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(54) Title: THERAPEUTIC AND DIAGNOSTIC METHODS FOR CANCER

(57) Abstract: The present invention provides therapeutic and diagnostic methods and compositions for cancer, for example, non-small cell lung cancer (NSCLC). The invention provides methods of treating NSCLC, methods of determining whether a patient suffering from NSCLC is likely to respond to treatment comprising a PD-L1 axis binding antagonist, methods of predicting responsiveness of a patient suffering from NSCLC to treatment comprising a PD-L1 axis binding antagonist, and methods of selecting a therapy for a patient suffering from NSCLC, based on expression levels of a biomarker of the invention (e.g., PD-L1 expression levels in tumor cells and/or tumor-infiltrating immune cells).

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THERAPEUTIC AND DIAGNOSTIC METHODS FOR CANCER

SEQUENCE LISTING

The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on May 11, 2016, is named 50474-111WO3_Sequence_Listing_5_11_16_ST25 and is 23,626 bytes in size.

FIELD OF THE INVENTION

Provided herein are therapeutic and diagnostic methods and compositions for pathological conditions, such as cancer (e.g., non-small cell lung cancer), and methods of using PD-L1 axis binding antagonists. In particular, the invention provides biomarkers for patient selection and prognosis, methods of treatment, articles of manufacture, diagnostic kits, and methods of detection.

BACKGROUND

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

Programmed death-ligand 1 (PD-L1) is a protein that has been implicated in the suppression of immune system responses during chronic infections, pregnancy, tissue allografts, autoimmune diseases, and cancer. PD-L1 regulates the immune response by binding to an inhibitory receptor, known as programmed death 1 (PD-1), which is expressed on the surface of T-cells, B cells, and monocytes. PD-L1 negatively regulates T-cell function also through interaction with another receptor, B7-1. Formation of the PD-L1/PD-1 and PD-L1/B7-1 complexes negatively regulates T-cell receptor signaling, resulting in the subsequent downregulation of T-cell activation and suppression of anti-tumor immune activity.

Despite the significant advancement in the treatment of cancer, improved therapies and diagnostic methods are still being sought.

SUMMARY OF THE INVENTION

The present invention provides therapeutic and diagnostic methods and compositions for cancer, for example, non-small cell lung cancer (NSCLC).

In one aspect, the invention features a method of treating a patient suffering from a non-small cell lung cancer, the method comprising administering to the patient a therapeutically effective amount of a PD-L1 axis binding antagonist, wherein a tumor sample obtained from the patient has been determined to have a detectable expression level of PD-L1 in 5% or more of the tumor cells in the tumor sample. In

some embodiments, the tumor sample obtained from the patient has been determined to have a detectable expression level of PD-L1 in 10% or more of the tumor cells in the tumor sample. In some embodiments, the tumor sample obtained from the patient has been determined to have a detectable expression level of PD-L1 in 20% or more of the tumor cells in the tumor sample. In some embodiments, the tumor sample obtained from the patient has been determined to have a detectable expression level of PD-L1 in 50% or more of the tumor cells in the tumor sample. In some embodiments, the tumor sample obtained from the patient has a detectable expression level of PD-L1 in tumor-infiltrating immune cells that comprise less than 10% of the sample.

In another aspect, the invention features a method of treating a patient suffering from a non-small cell lung cancer, the method comprising administering to the patient a therapeutically effective amount of a PD-L1 axis binding antagonist, wherein a tumor sample obtained from the patient has been determined to have a detectable expression level of PD-L1 in tumor-infiltrating immune cells that comprise 5% or more of the tumor sample, and a detectable expression level of PD-L1 in less than 50% of the tumor cells in the tumor sample. In some embodiments, the tumor sample obtained from the patient has been determined to have a detectable expression level of PD-L1 in tumor-infiltrating immune cells that comprise 10% or more of the tumor sample.

In another aspect, the invention features a method for determining whether a patient suffering from a non-small cell lung cancer is likely to respond to treatment comprising a PD-L1 axis binding antagonist, the method comprising: determining the expression level of PD-L1 in tumor cells in a tumor sample obtained from the patient, wherein a detectable expression level of PD-L1 in 5% or more of the tumor cells in the tumor sample indicates that the patient is likely to respond to treatment comprising a PD-L1 axis binding antagonist.

In another aspect, the invention features a method for determining whether a patient suffering from a non-small cell lung cancer is likely to respond to treatment comprising a PD-L1 axis binding antagonist, the method comprising: determining the expression level of PD-L1 in tumor-infiltrating immune cells and in tumor cells in a tumor sample obtained from the patient, wherein a detectable expression level of PD-L1 in tumor-infiltrating immune cells that comprise 5% or more of the tumor sample, and a detectable expression level of PD-L1 in less than 50% of the tumor cells in the tumor sample, indicates that the patient is likely to respond to treatment comprising a PD-L1 axis binding antagonist.

In another aspect, the invention features a method for predicting responsiveness of a patient suffering from a non-small cell lung cancer to treatment comprising a PD-L1 axis binding antagonist, the method comprising: determining the expression level of PD-L1 in tumor cells in a tumor sample obtained from the patient, wherein a detectable expression level of PD-L1 in 5% or more of the tumor cells in the tumor sample indicates that the patient is likely to respond to treatment comprising a PD-L1 axis binding antagonist.

In another aspect, the invention features a method for predicting responsiveness of a patient suffering from a non-small cell lung cancer to treatment comprising a PD-L1 axis binding antagonist, the method comprising: determining the expression level of PD-L1 in tumor-infiltrating immune cells and in tumor cells in a tumor sample obtained from the patient, wherein a detectable expression level of PD-L1 in tumor-infiltrating immune cells that comprise 5% or more of the tumor sample, and a detectable

expression level of PD-L1 in less than 50% of the tumor cells in the tumor sample, indicates that the patient is likely to respond to treatment comprising a PD-L1 axis binding antagonist.

In some embodiments of any of the preceding aspects, a detectable expression level of PD-L1 in 10% or more of the tumor cells in the tumor sample indicates that the patient is likely to respond to treatment comprising a PD-L1 axis binding antagonist. In some embodiments, a detectable expression level of PD-L1 in 20% or more of the tumor cells in the tumor sample indicates that the patient is likely to respond to treatment comprising a PD-L1 axis binding antagonist. In some embodiments, a detectable expression level of PD-L1 in 50% or more of the tumor cells in the tumor sample indicates that the patient is likely to respond to treatment comprising a PD-L1 axis binding antagonist. In some embodiments, the method further comprises determining the expression level of PD-L1 in tumor-infiltrating immune cells in the tumor sample obtained from the patient. In some embodiments, the tumor sample obtained from the patient has a detectable expression level of PD-L1 in tumor-infiltrating immune cells that comprise less than 10% of the sample.

In some embodiments of any of the preceding aspects, the tumor sample obtained from the patient has been determined to have a detectable expression level of PD-L1 in tumor-infiltrating immune cells that comprise 10% or more of the tumor sample.

In another aspect, the invention features a method for selecting a therapy for a patient suffering from a non-small cell lung cancer, the method comprising: determining the expression level of PD-L1 in tumor cells in a tumor sample obtained from the patient, and selecting a therapy comprising a PD-L1 axis binding antagonist for the patient based on a detectable expression level of PD-L1 in 5% or more of the tumor cells in the tumor sample. In some embodiments, the method comprises selecting a therapy comprising a PD-L1 axis binding antagonist for the patient based on a detectable expression level of PD-L1 in 10% or more of the tumor cells in the tumor sample. In some embodiments, the method comprises selecting a therapy comprising a PD-L1 axis binding antagonist for the patient based on a detectable expression level of PD-L1 in 20% or more of the tumor cells in the tumor sample. In some embodiments, the method comprises selecting a therapy comprising a PD-L1 axis binding antagonist for the patient based on a detectable expression level of PD-L1 in 50% or more of the tumor cells in the tumor sample. In some embodiments, the method further comprises determining the expression level of PD-L1 in tumor-infiltrating immune cells in the tumor sample obtained from the patient. In some embodiments, the tumor sample obtained from the patient has a detectable expression level of PD-L1 in tumor-infiltrating immune cells that comprise less than 10% of the sample.

In another aspect, the invention features a method for selecting a therapy for a patient suffering from a non-small cell lung cancer, the method comprising: determining the expression level of PD-L1 in tumor-infiltrating immune cells and in tumor cells in a tumor sample obtained from the patient, and selecting a therapy comprising a PD-L1 axis binding antagonist for the patient based on a detectable expression level of PD-L1 in tumor-infiltrating immune cells that comprise 5% or more of the tumor sample, and a detectable expression level of PD-L1 in less than 50% of the tumor cells in the tumor sample. In some embodiments, the expression level of PD-L1 in tumor-infiltrating immune cells is determined to be detectable in tumor-infiltrating cells that comprise at least 10% of the tumor sample.

In some embodiments of any of the preceding aspects, the tumor sample obtained from the patient comprises an increased number of intra-epithelial and/or stromal immune cells relative to a reference tumor sample. In some embodiments, the tumor sample obtained from the patient comprises an increased number of CD8+ T-cells relative to a reference tumor sample. In some embodiments, the tumor sample obtained from the patient has an increased expression level of one or more B-cell-related genes or natural killer (NK) cell-related genes relative to a reference tumor sample. In some embodiments, the one or more B-cell-related genes is selected from the group consisting of *CD19*, *MS4A1*, and *CD79A*. In some embodiments, the one or more NK cell-related genes is selected from the group consisting of *KLRB1*, *KLRC1*, *KLRC2*, *KLRC3*, *KLRD1*, *KLRF1*, *KLRG1*, *KLRK1*, *NCAM1*, *PRF1*, *NCR1*, *KIR2DL2*, *KIR2DL3*, *KIR2DL4*, *KIR2DS2*, *KIR3DL1*, *FCGR3A*, *MICA*, and *MICB*.

In some embodiments of any of the preceding aspects, the tumor sample obtained from the patient comprises a population of fibroblasts and/or myofibroblasts. In some embodiments, the tumor sample obtained from the patient comprises a cell-poor and/or collagenized stroma. In some embodiments, the tumor sample has an increased expression level of collagen, STAT1, or MEK relative to a reference tumor sample.

In some embodiments of any of the preceding aspects, the method further comprises administering to the patient a therapeutically effective amount of a PD-L1 axis binding antagonist based on the expression level of PD-L1 in tumor cells or in tumor-infiltrating immune cells in the tumor sample.

