VH region produced by the expression vector with ATCC Accession No. PTA-121183 and having the VL region produced by the expression vector with ATCC Accession No. PTA-121182.

14. The method of Claim 4, wherein the PD-1 axis binding antagonist is a PD-L1 binding antagonist.

15. The method of Claim 14, wherein the PD-L1 binding antagonist is an anti PD-L1 antibody or antibody fragment thereof.
16. The method of Claim 14, wherein the PD-L1 binding antagonist inhibits the binding of PD-L1 to PD-1.

17. The method of Claim 14, wherein the PD-L1 binding antagonist inhibits the binding of PD-L1 to B7-1.

18. The method of Claim 14, wherein the PD-L1 binding antagonist inhibits the binding of PD-L1 to both PD-1 and B7-1.

19. The method of Claim 14, wherein the PD-L1 binding antagonist is selected from the group consisting of: MPDL3280A, MDX-1 105, MEDI4736 and MSB001 071 8C.

20. The method of Claim 19, wherein the antibody or antibody fragment is mAb7 and comprises a heavy chain selected from the group consisting of SEQ ID NO: 4 or SEQ ID NO: 5 and a light chain selected from the group consisting of SEQ ID NO: 6 or SEQ ID NO: 7.

21. The method of Claim 19, wherein the antibody or antibody fragment is mAb1 5 and comprises a heavy chain of SEQ ID NO: 8 and a light chain of SEQ ID NO: 9.

22. The method of Claim 4, wherein the PD-1 axis binding antagonist is a PD-L2 binding antagonist.

23. The method of Claim 22, wherein the PD-L2 binding antagonist is an antibody or antibody fragment thereof.

24. The method of any one of claims 1-23, wherein the ALK inhibitor is a competitive inhibitor of ALK.

25. The method of claim 24, wherein the ALK inhibitor is selected from the group consisting of: crizotinib, ceritinib, PF-06463922, NVP-TAE684, AP261 13, TSR-01 1, X-396, CEP-37440, and RXDX-1 01.

26. The method of claim 25, wherein the ALK inhibitor is selected from the group consisting of PF-064609322 or crizotinib, or a pharmaceutically acceptable salt or solvate thereof.

27. The method of any one of claims 1-26, wherein the ALK-negative cancer is a solid tumor.

28. The method of claim 27, wherein the cancer does not test positive for an EML4 - ALK fusion.

29. The method of claim 28, wherein the ALK-negative cancer is selected from the group consisting of melanoma, colon cancer, bladder cancer, breast cancer, clear cell kidney cancer, head/neck squamous cell carcinoma, rectal cancer, lung squamous cell
carcinoma, thyroid cancer, bladder cancer, cervical cancer, uterine cancer, endometrial cancer, lung adenocarcinoma, ovarian cancer, papillary kidney cancer, non-small-cell lung cancer (NSCLC), ovarian cancer, pancreatic cancer, prostate cancer, renal cell cancer, small-cell lung cancer (SCLC) and triple negative breast cancer.

30. The method of any one of claims 1-29, wherein the treatment results in a sustained response in the individual after cessation of the treatment.

31. The method of any of claims 1-30, wherein the ALK inhibitor is administered continuously.

32. The method of any of claims 1-30, wherein the ALK inhibitor is administered intermittently.

33. The method of any of claims 1-30, wherein the ALK inhibitor is administered before the PD-1 axis binding antagonist.

34. The method of any of claims 1-30, wherein the ALK inhibitor is administered simultaneous with the PD-1 axis binding antagonist.

35. The method of any of claims 1-30, wherein the ALK inhibitor is administered after the PD-1 axis binding antagonist.

36. The method of any one of claims 1-30, wherein the individual has an ALK-negative cancer selected from the group consisting of colorectal cancer, melanoma, non-small cell lung cancer, ovarian cancer, breast cancer, pancreatic cancer, hematological malignancy, renal cell carcinoma.

37. A method of enhancing immune function in an individual having an ALK-negative cancer comprising administering an effective amount of a combination of a PD-1 axis binding antagonist and a ALK inhibitor.

38. The method of claim 37, wherein PD-L1 on the cancer cell surface is inhibited from transducing a signal to the intracellular pathway.

39. The method of claim 15, wherein the anti-PD-L1 antibody or antibody fragment is capable of inhibiting binding between PD-L1 and PD-1 and/or between PD-L1 and B7-I.

40. The method of claim 15, wherein the anti-PD-L1 antibody is an antibody fragment selected from the group consisting of Fab, Fab’-SH, Fv, scFv, and (Fab’)2 fragments.

41. The method of claim 40, wherein the anti-PD-L1 antibody is a humanized antibody or antibody fragment thereof.

42. The method of claim 40, wherein the anti-PD-L1 antibody is a human antibody or antibody fragment thereof.
43. The method of Claim 42, wherein the antibody or antibody fragment has at least 85% sequence identity to the full length heavy chain sequence of SEQ ID NO: 4, and at least 85% sequence identity to the full length light chain sequence of SEQ ID NO: 6.

44. The method of Claim 43, wherein the antibody or antibody fragment is mAb7 or mAb 15 and comprises a heavy chain selected from the group consisting of SEQ ID NOS: 4, 5 or 8.

45. A medicament comprising a PD1 axis binding antagonist, for use in combination with an ALK inhibitor for treating ALK-negative cancer in an individual.

46. A medicament comprising an ALK inhibitor, for use in combination with a PD-1 axis binding antagonist for treating ALK-negative cancer in an individual.