In some embodiments of any of the preceding aspects, the PD-L1 axis binding antagonist is selected from the group consisting of a PD-L1 binding antagonist, a PD-1 binding antagonist, and a PD-L2 binding antagonist. In some embodiments, the PD-L1 axis binding antagonist is a PD-L1 binding antagonist. In some embodiments, the PD-L1 binding antagonist inhibits the binding of PD-L1 to one or more of its ligand binding partners. In some embodiments, the PD-L1 binding antagonist inhibits the binding of PD-L1 to PD-1. In some embodiments, the PD-L1 binding antagonist inhibits the binding of PD-L1 to B7-1. In some embodiments, the PD-L1 binding antagonist inhibits the binding of PD-L1 to both PD-1 and B7-1. In some embodiments, the PD-L1 binding antagonist is an antibody. In some embodiments, the antibody is selected from the group consisting of: YW243.55.S70, MPDL3280A (atezolizumab), MDX-1105, MEDI4736 (durvalumab), and MSB0010718C (avelumab). In some embodiments, the antibody comprises a heavy chain comprising HVR-H1 sequence of SEQ ID NO:19, HVR-H2 sequence of SEQ ID NO:20, and HVR-H3 sequence of SEQ ID NO:21; and a light chain comprising HVR-L1 sequence of SEQ ID NO:22, HVR-L2 sequence of SEQ ID NO:23, and HVR-L3 sequence of SEQ ID NO:24. In some embodiments, the antibody comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:26 and a light chain variable region comprising the amino acid sequence of SEQ ID NO:4. In some embodiments, the PD-L1 axis binding antagonist is a PD-1 binding antagonist. In some embodiments, the PD-1 binding antagonist inhibits the binding of PD-1 to one or more of its ligand binding partners. In some embodiments, the PD-1 binding antagonist inhibits the binding of PD-1 to PD-L1. In some embodiments, the PD-1 binding antagonist inhibits the binding of PD-1 to PD-L2. In some embodiments, the PD-1 binding antagonist inhibits the binding of PD-1 to both PD-L1 and PD-L2. In some embodiments, the PD-1 binding antagonist is an antibody. In some embodiments, the antibody is selected from the group consisting of: MDX-1106 (nivolumab), MK-3475

(pembrolizumab), CT-011 (pidilizumab), MEDI-0680 (AMP-514), PDR001, REGN2810, and BGB-108. In some embodiments, the PD-1 binding antagonist is an Fc-fusion protein. In some embodiments, the Fc-fusion protein is AMP-224.

In some embodiments of any of the preceding methods, the method further comprises administering to the patient an effective amount of a second therapeutic agent. In some embodiments, the second therapeutic agent is selected from the group consisting of a cytotoxic agent, a growth-inhibitory agent, a radiation therapy agent, an anti-angiogenic agent, and combinations thereof. In some embodiments, the non-small cell lung cancer is a locally advanced or metastatic non-small cell lung cancer.

In some embodiments of any of the preceding aspects, the tumor sample is a formalin-fixed and paraffin-embedded (FFPE) tumor sample, an archival tumor sample, a fresh tumor sample, or a frozen tumor sample. In some embodiments, the expression level of PD-L1 is a protein expression level. In some embodiments, the protein expression level of PD-L1 is determined using a method selected from the group consisting of immunohistochemistry (IHC), immunofluorescence, flow cytometry, and Western blot. In some embodiments, the protein expression level of PD-L1 is determined using IHC. In some embodiments, the protein expression level of PD-L1 is detected using an anti-PD-L1 antibody.

In some embodiments of any of the preceding aspects, the expression level of PD-L1 is an mRNA expression level. In some embodiments, the mRNA expression level of PD-L1 is determined using a method selected from the group consisting of quantitative polymerase chain reaction (qPCR), reverse transcription qPCR (RT-qPCR), RNA sequencing, microarray analysis, in situ hybridization, and serial analysis of gene expression (SAGE).

In another aspect, the invention features a PD-L1 axis binding antagonist for use in treating a patient suffering from a non-small cell lung cancer, wherein a tumor sample obtained from the patient has been determined to have a detectable expression level of PD-L1 in 5% or more of the tumor cells in the tumor sample.

In another aspect, the invention features the use of an effective amount of a PD-L1 axis binding antagonist in the manufacture of a medicament for use in treating a patient suffering from a non-small cell lung cancer, wherein a tumor sample obtained from the patient has been determined to have a detectable expression level of PD-L1 in 5% or more of the tumor cells in the tumor sample.

In another aspect, the invention features a composition comprising an effective amount of a PD-L1 axis binding antagonist for use in a method of treating a patient suffering from a non-small cell lung cancer, wherein a tumor sample obtained from the patient has been determined to have a detectable expression level of PD-L1 in 5% or more of the tumor cells in the tumor sample.

In another aspect, the invention features a PD-L1 axis binding antagonist for use in treating a patient suffering from a non-small cell lung cancer, wherein a tumor sample obtained from the patient has been determined to have a detectable expression level of PD-L1 in tumor-infiltrating immune cells that comprise 5% or more of the tumor sample, and a detectable expression level of PD-L1 in less than 50% of the tumor cells in the tumor sample.

In another aspect, the invention features the use of an effective amount of a PD-L1 axis binding antagonist in the manufacture of a medicament for use in treating a patient suffering from a non-small cell

lung cancer, wherein a tumor sample obtained from the patient has been determined to have a detectable expression level of PD-L1 in tumor-infiltrating immune cells that comprise 5% or more of the tumor sample, and a detectable expression level of PD-L1 in less than 50% of the tumor cells in the tumor sample.

In another aspect, the invention features a composition comprising an effective amount of a PD-L1 axis binding antagonist for use in a method of treating a patient suffering from a non-small cell lung cancer, wherein a tumor sample obtained from the patient has been determined to have a detectable expression level of PD-L1 in tumor-infiltrating immune cells that comprise 5% or more of the tumor sample, and a detectable expression level of PD-L1 in less than 50% of the tumor cells in the tumor sample.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGURE 1A is a table showing that PD-L1 is broadly expressed in various human cancers. PD-L1 expression was assessed by immunohistochemistry (IHC) in tumor-infiltrating immune cells ("IC") and in tumor cells ("TC"). IC2/3 indicates an IC IHC score of 2 or 3 (see Table 2). TC2/3 indicates a TC IHC score of 2 or 3 (see Table 3).

FIGURES 1B-1D are images showing representative non-small cell lung cancer (NSCLC) tumor sample sections analyzed by IHC for PD-L1 expression. Figure 1B shows a sample that is PD-L1-positive in tumor cells, Figure 1C shows a sample that is PD-L1-positive in tumor cells and in tumor-infiltrating immune cells, and Figure 1D shows a sample that is PD-L1-positive in tumor-infiltrating immune cells.

FIGURE 2A is a table showing that there was minimal overlap of the IC3 and TC3 IHC diagnostic criteria (see Table 2 and Table 3, respectively) in NSCLC tumor samples as determined in an ongoing phase II clinical trial. N=287.

FIGURE 2B is a series of Venn diagrams showing the prevalence and overlap of IC and TC IHC diagnostic criteria at different cut-offs for NSCLC tumor samples in an ongoing phase II trial (phase II-2). IC2/3 indicates an IC IHC score of 2 or 3 (see Table 2). TC2/3 indicates a TC IHC score of 2 or 3 (see Table 3). IC1/2/3 indicates an IC IHC score of 1, 2, or 3 (see Table 2). TC1/2/3 indicates a TC IHC score of 1, 2, or 3 (see Table 3).

FIGURE 3A is a table showing that PD-L1 expression (as assessed by IHC using a diagnostic anti-PD-L1 antibody) in immune-infiltrating tumor cells or in tumor cells independently predicted outcomes to treatment with the anti-PD-L1 antibody MPDL3280A in non-small cell lung cancer in an ongoing phase Ia clinical trial . The overall response rate (ORR) for patients having the indicated PD-L1 IHC score is shown. "IC3" indicates patients whose tumors were scored as IC3 and may include any TC value (i.e., TC0/1/2/3). "TC3" indicates patients whose tumors were scored as TC3 and may include any IC value (i.e., IC0/1/2/3). "TC3 or IC3" indicates patients whose tumors were scored as either TC3 or IC3. One patient's tumor sample had both a TC3 and IC3 scoring.

FIGURE 3B is a table showing efficacy results from an ongoing phase II clinical trial (phase II-1) as determined by mRECIST. The confirmed ORR, duration of response (DOR), and 6-month

progression-free survival (PFS) for the indicated cohorts are shown. [a]Two patients had unknown IC/TC status. [b]Unless indicated, the median has not yet been reached.

FIGURE 3C is a table showing efficacy results from an ongoing phase II clinical trial (phase II-1) as determined by RECIST v1.1. The confirmed ORR, duration of response (DOR), and 6-month progression-free survival (PFS) for the indicated cohorts are shown. [a]Two patients had unknown IC/TC status. [b] Medians have not yet been reached. Mets, metastases.

FIGURE 3D is a table showing that PD-L1 expression (as assessed by IHC using a diagnostic anti-PD-L1 antibody) in immune-infiltrating tumor cells or in tumor cells predicted improved overall survival in NSCLC patients treated with atezolizumab (MDPL3280A) in an ongoing phase II clinical trial (phase II-2). ITT, intention-to-treat.

FIGURE 3E is a table showing that PD-L1 expression (as assessed by IHC using a diagnostic anti-PD-L1 antibody) in immune-infiltrating tumor cells or in tumor cells predicted improved progression-free survival in NSCLC patients treated with atezolizumab (MPDL3280A). A comparison to NSCLC patients in the trial that were treated with docetaxel is shown.

FIGURE 3F is a table showing that PD-L1 expression in immune-infiltrating tumor cells or in tumor cells in NSCLC patient tumor samples predicted response (as assessed by ORR) of NSCLC patients to treatment with the anti-PD-L1 antibody MPDL3280A in an ongoing phase II clinical trial (phase II-2).

FIGURE 4 is a graph showing that the presence of tumor-infiltrating immune cells is necessary but not sufficient to reflect PD-L1 positivity in the IC IHC diagnostic criteria.

FIGURE 5A is a graph showing that immune infiltrate is present in TC3 patients but is largely PD-L1-negative by IHC. The graph shows the percentage of total immune cell infiltrate per tumor mass. The image to the left is a representative section that was scored TC3, while the image to the right of the graph is a representative section that was scored IC3.

FIGURES 5B-5C are graphs showing the mRNA expression level of *CD68* (Figure 5B) and *CD8* (Figure 5C) in NSCLC patients enrolled in an ongoing phase Ia clinical trial and an ongoing phase 2 clinical trial. Neg, PD-L1-negative.

FIGURES 6A-6B are graphs showing that TC3 and IC3 NSCLC tumors had distinct histopathology as assessed by hematoxylin and eosin (H&E) staining. Figure 6A shows histopathological scoring for TC3 tumors, while Figure 6B shows histopathological scoring for IC3 tumors. TC3 tumors exhibited a desmoplastic tumor microenvironment with low intra-epithelial and stromal tumor-infiltrating immune cells, while IC3 tumors exhibited the presence of intra-epithelial or stromal tumor-infiltrating immune cells. "IC interface activity" indicates immune cell infiltrates at the tumor/stroma interface. "IC intra-epithelial indicates the presence of intraepithelial immune cell infiltrates. "IC TLS" indicates the presence of tertiary lymphoid follicles. "Other stromal IC" indicates the presence of immune cells in the stroma. "Desmoplasia" indicates the presence of a cellular and "activated" fibroblast population (myofibroblasts). "Sclerotic reaction" indicates the presence of a cell-poor/collagenized stroma. "Increased vessels/qualitative" indicates the presence of blood vessels scored qualitatively. The samples were from an ongoing phase II clinical trial.

FIGURE 7 is a graph showing that TC3 NSCLC tumors had higher expression of collagen (*COL6A1*) compared to PD-L1-negative ("negative") tumors, IC3 tumors, or TC3 and IC3 tumors. The images below the graph are representative sections of PD-L1 TC3 tumor samples.

FIGURE 8 is a heatmap showing hierarchical clustering of immune gene set expression across PD-L1 TC/IC subtypes.

FIGURES 9A-9B are a series of graphs showing increased mRNA expression levels of *CD8* (Figure 9A) and *CXCL9* (Figure 9B) in IC3 NSCLC tumors compared to TC3 tumors and PD-L1-negative (TC0 and IC0) tumors.

FIGURES 10A-10B are a series of graphs showing increased expression levels of STAT1 and MEK in TC3 tumors compared to PD-L1-negative tumors, IC3 tumors, and TC3 and IC3 tumors. The data are from patients enrolled in an ongoing phase Ia clinical trial and an ongoing phase II clinical trial.

FIGURE 10C is a graph showing JAK1 expression levels were higher in PD-L1-positive tumors compared to PD-L1-negative tumors.

FIGURE 10D is an image of a western blot showing that despite active STAT signaling, some tumor cell lines did not upregulate PD-L1 in response to interferon gamma (IFNγ).

FIGURE 11 is a table showing the prevalence of the indicated PD-L1 IHC diagnostic criteria (see Table 2 and Table 3) in adjuvant, 1L, and 2L+ NSCLC. The Venn diagram below the graph shows that TC3 and IC3 tumors represent distinct sub-populations in NSCLC, with less than 1% overlap.

FIGURES 12A-12B are graphs showing that IC3 NSCLC tumors were characterized by higher expression of B-cell signatures (Figure 12A) and natural killer (NK) cell signatures (Figure 12B). These data are from patients in an ongoing phase Ia clinical trial and an ongoing phase II clinical trial.

FIGURES 13A-13C are graphs showing that PD-L1-positive NSCLC tumors showed increased expression of effector T cell (T_{eff}) gene signatures compared to PD-L1-negative tumors.

FIGURE 13D is a graph showing that IC3 NSCLC tumors were characterized by increased expression of the T_{eff} gene signature as determined by RNA sequencing. In this analysis, the TC3 subgroup excluded IC2/3 tumors, whereas the IC3 subgroup excluded TC2/3 tumors.

FIGURES 13E-13G are a series of graphs showing that IC3 NSCLC tumors were characterized by increased expression of *IFNG* (Figure 13E), *GZMB* (Figure 13F), and *CXCL9* (Figure 13G) as determined by RNA sequencing. In this analysis, the TC3 subgroup excluded IC2/3 tumors, whereas the IC3 subgroup excluded TC2/3 tumors.

FIGURES 14A-14B are graphs showing that treatment of NSCLC patients with the anti-PD-L1 antibody MPDL3280A resulted in an increased plasma level of the IFNγ-associated markers interleukin-18 (IL-18) (Figure 14A) and ITAC (Figure 14B). The graphs show the $\log_2$ fold change relative to C1D1 pre-dose. C indicates cycle, and D indicates day.

FIGURES 15A-15C are graphs showing a comparison between increased baseline PD-L1 (Figure 15A), PD-L2 (Figure 15B), and PD-1 (Figure 15C) mRNA expression levels (as determined using a NanoString assay) in peripheral blood mononuclear cells (PMBCs) and response of NSCLC patients to treatment with the anti-PD-L1 antibody MPDL3280A.

FIGURES 16A-16B are graphs showing that baseline mRNA expression levels of NK cell (Figure 16A) and myeloid cell (Figure 16B) signature genes in PBMCs were associated with response of NSCLC

patients to treatment with the anti-PD-L1 antibody MPDL3280A. Increased expression levels of immuno-suppressive myeloid cell gene signatures were associated with progression, while increased expression levels of cytotoxic NK cells were associated with response to MPDL3280A.

FIGURES 17A-17B are graphs showing expression of PD-L1 (Figure 17A) and PD-L2 (Figure 17B) in NSCLC tumor samples in patients with TC0 and IC0 tumors, IC3 tumors, and in TC3 tumors as determined by RNA sequencing. The patients were from the phase II-2 trial and a non-trial cohort (N=162). RPKM, Reads per kilobase per million mapped reads.

FIGURES 18A-18B are graphs showing that TC3 tumors express high levels of molecular markers of desmoplastic/sclerotic stroma as determined by RNA sequencing. Figure 18A shows increased expression of the collagen gene *COL6A1* in TC3 tumors compared to IC3 tumors and TC0 and IC0 tumors. Figure 18B shows increased expression of collagen gene *COL6A2* in TC3 tumors compared to IC3 tumors and TC0 and IC0 tumors. In this analysis, the TC3 subgroup excluded IC2/3 tumors, whereas the IC3 subgroup excluded TC2/3 tumors.

DETAILED DESCRIPTION OF EMBODIMENTS OF THE INVENTION

I. Introduction

The present invention provides therapeutic and diagnostic methods and compositions for cancer, for example, non-small cell lung cancer. The invention is based, at least in part, on the discovery that determination of expression levels of biomarkers of the invention, for example, PD-L1, in samples obtained from a patient (e.g., in tumor cells and in tumor-infiltrating immune cells in a tumor sample obtained from the patient) is useful in treatment of a patient suffering from cancer, for diagnosing a patient suffering from cancer, for determining whether a patient having a cancer is likely to respond to treatment with an anti-cancer therapy that includes a PD-L1 axis binding antagonist (e.g., an anti-PD-L1 antibody, e.g., MPDL3280A), for optimizing therapeutic efficacy of an anti-cancer therapy that includes a PD-L1 axis binding antagonist (e.g., an anti-PD-L1 antibody, e.g., MPDL3280A), and/or for patient selection for an anti-cancer therapy comprising a PD-L1 axis binding antagonist (e.g., an anti-PD-L1 antibody, e.g., MPDL3280A).

II. Definitions

It is to be understood that aspects and embodiments of the invention described herein include "comprising," "consisting," and "consisting essentially of" aspects and embodiments. As used herein, the singular form "a," "an," and "the" includes plural references unless indicated otherwise.

The term "about" as used herein refers to the usual error range for the respective value readily known to the skilled person in this technical field. Reference to "about" a value or parameter herein includes (and describes) embodiments that are directed to that value or parameter per se. For example, description referring to "about X" includes description of "X".

The term "PD-L1 axis binding antagonist" refers to a molecule that inhibits the interaction of a PD-L1 axis binding partner with one or more of its binding partners, so as to remove T-cell dysfunction resulting from signaling on the PD-1 signaling axis, with a result being restored or enhanced T-cell function. As used herein, a PD-L1 axis binding antagonist includes a PD-L1 binding antagonist and a PD-

1 binding antagonist as well as molecules that interfere with the interaction between PD-L1 and PD-1 (e.g., a PD-L2-Fc fusion).

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 down-stream 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 co-stimulation. The unresponsive state can often be overriden 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%, or 200% enhancement. The manner of measuring this enhancement is known to one of ordinary skill in the art.

"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 a PD-L1 axis binding antagonist.

As used herein, a "PD-L1 binding antagonist" 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 and/or 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, PD-L1 binding antagonists include anti-PD-L1 antibodies and antigen-binding fragments thereof, immunoadhesins, fusion proteins, oligopeptides, small molecule antagonist, polynucleotide antagonists, 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 and/or B7-1. In one embodiment, a PD-L1 binding antagonist reduces the negative signal mediated by or through cell surface proteins expressed on T lymphocytes, and other cells, mediated signaling through PD-L1 or PD-1 so as render a dysfunctional T-cell less dysfunctional. In some embodiments, a PD-L1 binding antagonist is an anti-PD-L1 antibody. In a specific aspect, an anti-PD-L1 antibody is YW243.55.S70 described herein. In another specific aspect, an anti-PD-L1 antibody is MDX-1105 described herein. In still another specific aspect, an anti-PD-L1 antibody is MPDL3280A (atezolizumab) described herein. In still another specific aspect, an anti-PD-L1 antibody is MEDI4736 (durvalumab) described herein. In still another specific aspect, an anti-PD-L1 antibody is MSB0010718C (avelumab) described herein.

As used herein, a "PD-1 binding antagonist" 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 and/or 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 and antigen-binding fragments thereof, immunoadhesins, fusion proteins, oligopeptides, small molecule antagonist, polynucleotide antagonists, 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 signal mediated by or through cell surface proteins expressed on T lymphocytes, and other cells, mediated signaling through PD-1 or PD-L1 so as render a dysfunctional T-cell less dysfunctional. In some embodiments, the PD-1 binding antagonist is an anti-PD-1 antibody. In a specific aspect, a PD-1 binding antagonist is MDX-1106 (nivolumab) described herein. In another specific aspect, a PD-1 binding antagonist is MK-3475 (pembrolizumab) described herein. In another specific aspect, a PD-1 binding antagonist is CT-011 (pidilizumab) described herein. In another specific aspect, a PD-1 binding antagonist is MEDI-0680 (AMP-514) described herein. In another specific aspect, a PD-1 binding antagonist is PDR001 described herein. In another specific aspect, a PD-1 binding antagonist is REGN2810 described herein. In another specific aspect, a PD-1 binding antagonist is BGB-108 described herein. In another specific aspect, a PD-1 binding antagonist is AMP-224 described herein.

The terms "Programmed Death Ligand 1" and "PD-L1" refer herein to a native sequence PD-L1 polypeptide, polypeptide variants, and fragments of a native sequence polypeptide and polypeptide variants (which are further defined herein). The PD-L1 polypeptide described herein may be that which is

isolated from a variety of sources, such as from human tissue types or from another source, or prepared by recombinant or synthetic methods.

A "native sequence PD-L1 polypeptide" comprises a polypeptide having the same amino acid sequence as the corresponding PD-L1 polypeptide derived from nature.

A "PD-L1 polypeptide variant," or variations thereof, means a PD-L1 polypeptide, generally an active PD-L1 polypeptide, as defined herein having at least about 80% amino acid sequence identity with any of the native sequence PD-L1 polypeptide sequences as disclosed herein. Such PD-L1 polypeptide variants include, for instance, PD-L1 polypeptides wherein one or more amino acid residues are added, or deleted, at the N- or C-terminus of a native amino acid sequence. Ordinarily, a PD-L1 polypeptide variant will have at least about 80% amino acid sequence identity, alternatively at least about 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% amino acid sequence identity, to a native sequence PD-L1 polypeptide sequence as disclosed herein. Ordinarily, PD-L1 variant polypeptides are at least about 10 amino acids in length, alternatively at least about 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 260, 270, 280, 281, 282, 283, 284, 285, 286, 287, 288, or 289 amino acids in length, or more. Optionally, PD-L1 variant polypeptides will have no more than one conservative amino acid substitution as compared to a native PD-L1 polypeptide sequence, alternatively no more than 2, 3, 4, 5, 6, 7, 8, 9, or 10 conservative amino acid substitutions as compared to the native PD-L1 polypeptide sequence.

"Polynucleotide," or "nucleic acid," as used interchangeably herein, refer to polymers of nucleotides of any length, and include DNA and RNA. The nucleotides can be deoxyribonucleotides, ribonucleotides, modified nucleotides or bases, and/or their analogs, or any substrate that can be incorporated into a polymer by DNA or RNA polymerase, or by a synthetic reaction. Thus, for instance, polynucleotides as defined herein include, without limitation, single- and double-stranded DNA, DNA including single- and double-stranded regions, single- and double-stranded RNA, and RNA including single- and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded or include single- and double-stranded regions. In addition, the term "polynucleotide" as used herein refers to triple-stranded regions comprising RNA or DNA or both RNA and DNA. The strands in such regions may be from the same molecule or from different molecules. The regions may include all of one or more of the molecules, but more typically involve only a region of some of the molecules. One of the molecules of a triple-helical region often is an oligonucleotide. The term "polynucleotide" specifically includes cDNAs.