47. The method of any one of claims 1-46, wherein the PD-1 axis binding antagonist is administered intravenously, intramuscularly, subcutaneously, topically, orally, transdermal\textsuperscript{a}, intraperitoneal\textsuperscript{a}, intraorbital\textsuperscript{a}, by implantation, by inhalation, intrathecal\textsuperscript{a}, intraventricularly, or intranasally.

48. 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 medicament comprising an anti-PD-1 axis binding antagonist, the second container comprises at least one dose of a medicament comprising an ALK antagonist, and the package insert comprises instructions for treating an individual for cancer using the medicaments.

49. A kit comprising a PD-1 axis binding antagonist and a package insert comprising instructions for using the PD-1 axis binding antagonist in combination with an ALK inhibitor to treat or delay progression of an ALK-negative cancer in an individual.

50. A kit comprising a PD-1 axis binding antagonist and an ALK inhibitor, and a package insert comprising instructions for using the PD-1 axis binding antagonist and the ALK inhibitor to treat or delay progression of an ALK-negative cancer in an individual.

51. A kit comprising an ALK inhibitor and a package insert comprising instructions for using the ALK inhibitor in combination with a PD-1 axis binding antagonist to treat or delay progression of an ALK-negative cancer in an individual.

52. The kit of claim 48, wherein the instructions state that the medicaments are intended for use in treating an individual having a cancer that does not test positive for an ALK fusion event or an ALK oncogenic mutation selected from the group consisting of EML4-ALK, KIF5B-ALK, TFG-ALK, KLC1-ALK, NPM-ALK, TMP3-ALK, TPM4-ALK, ATIC-ALK and CLTC-ALK.
53. The kit of any one of claims 48-52, wherein the PD-1 axis binding antagonist is an anti-PD-L1 antibody or antibody fragment thereof.

54. The kit of any one of claims 48-52, wherein the PD-1 axis binding antagonist is an anti-PD-1 antibody or antibody fragment thereof.

55. The kit of any one of claims 48-52, wherein the ALK-negative cancer is selected from the group consisting of melanoma, colon cancer, bladder cancer, breast cancer, clear cell kidney cancer, head/neck squamous cell carcinoma, rectal cancer, lung squamous cell carcinoma, thyroid cancer, bladder cancer, cervical cancer, uterine cancer, endometrial cancer, lung adenocarcinoma, ovarian cancer, papillary kidney cancer, non-small-cell lung cancer (NSCLC), ovarian cancer, pancreatic cancer, prostate cancer, renal cell cancer, small-cell lung cancer (SCLC) and triple negative breast cancer.

56. The method, use or kit of claim 55, wherein the ALK-negative cancer is an advanced or metastatic solid tumor.

57. The method, use or kit of any of the preceding claims, wherein the ALK-negative cancer does not overexpress c-Met and/or ROS1 and/or have no known alterations in either c-Met or Ros1.
human Pan T cells: CD3-CD28 stimulation in the presence of compounds for 3 days; Cell proliferation is measured by thymidine incorporation.
T Cell Viability

human Pan T cells; CD3-CD28 stimulation in the presence of compounds for 3 days; Viability is measured by Cell-Titer Glo Luminescent Cell Viability assay
PF-06463922 in CT26 Model

Days After Tumor Inoculation

FIGURE 2C

anti-mPDL1 and PF-06463922 CT26 Model

Days After Tumor Inoculation

FIGURE 2D
Rat IgG2a in B16 Model

Days After Tumor Inoculation

Antibody dosing:
10mg/kg, IP

Anti-mPDL1 in B16 Model

Days After Tumor Inoculation

Antibody dosing:
10mg/kg, IP

FIGURE 3A

FIGURE 3B
PF-06463922 in B16 Model

Days After Tumor Inoculation

FIGURE 3C

Anti-mPDL1 and PF-06463922 in B16 Model

Days After Tumor Inoculation

FIGURE 3D
CT26 Syngeneic Model: Anti-mPDL1 in Combination with Escalating Crizotinib Doses

FIGURE 4A
CT26 Syngeneic Model: Anti-mPDL1 in Combination with Escalating Crizotinib Doses

FIGURE 4B
MC38 Syngeneic Model: Anti-mPDL1 in Combination with Crizotinib

![Graph showing tumor volume over time with different treatments: Rat IgG2b, Anti-mPDL1, Crizotinib, and Anti-mPDL1+ Crizotinib.](image)

- Rat IgG2b
- Anti-mPDL1
- Crizotinib
- Anti-mPDL1+ Crizotinib

**Days After Tumor Inoculation**

- Day 7
- Day 14
- Day 19
- Day 24
- Day 27

**Antibody dosing:**
- 10mg/kg, IP

**12 days of Crizotinib dosing, 40mg/kg, QD**

**FIGURE 4C**
CT26 Syngeneic Model: Anti-mPDL1 in Combination with Alectinib

- Rat IgG2a
- Anti-mPDL1
- Alectinib
- Anti-mPDL1 + Alectinib

Days After Tumor Inoculation

FIGURE 4D
Impact of CD4+ and CD8+ T Cell Depletion

FIGURE 5
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. [X] 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 13fer1 (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:
      - □ in the form of an Annex C/ST.25 text file (Rule 13fer1 (a)).
      - □ on paper or in the form of an image file (Rule 13fer1 (b) and Administrative Instructions, Section 7:13).

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 forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:
**INTERNATIONAL SEARCH REPORT**

**INTERNATIONAL APPLICATION**

**PCT/US2016/053939**

A. CLASSIFICATION OF SUBJECT MATTER

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

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

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

**EPO-Internal**

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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

* Special categories of cited documents:

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Date of the actual completion of the international search: 15 December 2016

Date of mailing of the international search report: 04/01/2017

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Authorized officer:
Vadot, Pierre
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