A polynucleotide may comprise modified nucleotides, such as methylated nucleotides and their analogs. If present, modification to the nucleotide structure may be imparted before or after assembly of the polymer. The sequence of nucleotides may be interrupted by non-nucleotide components. A polynucleotide may be further modified after synthesis, such as by conjugation with a label. Other types of modifications include, for example, "caps," substitution of one or more of the naturally-occurring nucleotides with an analog, internucleotide modifications such as, for example, those with uncharged linkages (e.g., methyl phosphonates, phosphotriesters, phosphoamidates, carbamates, and the like) and with charged linkages (e.g., phosphorothioates, phosphorodithioates, and the like), those containing

pendant moieties, such as, for example, proteins (e.g., nucleases, toxins, antibodies, signal peptides, poly-L-lysine, and the like), those with intercalators (e.g., acridine, psoralen, and the like), those containing chelators (e.g., metals, radioactive metals, boron, oxidative metals, and the like), those containing alkylators, those with modified linkages (e.g., alpha anomeric nucleic acids), as well as unmodified forms of the polynucleotide(s). Further, any of the hydroxyl groups ordinarily present in the sugars may be replaced, for example, by phosphonate groups, phosphate groups, protected by standard protecting groups, or activated to prepare additional linkages to additional nucleotides, or may be conjugated to solid or semi-solid supports. The 5' and 3' terminal OH can be phosphorylated or substituted with amines or organic capping group moieties of from 1 to 20 carbon atoms. Other hydroxyls may also be derivatized to standard protecting groups. Polynucleotides can also contain analogous forms of ribose or deoxyribose sugars that are generally known in the art, including, for example, 2'-O-methyl-, 2'-O-allyl-, 2'-fluoro-, or 2'-azido-ribose, carbocyclic sugar analogs, α-anomeric sugars, epimeric sugars such as arabinose, xyloses or lyxoses, pyranose sugars, furanose sugars, sedoheptuloses, acyclic analogs, and abasic nucleoside analogs such as methyl riboside. One or more phosphodiester linkages may be replaced by alternative linking groups. These alternative linking groups include, but are not limited to, embodiments wherein phosphate is replaced by P(O)S ("thioate"), P(S)S ("dithioate"), "(O)NR_2 ("amidate"), P(O)R, P(O)OR', CO or CH_2 ("formacetal"), in which each R or R' is independently H or substituted or unsubstituted alkyl (1-20 C) optionally containing an ether (-O-) linkage, aryl, alkenyl, cycloalkyl, cycloalkenyl or araldyl. Not all linkages in a polynucleotide need be identical. The preceding description applies to all polynucleotides referred to herein, including RNA and DNA.

"Oligonucleotide," as used herein, generally refers to short, single stranded, polynucleotides that are, but not necessarily, less than about 250 nucleotides in length. Oligonucleotides may be synthetic. The terms "oligonucleotide" and "polynucleotide" are not mutually exclusive. The description above for polynucleotides is equally and fully applicable to oligonucleotides.

The term "primer" refers to a single-stranded polynucleotide that is capable of hybridizing to a nucleic acid and allowing polymerization of a complementary nucleic acid, generally by providing a free 3'-OH group.

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

The terms "host cell," "host cell line," and "host cell culture" are used interchangeably and refer to cells into which exogenous nucleic acid has been introduced, including the progeny of such cells. Host cells include "transformants" and "transformed cells," which include the primary transformed cell and progeny derived therefrom without regard to the number of passages. Progeny may not be completely identical in nucleic acid content to a parent cell, but may contain mutations. Mutant progeny that have the same function or biological activity as screened or selected for in the originally transformed cell are included herein.

The term "vector," as used herein, refers to a nucleic acid molecule capable of propagating another nucleic acid to which it is linked. The term includes the vector as a self-replicating nucleic acid structure as well as the vector incorporated into the genome of a host cell into which it has been

introduced. Certain vectors are capable of directing the expression of nucleic acids to which they are operatively linked. Such vectors are referred to herein as "expression vectors."

An "isolated" nucleic acid refers to a nucleic acid molecule that has been separated from a component of its natural environment. An isolated nucleic acid includes a nucleic acid molecule contained in cells that ordinarily contain the nucleic acid molecule, but the nucleic acid molecule is present extrachromosomally or at a chromosomal location that is different from its natural chromosomal location.

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

An "isolated" antibody is one which has been identified and separated and/or recovered from a component of its natural environment. Contaminant components of its natural environment are materials which would interfere with research, diagnostic, and/or therapeutic uses for the antibody, and may include enzymes, hormones, and other proteinaceous or nonproteinaceous solutes. In some embodiments, an antibody is purified (1) to greater than 95% by weight of antibody as determined by, for example, the Lowry method, and in some embodiments, to greater than 99% by weight; (2) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by use of, for example, a spinning cup sequenator, or (3) to homogeneity by SDS-PAGE under reducing or nonreducing conditions using, for example, Coomassie blue or silver stain. An isolated antibody includes the antibody *in situ* within recombinant cells since at least one component of the antibody's natural environment will not be present. Ordinarily, however, an isolated antibody will be prepared by at least one purification step.

"Native antibodies" are usually heterotetrameric glycoproteins of about 150,000 daltons, composed of two identical light (L) chains and two identical heavy (H) chains. Each light chain is linked to a heavy chain by one covalent disulfide bond, while the number of disulfide linkages varies among the heavy chains of different immunoglobulin isotypes. Each heavy and light chain also has regularly spaced intrachain disulfide bridges. Each heavy chain has at one end a variable domain (VH) followed by a number of constant domains. Each light chain has a variable domain at one end (VL) and a constant domain at its other end; the constant domain of the light chain is aligned with the first constant domain of the heavy chain, and the light chain variable domain is aligned with the variable domain of the heavy chain. Particular amino acid residues are believed to form an interface between the light chain and heavy chain variable domains.

The "light chains" of antibodies (immunoglobulins) from any mammalian species can be assigned to one of two clearly distinct types, called kappa ("κ") and lambda ("λ"), based on the amino acid sequences of their constant domains.

The term "constant domain" refers to the portion of an immunoglobulin molecule having a more conserved amino acid sequence relative to the other portion of the immunoglobulin, the variable domain, which contains the antigen binding site. The constant domain contains the CH1, CH2 and CH3 domains (collectively, CH) of the heavy chain and the CHL (or CL) domain of the light chain.

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 domain of the heavy chain may be referred to as "VH." The variable domain of the light chain may be referred to as "VL." These domains are generally the most variable parts of an antibody and contain the antigen-binding sites.

The term "variable" refers to the fact that certain portions of the variable domains differ extensively in sequence among antibodies and are used in the binding and specificity of each particular antibody for its particular antigen. However, the variability is not evenly distributed throughout the variable domains of antibodies. 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 Proteins of Immunological Interest*, Fifth Edition, National Institute of Health, Bethesda, Md. (1991)). The constant domains are not involved directly in the binding of an antibody to an antigen, but exhibit various effector functions, such as participation of the antibody in antibody-dependent cellular toxicity.

The term "hypervariable region," "HVR," or "HV," as 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, for example, Xu et al., *Immunity* 13:37-45 (2000); Johnson and Wu, in *Methods in Molecular Biology* 248:1-25 (Lo, ed., Human Press, Totowa, N.J., 2003). Indeed, naturally occurring camelid antibodies consisting of a heavy chain only are functional and stable in the absence of light chain. See, for example, Hamers-Casterman et al., *Nature* 363:446-448 (1993); Sheriff et al., *Nature Struct. Biol.* 3:733-736 (1996).

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.

Loop	*Kabat*	*AbM*	*Chothia*	*Contact*
L1	L24-L34	L24-L34	L26-L32	L30-L36
L2	L50-L56	L50-L56	L50-L52	L46-L55
L3	L89-L97	L89-L97	L91-L96	L89-L96
H1	H31-H35b	H26-H35b	H26-H32	H30-H35b (Kabat Numbering)
H1	H31-H35	H26-H35	H26-H32	H30-H35 (Chothia Numbering)
H2	H50-H65	H50-H58	H53-H55	H47-H58
H3	H95-H102	H95-H102	H96-H101	H93-H101

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.

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

The term "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.

The Kabat numbering system is generally used when referring to a residue in the variable domain (approximately residues 1-107 of the light chain and residues 1-113 of the heavy chain) (e.g., Kabat et al., *Sequences of Immunological Interest*. 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991)). The "EU numbering system" or "EU index" is generally used when referring to a residue in an immunoglobulin heavy chain constant region (e.g., the EU index reported in Kabat et al., *supra*). The "EU index as in Kabat" refers to the residue numbering of the human IgG1 EU antibody.

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

"Antibody fragments" comprise a portion of an intact antibody, preferably comprising the antigen-binding region thereof. In some embodiments, the antibody fragment described herein is an antigen-binding fragment. Examples of antibody fragments include Fab, Fab', $F(ab')_2$, and Fv fragments; diabodies; linear antibodies; single-chain antibody molecules; and multispecific antibodies formed from antibody fragments.

Papain digestion of antibodies produces two identical antigen-binding fragments, called "Fab" fragments, each with a single antigen-binding site, and a residual "Fc" fragment, whose name reflects its ability to crystallize readily. Pepsin treatment yields an $F(ab')_2$ fragment that has two antigen-combining sites and is still capable of cross-linking antigen.

"Fv" is the minimum antibody fragment which contains a complete antigen-binding site. In one embodiment, a two-chain Fv species consists of a dimer of one heavy- and one light-chain variable domain in tight, non-covalent association. In a single-chain Fv (scFv) species, one heavy- and one light-chain variable domain can be covalently linked by a flexible peptide linker such that the light and heavy chains can associate in a "dimeric" structure analogous to that in a two-chain Fv species. It is in this configuration that the three HVRs of each variable domain interact to define an antigen-binding site on the surface of the VH-VL dimer. Collectively, the six HVRs 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.

The Fab fragment contains the heavy- and light-chain variable domains and also contains the constant domain of the light chain and the first constant domain (CH1) of the heavy chain. Fab' fragments differ from Fab fragments by the addition of a few residues at the carboxy terminus of the heavy chain 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.

"Single-chain Fv" or "scFv" antibody fragments comprise the VH and VL domains of antibody, wherein these domains are present in a single polypeptide chain. Generally, the scFv polypeptide further comprises a polypeptide linker between the VH and VL domains which enables the scFv to form the desired structure for antigen binding. For a review of scFv, see, e.g., Pluckthün, in *The Pharmacology of Monoclonal Antibodies*, vol. 113, Rosenburg and Moore eds., (Springer-Verlag, New York, 1994), pp. 269-315.

The term "diabodies" refers to antibody fragments with two antigen-binding sites, which fragments comprise a heavy-chain variable domain (VH) connected to a light-chain variable domain (VL) in the same polypeptide chain (VH-VL). By using a linker that is too short to allow pairing between the two domains on the same chain, the domains are forced to pair with the complementary domains of another chain and create two antigen-binding sites. Diabodies may be bivalent or bispecific. Diabodies are described more fully in, for example, EP 404,097; WO 1993/01161; Hudson et al., *Nat. Med.* 9:129-134 (2003); and Hollinger et al., *Proc. Natl. Acad. Sci. USA* 90: 6444-6448 (1993). Triabodies and tetrabodies are also described in Hudson et al., *Nat. Med.* 9:129-134 (2003).

The "class" of an antibody refers to the type of constant domain or constant region possessed by its heavy chain. There are five major classes of antibodies: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), e.g., IgG1, IgG2, IgG3, IgG4, IgA1, and IgA2. The heavy chain constant domains that correspond to the different classes of antibodies are called α, δ, ε, γ, and μ, respectively.

The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, e.g., the individual antibodies comprising the population are identical except for possible mutations, e.g., naturally occurring mutations, that may be present in minor amounts. Thus, the modifier "monoclonal" indicates the character of the antibody as not being a mixture of discrete antibodies. In certain embodiments, such a monoclonal antibody typically includes an antibody comprising a polypeptide sequence that binds a target, wherein the target-binding polypeptide sequence was obtained by a process that includes the selection of a single target binding polypeptide sequence from a plurality of polypeptide sequences. For example, the selection process can be the selection of a unique clone from a plurality of clones, such as a pool of hybridoma clones, phage clones, or recombinant DNA clones. It should be understood that a selected target binding sequence can be further altered, for example, to improve affinity for the target, to humanize the target binding sequence, to improve its production in cell culture, to reduce its immunogenicity *in vivo*, to create a multispecific antibody, etc., and that an antibody comprising the altered target binding sequence is also a monoclonal antibody of this invention. In contrast to polyclonal antibody preparations, which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody of a monoclonal antibody preparation is directed against a single determinant on an antigen. In addition to their specificity, monoclonal antibody preparations are advantageous in that they are typically 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 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. Pat. 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; WO 1996/34096; WO 1996/33735; WO 1991/10741; Jakobovits et al., *Proc. Natl. Acad. Sci. USA* 90: 2551 (1993); Jakobovits et al., *Nature* 362: 255-258 (1993); Bruggemann et al., *Year in Immunol.* 7:33 (1993); U.S. Pat. Nos. 5,545,807; 5,545,806; 5,569,825; 5,625,126; 5,633,425; and 5,661,016; Marks et al., *Bio/Technology* 10: 779-783 (1992); Lonberg et al., *Nature* 368: 856-859 (1994); Morrison, *Nature* 368: 812-813 (1994); Fishwild et al., *Nature Biotechnol.* 14: 845-851 (1996); Neuberger, *Nature Biotechnol.* 14: 826 (1996); and Lonberg et al., *Intern. Rev. Immunol.* 13: 65-93 (1995).

The monoclonal antibodies herein specifically include "chimeric" antibodies 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 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 (see, e.g., U.S. Pat. No. 4,816,567; and Morrison et al., *Proc. Natl. Acad. Sci. USA* 81:6851-6855 (1984)). Chimeric antibodies include PRIMATIZED® antibodies wherein the antigen-binding region of the antibody is derived from an antibody produced by, e.g., immunizing macaque monkeys with the antigen of interest.

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

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

The terms "anti-PD-L1 antibody" and "an antibody that binds to PD-L1" refer to an antibody that is capable of binding PD-L1 with sufficient affinity such that the antibody is useful as a diagnostic and/or therapeutic agent in targeting PD-L1. In one embodiment, the extent of binding of an anti-PD-L1 antibody to an unrelated, non-PD-L1 protein is less than about 10% of the binding of the antibody to PD-L1 as measured, for example, by a radioimmunoassay (RIA). In certain embodiments, an anti-PD-L1 antibody binds to an epitope of PD-L1 that is conserved among PD-L1 from different species.

The terms "anti-PD-1 antibody" and "an antibody that binds to PD-1" refer to an antibody that is capable of binding PD-1 with sufficient affinity such that the antibody is useful as a diagnostic and/or therapeutic agent in targeting PD-1. In one embodiment, the extent of binding of an anti-PD-1 antibody to an unrelated, non-PD-1 protein is less than about 10% of the binding of the antibody to PD-1 as measured, for example, by a radioimmunoassay (RIA). In certain embodiments, an anti-PD-1 antibody binds to an epitope of PD-1 that is conserved among PD-1 from different species.

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

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

As used herein, the term "binds", "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 binds to or 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 ≤ 1μM, ≤ 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.

An "affinity matured" antibody refers to an antibody with one or more alterations in one or more hypervariable regions (HVRs), compared to a parent antibody which does not possess such alterations, such alterations resulting in an improvement in the affinity of the antibody for antigen.

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

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

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 IgG1, IgG2 (including IgG2A and IgG2B), IgG3, or IgG4 subtypes, IgA (including IgA1 and IgA2), 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 IgG1molecule. For the production of immunoglobulin fusions see also US Patent No. 5,428,130. For example, useful immunoadhesins as medicaments useful for therapy herein include polypeptides that comprise the extracellular domain (ECD) 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, and the like. 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.

"Percent (%) amino acid sequence identity" with respect to the polypeptide sequences identified herein is defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the polypeptide being compared, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN or Megalign (DNASTAR) software. Those skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full-length of the sequences being compared. For purposes herein, however, % amino acid sequence identity values are generated using the sequence comparison computer program ALIGN-2. The ALIGN-2 sequence comparison computer program was authored by Genentech, Inc. and the source code has been filed with user documentation in the U.S. Copyright Office, Washington D.C., 20559, where it is registered under U.S. Copyright Registration No. TXU510087. The ALIGN-2 program is publicly available through Genentech, Inc., South San Francisco, California. The ALIGN-2 program should be compiled for use on a UNIX operating system, preferably digital UNIX V4.0D. All sequence comparison parameters are set by the ALIGN-2 program and do not vary.

In situations where ALIGN-2 is employed for amino acid sequence comparisons, the % amino acid sequence identity of a given amino acid sequence A to, with, or against a given amino acid sequence B (which can alternatively be phrased as a given amino acid sequence A that has or comprises a certain % amino acid sequence identity to, with, or against a given amino acid sequence B) is calculated as follows:

100 times the fraction X/Y

where X is the number of amino acid residues scored as identical matches by the sequence alignment program ALIGN-2 in that program's alignment of A and B, and where Y is the total number of amino acid residues in B. It will be appreciated that where the length of amino acid sequence A is not equal to the length of amino acid sequence B, the % amino acid sequence identity of A to B will not equal the % amino acid sequence identity of B to A. Unless specifically stated otherwise, all % amino acid sequence identity values used herein are obtained as described in the immediately preceding paragraph using the ALIGN-2 computer program.

The term "detection" includes any means of detecting, including direct and indirect detection.

The term "biomarker" as used herein refers to an indicator, e.g., predictive, diagnostic, and/or prognostic, which can be detected in a sample. The biomarker may serve as an indicator of a particular

subtype of a disease or disorder (e.g., cancer) characterized by certain, molecular, pathological, histological, and/or clinical features. In some embodiments, a biomarker is a gene. Biomarkers include, but are not limited to, polynucleotides (e.g., DNA and/or RNA), polynucleotide copy number alterations (e.g., DNA copy numbers), polypeptides, polypeptide and polynucleotide modifications (e.g., post-translational modifications), carbohydrates, and/or glycolipid-based molecular markers.

The terms "biomarker signature," "signature," "biomarker expression signature," or "expression signature" are used interchangeably herein and refer to one or a combination of biomarkers whose expression is an indicator, e.g., predictive, diagnostic, and/or prognostic. The biomarker signature may serve as an indicator of a particular subtype of a disease or disorder (e.g., cancer) characterized by certain molecular, pathological, histological, and/or clinical features. In some embodiments, the biomarker signature is a "gene signature." The term "gene signature" is used interchangeably with "gene expression signature" and refers to one or a combination of polynucleotides whose expression is an indicator, e.g., predictive, diagnostic, and/or prognostic. In some embodiments, the biomarker signature is a "protein signature." The term "protein signature" is used interchangeably with "protein expression signature" and refers to one or a combination of polypeptides whose expression is an indicator, e.g., predictive, diagnostic, and/or prognostic.

The "amount" or "level" of a biomarker associated with an increased clinical benefit to an individual is a detectable level in a biological sample. These can be measured by methods known to one skilled in the art and also disclosed herein. The expression level or amount of biomarker assessed can be used to determine the response to the treatment.

The terms "level of expression" or "expression level" in general are used interchangeably and generally refer to the amount of a biomarker in a biological sample. "Expression" generally refers to the process by which information (e.g., gene-encoded and/or epigenetic information) is converted into the structures present and operating in the cell. Therefore, as used herein, "expression" may refer to transcription into a polynucleotide, translation into a polypeptide, or even polynucleotide and/or polypeptide modifications (e.g., posttranslational modification of a polypeptide). Fragments of the transcribed polynucleotide, the translated polypeptide, or polynucleotide and/or polypeptide modifications (e.g., posttranslational modification of a polypeptide) shall also be regarded as expressed whether they originate from a transcript generated by alternative splicing or a degraded transcript, or from a post-translational processing of the polypeptide, e.g., by proteolysis. "Expressed genes" include those that are transcribed into a polynucleotide as mRNA and then translated into a polypeptide, and also those that are transcribed into RNA but not translated into a polypeptide (for example, transfer and ribosomal RNAs).

"Elevated expression," "elevated expression levels," or "elevated levels" refers to an increased expression or increased levels of a biomarker in an individual relative to a control, such as an individual or individuals who are not suffering from the disease or disorder (e.g., cancer) or an internal control (e.g., a housekeeping biomarker).

"Reduced expression," "reduced expression levels," or "reduced levels" refers to a decrease expression or decreased levels of a biomarker in an individual relative to a control, such as an individual or individuals who are not suffering from the disease or disorder (e.g., cancer) or an internal control (e.g., a housekeeping biomarker). In some embodiments, reduced expression is little or no expression.

The term "housekeeping biomarker" refers to a biomarker or group of biomarkers (e.g., polynucleotides and/or polypeptides) which are typically similarly present in all cell types. In some embodiments, the housekeeping biomarker is a "housekeeping gene." A "housekeeping gene" refers herein to a gene or group of genes which encode proteins whose activities are essential for the maintenance of cell function and which are typically similarly present in all cell types.

"Amplification," as used herein generally refers to the process of producing multiple copies of a desired sequence. "Multiple copies" mean at least two copies. A "copy" does not necessarily mean perfect sequence complementarity or identity to the template sequence. For example, copies can include nucleotide analogs such as deoxyinosine, intentional sequence alterations (such as sequence alterations introduced through a primer comprising a sequence that is hybridizable, but not complementary, to the template), and/or sequence errors that occur during amplification.

The term "multiplex-PCR" refers to a single PCR reaction carried out on nucleic acid obtained from a single source (e.g., an individual) using more than one primer set for the purpose of amplifying two or more DNA sequences in a single reaction.

The technique of "polymerase chain reaction" or "PCR" as used herein generally refers to a procedure wherein minute amounts of a specific piece of nucleic acid, RNA and/or DNA, are amplified as described, for example, in U.S. Pat. No. 4,683,195. Generally, sequence information from the ends of the region of interest or beyond needs to be available, such that oligonucleotide primers can be designed; these primers will be identical or similar in sequence to opposite strands of the template to be amplified. The 5' terminal nucleotides of the two primers may coincide with the ends of the amplified material. PCR can be used to amplify specific RNA sequences, specific DNA sequences from total genomic DNA, and cDNA transcribed from total cellular RNA, bacteriophage, or plasmid sequences, etc. See generally Mullis et al., *Cold Spring Harbor Symp. Quant. Biol.* 51:263 (1987) and Erlich, ed., *PCR Technology*, (Stockton Press, NY, 1989). As used herein, PCR is considered to be one, but not the only, example of a nucleic acid polymerase reaction method for amplifying a nucleic acid test sample, comprising the use of a known nucleic acid (DNA or RNA) as a primer and utilizes a nucleic acid polymerase to amplify or generate a specific piece of nucleic acid or to amplify or generate a specific piece of nucleic acid which is complementary to a particular nucleic acid.

"Quantitative real-time polymerase chain reaction" or "qRT-PCR" refers to a form of PCR wherein the amount of PCR product is measured at each step in a PCR reaction. This technique has been described in various publications including, for example, Cronin et al., *Am. J. Pathol.* 164(1):35-42 (2004) and Ma et al., *Cancer Cell* 5:607-616 (2004).

The term "microarray" refers to an ordered arrangement of hybridizable array elements, preferably polynucleotide probes, on a substrate.

The term "diagnosis" is used herein to refer to the identification or classification of a molecular or pathological state, disease or condition (e.g., cancer). For example, "diagnosis" may refer to identification of a particular type of cancer. "Diagnosis" may also refer to the classification of a particular subtype of cancer, for instance, by histopathological criteria, or by molecular features (e.g., a subtype characterized by expression of one or a combination of biomarkers (e.g., particular genes or proteins encoded by said genes)).

The term "aiding diagnosis" is used herein to refer to methods that assist in making a clinical determination regarding the presence, or nature, of a particular type of symptom or condition of a disease or disorder (e.g., cancer). For example, a method of aiding diagnosis of a disease or condition (e.g., cancer) can comprise measuring certain biomarkers (e.g., PD-L1) in a biological sample from an individual.

The term "sample," as used herein, refers to a composition that is obtained or derived from a subject and/or individual of interest that contains a cellular and/or other molecular entity that is to be characterized and/or identified, for example, based on physical, biochemical, chemical, and/or physiological characteristics. For example, the phrase "disease sample" and variations thereof refers to any sample obtained from a subject of interest that would be expected or is known to contain the cellular and/or molecular entity that is to be characterized. Samples include, but are not limited to, tissue samples, primary or cultured cells or cell lines, cell supernatants, cell lysates, platelets, serum, plasma, vitreous fluid, lymph fluid, synovial fluid, follicular fluid, seminal fluid, amniotic fluid, milk, whole blood, blood-derived cells, urine, cerebro-spinal fluid, saliva, sputum, tears, perspiration, mucus, tumor lysates, and tissue culture medium, tissue extracts such as homogenized tissue, tumor tissue, cellular extracts, and combinations thereof.

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

A "tumor-infiltrating immune cell," as used herein, refers to any immune cell present in a tumor or a sample thereof. Tumor-infiltrating immune cells include, but are not limited to, intratumoral immune cells, peritumoral immune cells, other tumor stroma cells (e.g., fibroblasts), or any combination thereof. Such tumor-infiltrating immune cells can be, for example, T lymphocytes (such as CD8+ T lymphocytes and/or CD4+ T lymphocytes), B lymphocytes, or other bone marrow-lineage cells, including granulocytes (e.g., neutrophils, eosinophils, and basophils), monocytes, macrophages, dendritic cells (e.g., interdigitating dendritic cells), histiocytes, and natural killer cells.

A "tumor cell" as used herein, refers to any tumor cell present in a tumor or a sample thereof. Tumor cells may be distinguished from other cells that may be present in a tumor sample, for example, stromal cells and tumor-infiltrating immune cells, using methods known in the art and/or described herein.

A "reference sample," "reference cell," "reference tissue," "control sample," "control cell," or "control tissue," as used herein, refers to a sample, cell, tissue, standard, or level that is used for comparison purposes. In one embodiment, a reference sample, reference cell, reference tissue, control sample, control cell, or control tissue is obtained from a healthy and/or non-diseased part of the body (e.g., tissue or cells) of the same subject or individual. For example, the reference sample, reference cell,

reference tissue, control sample, control cell, or control tissue may be healthy and/or non-diseased cells or tissue adjacent to the diseased cells or tissue (e.g., cells or tissue adjacent to a tumor). In another embodiment, a reference sample is obtained from an untreated tissue and/or cell of the body of the same subject or individual. In yet another embodiment, a reference sample, reference cell, reference tissue, control sample, control cell, or control tissue is obtained from a healthy and/or non-diseased part of the body (e.g., tissues or cells) of an individual who is not the subject or individual. In even another embodiment, a reference sample, reference cell, reference tissue, control sample, control cell, or control tissue is obtained from an untreated tissue and/or cell of the body of an individual who is not the subject or individual.

For the purposes herein a "section" of a tissue sample is meant a single part or piece of a tissue sample, for example, a thin slice of tissue or cells cut from a tissue sample (e.g., a tumor sample). It is to be understood that multiple sections of tissue samples may be taken and subjected to analysis, provided that it is understood that the same section of tissue sample may be analyzed at both morphological and molecular levels, or analyzed with respect to polypeptides (e.g., by immunohistochemistry) and/or polynucleotides (e.g., by *in situ* hybridization).

By "correlate" or "correlating" is meant comparing, in any way, the performance and/or results of a first analysis or protocol with the performance and/or results of a second analysis or protocol. For example, one may use the results of a first analysis or protocol in carrying out a second protocols and/or one may use the results of a first analysis or protocol to determine whether a second analysis or protocol should be performed. With respect to the embodiment of polypeptide analysis or protocol, one may use the results of the polypeptide expression analysis or protocol to determine whether a specific therapeutic regimen should be performed. With respect to the embodiment of polynucleotide analysis or protocol, one may use the results of the polynucleotide expression analysis or protocol to determine whether a specific therapeutic regimen should be performed.

"Individual response" or "response" can be assessed using any endpoint indicating a benefit to the individual, including, without limitation, (1) inhibition, to some extent, of disease progression (e.g., cancer progression), including slowing down and complete arrest; (2) a reduction in tumor size; (3) inhibition (i.e., reduction, slowing down or complete stopping) of cancer cell infiltration into adjacent peripheral organs and/or tissues; (4) inhibition (i.e. reduction, slowing down or complete stopping) of metatasis; (5) relief, to some extent, of one or more symptoms associated with the disease or disorder (e.g., cancer); (6) increase or extend in the length of survival, including overall survival and progression free survival; and/or (9) decreased mortality at a given point of time following treatment.

An "effective response" of a patient or a patient's "responsiveness" to treatment with a medicament and similar wording refers to the clinical or therapeutic benefit imparted to a patient at risk for, or suffering from, a disease or disorder, such as cancer. In one embodiment, such benefit includes any one or more of: extending survival (including overall survival and progression-free survival); resulting in an objective response (including a complete response or a partial response); or improving signs or symptoms of cancer. In one embodiment, the biomarker (e.g., PD-L1 expression, for example, as determined using IHC) is used to identify the patient who is predicted to have an increased likelihood of being responsive to treatment with a medicament (e.g., treatment comprising a PD-L1 axis binding

antagonist, e.g., an anti-PD-L1 antibody), relative to a patient who does not express the biomarker. In one embodiment, the biomarker (e.g., PD-L1 expression, for example, as determined using IHC) is used to identify the patient who is predicted to have an increase likelihood of being responsive to treatment with a medicament (e.g., anti-PD-L1 antibody), relative to a patient who does not express the biomarker at the same level. In one embodiment, the presence of the biomarker is used to identify a patient who is more likely to respond to treatment with a medicament, relative to a patient that does not have the presence of the biomarker. In another embodiment, the presence of the biomarker is used to determine that a patient will have an increased likelihood of benefit from treatment with a medicament, relative to a patient that does not have the presence of the biomarker.

An "objective response" refers to a measurable response, including complete response (CR) or partial response (PR). In some embodiments, the "objective response rate (ORR)" refers to the sum of complete response (CR) rate and partial response (PR) rate.

By "complete response" or "CR" is intended the disappearance of all signs of cancer (e.g., disappearance of all target lesions) in response to treatment. This does not always mean the cancer has been cured.

"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, or longer.

As used herein, "reducing or inhibiting cancer relapse" means to reduce or inhibit tumor or cancer relapse or tumor or cancer progression. As disclosed herein, cancer relapse and/or cancer progression include, without limitation, cancer metastasis.

As used herein, "partial response" or "PR" refers to a decrease in the size of one or more tumors or lesions, or in the extent of cancer in the body, in response to treatment. For example, in some embodiments, 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.

As used herein, "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.

The term "survival" refers to the patient remaining alive, and includes overall survival as well as progression-free survival

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 survival" (OS) refers to the percentage of individuals in a group who are likely to be alive after a particular duration of time.

By "extending survival" is meant increasing overall or progression-free survival in a treated patient relative to an untreated patient (i.e. relative to a patient not treated with the medicament), or relative to a patient who does not express a biomarker at the designated level, and/or relative to a patient treated with an approved anti-tumor agent.

The term "substantially the same," as used herein, denotes a sufficiently high degree of similarity between two numeric values, such that one of skill in the art would consider the difference between the two values to be of little or no biological and/or statistical significance within the context of the biological characteristic measured by said values (e.g., Kd values or expression levels). 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.

The phrase "substantially different," as used herein, denotes a sufficiently high degree of difference between two numeric values such that one of skill in the art would consider the difference between the two values to be of statistical significance within the context of the biological characteristic measured by said values (e.g., Kd values or expression levels). 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 word "label" when used herein refers to a compound or composition that is conjugated or fused directly or indirectly to a reagent such as a polynucleotide probe or an antibody and facilitates detection of the reagent to which it is conjugated or fused. The label may itself be detectable (e.g., radioisotope labels or fluorescent labels) or, in the case of an enzymatic label, may catalyze chemical alteration of a substrate compound or composition which is detectable. The term is intended to encompass direct labeling of a probe or antibody by coupling (i.e., physically linking) a detectable substance to the probe or antibody, as well as indirect labeling of the probe or antibody by reactivity with another reagent that is directly labeled. Examples of indirect labeling include detection of a primary antibody using a fluorescently-labeled secondary antibody and end-labeling of a DNA probe with biotin such that it can be detected with fluorescently-labeled streptavidin.

A "therapeutically effective amount" refers to an amount of a therapeutic agent to treat or prevent a disease or disorder in a mammal. In the case of cancers, the therapeutically effective amount of the therapeutic agent may reduce the number of cancer cells; reduce the primary tumor size; inhibit (i.e., slow to some extent and preferably stop) cancer cell infiltration into peripheral organs; inhibit (i.e., slow to some extent and preferably stop) tumor metastasis; inhibit, to some extent, tumor growth; and/or relieve to some extent one or more of the symptoms associated with the disorder. To the extent the drug may prevent growth and/or kill existing cancer cells, it may be cytostatic and/or cytotoxic. For cancer therapy, efficacy in vivo can, for example, be measured by assessing the duration of survival, time to disease progression (TTP), response rates (e.g., CR and PR), duration of response, and/or quality of life.

A "disorder" is any condition that would benefit from treatment including, but not limited to, chronic and acute disorders or diseases including those pathological conditions which predispose the mammal to the disorder in question.

The terms "cancer" and "cancerous" refer to or describe the physiological condition in mammals that is typically characterized by unregulated cell growth. Included in this definition are benign and malignant cancers. By "early stage cancer" or "early stage tumor" is meant a cancer that is not invasive or metastatic or is classified as a Stage 0, 1, or II cancer. Examples of cancer include, but are not limited to, carcinoma, lymphoma, blastoma (including medulloblastoma and retinoblastoma), sarcoma (including liposarcoma and synovial cell sarcoma), neuroendocrine tumors (including carcinoid tumors, gastrinoma, and islet cell cancer), mesothelioma, schwannoma (including acoustic neuroma), meningioma, adenocarcinoma, melanoma, and leukemia or lymphoid malignancies. More particular examples of such cancers include squamous cell cancer (e.g., epithelial squamous cell cancer), lung cancer including small-cell lung cancer (SCLC), non-small cell lung cancer (NSCLC), adenocarcinoma of the lung and squamous carcinoma of the lung, cancer of the peritoneum, hepatocellular cancer, gastric or stomach cancer including gastrointestinal cancer, pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, breast cancer (including metastatic breast cancer), colon cancer, rectal cancer, colorectal cancer, endometrial or uterine carcinoma, salivary gland carcinoma, kidney or renal cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma, anal carcinoma, penile carcinoma, Merkel cell cancer, mycoses fungoids, testicular cancer, esophageal cancer, tumors of the biliary tract, as well as head and neck cancer and hematological malignancies. In some embodiments, the cancer is triple-negative metastatic breast cancer, including any histologically confirmed triple-negative (ER-, PR-, HER2-) adenocarcinoma of the breast with locally recurrent or metastatic disease (where the locally recurrent disease is not amenable to resection with curative intent). In particular embodiments, the cancer is NSCLC, including squamous NSCLC and non-squamous NSCLC.

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

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

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

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

and remission or improved prognosis. In some embodiments, antibodies (e.g., anti-PD-L1 antibodies and/or anti-PD-1 antibodies) are used to delay development of a disease or to slow the progression of a disease.

The term "anti-cancer therapy" refers to a therapy useful in treating cancer. Examples of anti-cancer therapeutic agents include, but are limited to, cytotoxic agents, chemotherapeutic agents, growth inhibitory agents, agents used in radiation therapy, anti-angiogenesis agents, apoptotic agents, anti-tubulin agents, and other agents to treat cancer, for example, anti-CD20 antibodies, platelet derived growth factor inhibitors (e.g., GLEEVEC™ (imatinib mesylate)), a COX-2 inhibitor (e.g., celecoxib), interferons, cytokines, antagonists (e.g., neutralizing antibodies) that bind to one or more of the following targets PDGFR-β, BlyS, APRIL, BCMA receptor(s), TRAIL/Apo2, other bioactive and organic chemical agents, and the like. Combinations thereof are also included in the invention.

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

A "chemotherapeutic agent" is a chemical compound useful in the treatment of cancer. Examples of chemotherapeutic agents include alkylating agents such as thiotepa and CYTOXAN® cyclosphosphamide; alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, trietylenephosphoramide, triethiylenethiophosphoramide and trimethylolomelamine; acetogenins (especially bullatacin and bullatacinone); delta-9-tetrahydrocannabinol (dronabinol, MARINOL®); beta-lapachone; lapachol; colchicines; betulinic acid; a camptothecin (including the synthetic analogue topotecan (HYCAMTIN®), CPT-11 (irinotecan, CAMPTOSAR®), acetylcamptothecin, scopolectin, and 9-aminocamptothecin); bryostatin; callystatin; CC-1065 (including its adozelesin, carzelesin and bizelesin synthetic analogues); podophyllotoxin; podophyllinic acid; teniposide; cryptophycins (particularly cryptophycin 1 and cryptophycin 8); dolastatin; duocarmycin (including the synthetic analogues, KW-2189 and CB1-TM1); eleutherobin; pancratistatin; a sarcodictyin; spongistatin; nitrogen mustards such as chlorambucil, chlornaphazine, cholophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, and ranimnustine; antibiotics such as the enediyne antibiotics (e.g., calicheamicin, especially calicheamicin γ1I and calicheamicin ω1I (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 antiobiotic chromophores), aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, carabicin,

carminomycin, carzinophilin, chromomycinis, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, ADRIAMYCIN® doxorubicin (including morpholino-doxorubicin, cyanomorpholino-doxorubicin, 2-pyrrolino-doxorubicin 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 and 5-fluorouracil (5-FU); folic acid analogues such as denopterin, methotrexate, pteropterin, trimetrexate; purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine; androgens such as calusterone, dromostanolone propionate, epitiostanol, mepitiostane, testolactone; anti-adrenals such as aminoglutethimide, mitotane, trilostane; folic acid replenisher such as frolinic acid; aceglatone; aldophosphamide glycoside; aminolevulinic acid; eniluracil; amsacrine; bestrabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elfornithine; elliptinium acetate; an epothilone; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidainine; maytansinoids such as maytansine and ansamitocins; mitoguazone; mitoxantrone; mopidanmol; nitraerine; pentostatin; phenamet; pirarubicin; losoxantrone; 2-ethylhydrazide; procarbazine; PSK® polysaccharide complex (JHS Natural Products, Eugene, OR); razoxane; rhizoxin; sizofiran; spirogermanium; tenuazonic acid; triaziquone; 2,2',2"-trichlorotriethylamine; trichothecenes (especially T-2 toxin, verracurin A, roridin A and anguidine); urethan; vindesine (ELDISINE®, FILDESIN®); dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); thiotepa; taxoids, for example taxanes including TAXOL® paclitaxel (Bristol-Myers Squibb Oncology, Princeton, N.J.), ABRAXANE™ Cremophor-free, albumin-engineered nanoparticle formulation of paclitaxel (American Pharmaceutical Partners, Schaumberg, Illinois), and TAXOTERE® docetaxel (Rhône-Poulenc Rorer, Antony, France); chloranbucil; gemcitabine (GEMZAR®); 6-thioguanine; mercaptopurine; methotrexate; platinum analogs such as cisplatin and carboplatin; vinblastine (VELBAN®); platinum; etoposide (VP-16); ifosfamide; mitoxantrone; vincristine (ONCOVIN®); oxaliplatin; leucovovin; vinorelbine (NAVELBINE®); novantrone; edatrexate; daunomycin; aminopterin; ibandronate; topoisomerase inhibitor RFS 2000; difluorometlhylornithine (DMFO); retinoids such as retinoic acid; capecitabine (XELODA®); 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 leucovorin. Additional chemotherapeutic agents include the cytotoxic agents useful as antibody drug conjugates, such as maytansinoids (DM1, for example) and the auristatins MMAE and MMAF, for example.

"Chemotherapeutic agents" also include "anti-hormonal agents" or "endocrine therapeutics" 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), EVISTA® raloxifene, droloxifene, 4-hydroxytamoxifen, trioxifene, keoxifene, LY117018, onapristone, and FARESTON® toremifene; anti-progesterones; estrogen receptor down-regulators (ERDs); agents that function to suppress or shut down

the ovaries, for example, leutinizing hormone-releasing hormone (LHRH) agonists such as LUPRON® and ELIGARD® leuprolide acetate, goserelin acetate, buserelin acetate and tripterelin; other 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, aminoglutethimide, MEGASE® megestrol acetate, AROMASIN® exemestane, formestanie, fadrozole, RIVISOR® vorozole, FEMARA® letrozole, and ARIMIDEX® anastrozole. In addition, such definition of chemotherapeutic agents includes bisphosphonates such as clodronate (for example, BONEFOS® or OSTAC®), DIDROCAL® etidronate, NE-58095, ZOMETA® zoledronic acid/zoledronate, FOSAMAX® alendronate, AREDIA® pamidronate, SKELID® tiludronate, or ACTONEL® risedronate; as well as troxacitabine (a 1,3-dioxolane nucleoside cytosine analog); antisense 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 (EGFR); vaccines such as THERATOPE® vaccine and gene therapy vaccines, for example, ALLOVECTIN® vaccine, LEUVECTIN® vaccine, and VAXID® vaccine; LURTOTECAN® topoisomerase 1 inhibitor; ABARELIX® rmRH; lapatinib ditosylate (an ErbB-2 and EGFR dual tyrosine kinase small-molecule inhibitor also known as GW572016); and pharmaceutically acceptable salts, acids or derivatives of any of the above.

Chemotherapeutic agents also include antibodies such as alemtuzumab (Campath), bevacizumab (AVASTIN®, Genentech); cetuximab (ERBITUX®, Imclone); panitumumab (VECTIBIX®, Amgen), rituximab (RITUXAN®, Genentech/Biogen Idec), pertuzumab (OMNITARG®, 2C4, Genentech), trastuzumab (HERCEPTIN®, Genentech), tositumomab (Bexxar, Corixia), and the antibody drug conjugate, gemtuzumab ozogamicin (MYLOTARG®, Wyeth). Additional humanized monoclonal antibodies with therapeutic potential as agents in combination with the compounds of the invention include: apolizumab, aselizumab, atlizumab, bapineuzumab, bivatuzumab mertansine, cantuzumab mertansine, cedelizumab, certolizumab pegol, cidfusituzumab, cidtuzumab, daclizumab, eculizumab, efalizumab, epratuzumab, erlizumab, felvizumab, fontolizumab, gemtuzumab ozogamicin, inotuzumab ozogamicin, ipilimumab, labetuzumab, lintuzumab, matuzumab, mepolizumab, motavizumab, motovizumab, natalizumab, nimotuzumab, nolovizumab, numavizumab, ocrelizumab, omalizumab, palivizumab, pascolizumab, pecfusituzumab, pectuzumab, pexelizumab, ralivizumab, ranibizumab, reslivizumab, reslizumab, resyvizumab, rovelizumab, ruplizumab, sibrotuzumab, siplizumab, sontuzumab, tacatuzumab tetraxetan, tadocizumab, talizumab, tefibazumab, tocilizumab, toralizumab, tucotuzumab celmoleukin, tucusituzumab, umavizumab, urtoxazumab, ustekinumab, visilizumab, and the anti–interleukin-12 (ABT-874/J695, Wyeth Research and Abbott Laboratories) which is a recombinant exclusively human-sequence, full-length IgG1 λ antibody genetically modified to recognize interleukin-12 p40 protein.

Chemotherapeutic agents also include "EGFR inhibitors," which refers to compounds that bind to or otherwise interact directly with EGFR and prevent or reduce its signaling activity, and is alternatively referred to as an "EGFR antagonist." Examples of such agents include antibodies and small molecules that bind to EGFR. Examples of antibodies which bind to EGFR include MAb 579 (ATCC CRL HB 8506), MAb 455 (ATCC CRL HB8507), MAb 225 (ATCC CRL 8508), MAb 528 (ATCC CRL 8509) (see, US

Patent No. 4,943, 533, Mendelsohn et al.) and variants thereof, such as chimerized 225 (C225 or Cetuximab; ERBUTIX®) and reshaped human 225 (H225) (see, WO 96/40210, Imclone Systems Inc.); IMC-11F8, a fully human, EGFR-targeted antibody (Imclone); antibodies that bind type II mutant EGFR (US Patent No. 5,212,290); humanized and chimeric antibodies that bind EGFR as described in US Patent No. 5,891,996; and human antibodies that bind EGFR, such as ABX-EGF or Panitumumab (see WO98/50433, Abgenix/Amgen); EMD 55900 (Stragliotto et al. *Eur. J. Cancer* 32A:636-640 (1996)); EMD7200 (matuzumab) a humanized EGFR antibody directed against EGFR that competes with both EGF and TGF-alpha for EGFR binding (EMD/Merck); human EGFR antibody, HuMax-EGFR (GenMab); fully human antibodies known as E1.1, E2.4, E2.5, E6.2, E6.4, E2.11, E6. 3, and E7.6. 3 and described in US 6,235,883; MDX-447 (Medarex Inc); and mAb 806 or humanized mAb 806 (Johns et al., *J. Biol. Chem.* 279(29):30375-30384 (2004)). The anti-EGFR antibody may be conjugated with a cytotoxic agent, thus generating an immunoconjugate (see, e.g., EP659,439A2, Merck Patent GmbH). EGFR antagonists include small molecules such as compounds described in US Patent Nos: 5,616,582, 5,457,105, 5,475,001, 5,654,307, 5,679,683, 6,084,095, 6,265,410, 6,455,534, 6,521,620, 6,596,726, 6,713,484, 5,770,599, 6,140,332, 5,866,572, 6,399,602, 6,344,459, 6,602,863, 6,391,874, 6,344,455, 5,760,041, 6,002,008, and 5,747,498, as well as the following PCT publications: WO98/14451, WO98/50038, WO99/09016, and WO99/24037. Particular small molecule EGFR antagonists include OSI-774 (CP-358774, erlotinib, TARCEVA® Genentech/OSI Pharmaceuticals); PD 183805 (CI 1033, 2-propenamide, N-[4-[(3-chloro-4-fluorophenyl)amino]-7-[3-(4-morpholinyl)propoxy]-6-quinazolinyl]-, dihydrochloride, Pfizer Inc.); ZD1839, gefitinib (IRESSA®) 4-(3'-Chloro-4'-fluoroanilino)-7-methoxy-6-(3-morpholinopropoxy)quinazoline, AstraZeneca); ZM 105180 ((6-amino-4-(3-methylphenyl-amino)-quinazoline, Zeneca); BIBX-1382 (N8-(3-chloro-4-fluoro-phenyl)-N2-(1-methyl-piperidin-4-yl)-pyrimido[5,4-d]pyrimidine-2,8-diamine, Boehringer Ingelheim); PKI-166 ((R)-4-[4-[(1-phenylethyl)amino]-1H-pyrrolo[2,3-d]pyrimidin-6-yl]-phenol); (R)-6-(4-hydroxyphenyl)-4-[(1-phenylethyl)amino]-7H-pyrrolo[2,3-d]pyrimidine); CL-387785 (N-[4-[(3-bromophenyl)amino]-6-quinazolinyl]-2-butynamide); EKB-569 (N-[4-[(3-chloro-4-fluorophenyl)amino]-3-cyano-7-ethoxy-6-quinolinyl]-4-(dimethylamino)-2-butenamide) (Wyeth); AG1478 (Pfizer); AG1571 (SU 5271; Pfizer); dual EGFR/HER2 tyrosine kinase inhibitors such as lapatinib (TYKERB®, GSK572016 or N-[3-chloro-4-[(3 fluorophenyl)methoxy]phenyl]-6[5[[[2methylsulfonyl)ethyl]amino]methyl]-2-furanyl]-4-quinazolinamine).

Chemotherapeutic agents also include "tyrosine kinase inhibitors" including the EGFR-targeted drugs noted in the preceding paragraph; small molecule HER2 tyrosine kinase inhibitor such as TAK165 available from Takeda; CP-724,714, an oral selective inhibitor of the ErbB2 receptor tyrosine kinase (Pfizer and OSI); dual-HER inhibitors such as EKB-569 (available from Wyeth) which preferentially binds EGFR but inhibits both HER2 and EGFR-overexpressing cells; lapatinib (GSK572016; available from Glaxo-SmithKline), an oral HER2 and EGFR tyrosine kinase inhibitor; PKI-166 (available from Novartis); pan-HER inhibitors such as canertinib (CI-1033; Pharmacia); Raf-1 inhibitors such as antisense agent ISIS-5132 available from ISIS Pharmaceuticals which inhibit Raf-1 signaling; non-HER targeted TK inhibitors such as imatinib mesylate (GLEEVEC®, available from Glaxo SmithKline); multi-targeted tyrosine kinase inhibitors such as sunitinib (SUTENT®, available from Pfizer); VEGF receptor tyrosine kinase inhibitors such as vatalanib (PTK787/ZK222584, available from Novartis/Schering AG); MAPK

extracellular regulated kinase I inhibitor CI-1040 (available from Pharmacia); quinazolines, such as PD 153035,4-(3-chloroanilino) quinazoline; pyridopyrimidines; pyrimidopyrimidines; pyrrolopyrimidines, such as CGP 59326, CGP 60261 and CGP 62706; pyrazolopyrimidines, 4-(phenylamino)-7H-pyrrolo[2,3-d] pyrimidines; curcumin (diferuloyl methane, 4,5-bis (4-fluoroanilino)phthalimide); tyrphostines containing nitrothiophene moieties; PD-0183805 (Warner-Lamber); antisense molecules (e.g., those that bind to HER-encoding nucleic acid); quinoxalines (US Patent No. 5,804,396); tryphostins (US Patent No. 5,804,396); ZD6474 (Astra Zeneca); PTK-787 (Novartis/Schering AG); pan-HER inhibitors such as CI-1033 (Pfizer); Affinitac (ISIS 3521; Isis/Lilly); imatinib mesylate (GLEEVEC®); PKI 166 (Novartis); GW2016 (Glaxo SmithKline); CI-1033 (Pfizer); EKB-569 (Wyeth); Semaxinib (Pfizer); ZD6474 (AstraZeneca); PTK-787 (Novartis/Schering AG); INC-1C11 (Imclone), rapamycin (sirolimus, RAPAMUNE®); or as described in any of the following patent publications: US Patent No. 5,804,396; WO 1999/09016 (American Cyanamid); WO 1998/43960 (American Cyanamid); WO 1997/38983 (Warner Lambert); WO 1999/06378 (Warner Lambert); WO 1999/06396 (Warner Lambert); WO 1996/30347 (Pfizer, Inc); WO 1996/33978 (Zeneca); WO 1996/3397 (Zeneca) and WO 1996/33980 (Zeneca).

Chemotherapeutic agents also include dexamethasone, interferons, colchicine, metoprine, cyclosporine, amphotericin, metronidazole, alemtuzumab, alitretinoin, allopurinol, amifostine, arsenic trioxide, asparaginase, BCG live, bevacuzimab, bexarotene, cladribine, clofarabine, darbepoetin alfa, denileukin, dexrazoxane, epoetin alfa, elotinib, filgrastim, histrelin acetate, ibritumomab, interferon alfa-2a, interferon alfa-2b, lenalidomide, levamisole, mesna, methoxsalen, nandrolone, nelarabine, nofetumomab, oprelvekin, palifermin, pamidronate, pegademase, pegaspargase, pegfilgrastim, pemetrexed disodium, plicamycin, porfimer sodium, quinacrine, rasburicase, sargramostim, temozolomide, VM-26, 6-TG, toremifene, tretinoin, ATRA, valrubicin, zoledronate, and zoledronic acid, and pharmaceutically acceptable salts thereof.

Chemotherapeutic agents also include hydrocortisone, hydrocortisone acetate, cortisone acetate, tixocortol pivalate, triamcinolone acetonide, triamcinolone alcohol, mometasone, amcinonide, budesonide, desonide, fluocinonide, fluocinolone acetonide, betamethasone, betamethasone sodium phosphate, dexamethasone, dexamethasone sodium phosphate, fluocortolone, hydrocortisone-17-butyrate, hydrocortisone-17-valerate, aclometasone dipropionate, betamethasone valerate, betamethasone dipropionate, prednicarbate, clobetasone-17-butyrate, clobetasol-17-propionate, fluocortolone caproate, fluocortolone pivalate and fluprednidene acetate; immune selective anti-inflammatory peptides (ImSAIDs) such as phenylalanine-glutamine-glycine (FEG) and its D-isomeric form (feG) (IMULAN BioTherapeutics, LLC); anti-rheumatic drugs such as azathioprine, ciclosporin (cyclosporine A), D-penicillamine, gold salts, hydroxychloroquine, leflunomideminocycline, sulfasalazine, tumor necrosis factor alpha (TNFα) blockers such as etanercept (ENBREL®), infliximab (REMICADE®), adalimumab (HUMIRA®), certolizumab pegol (CIMZIA®), golimumab (SIMPONI®), Interleukin 1 (IL-1) blockers such as anakinra (KINERET®), T-cell costimulation blockers such as abatacept (ORENCIA®), Interleukin 6 (IL-6) blockers such as tocilizumab (ACTEMERA®); Interleukin 13 (IL-13) blockers such as lebrikizumab; Interferon alpha (IFN) blockers such as rontalizumab; beta 7 integrin blockers such as rhuMAb Beta7; IgE pathway blockers such as Anti-M1 prime; Secreted homotrimeric LTa3 and membrane bound heterotrimer LTa1/β2 blockers such as Anti-lymphotoxin alpha (LTa); miscellaneous

investigational agents such as thioplatin, PS-341, phenylbutyrate, ET-18-OCH3, or farnesyl transferase inhibitors (L-739749, L-744832); polyphenols such as quercetin, resveratrol, piceatannol, epigallocatechine gallate, theaflavins, flavanols, procyanidins, betulinic acid and derivatives thereof; autophagy inhibitors such as chloroquine; delta-9-tetrahydrocannabinol (dronabinol, MARINOL®); beta-lapachone; lapachol; colchicines; betulinic acid; acetylcamptothecin, scopolectin, and 9-aminocamptothecin); podophyllotoxin; tegafur (UFTORAL®); bexarotene (TARGRETIN®); 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®); and epidermal growth factor receptor (EGF-R); vaccines such as THERATOPE® vaccine; perifosine, COX-2 inhibitor (e.g., celecoxib or etoricoxib), proteosome inhibitor (e.g., PS341); CCI-779; tipifarnib (R11577); orafenib, ABT510; Bcl-2 inhibitor such as oblimersen sodium (GENASENSE®); pixantrone; farnesyltransferase inhibitors such as lonafarnib (SCH 6636, SARASAR™); and pharmaceutically acceptable salts, acids or derivatives of any of the above; as well as combinations of two or more of the above.

The term "prodrug" as used herein refers to a precursor or derivative form of a pharmaceutically active substance that is less cytotoxic to tumor cells compared to the parent drug and is capable of being enzymatically activated or converted into the more active parent form. See, for example, Wilman, "Prodrugs in Cancer Chemotherapy" *Biochemical Society Transactions*, 14, pp. 375-382, 615th Meeting Belfast (1986) and Stella et al., "Prodrugs: A Chemical Approach to Targeted Drug Delivery," *Directed Drug Delivery*, Borchardt et al., (ed.), pp. 247-267, Humana Press (1985). The prodrugs of this invention include, but are not limited to, phosphate-containing prodrugs, thiophosphate-containing prodrugs, sulfate-containing prodrugs, peptide-containing prodrugs, D-amino acid-modified prodrugs, glycosylated prodrugs, β-lactam-containing prodrugs, optionally substituted phenoxyacetamide-containing prodrugs or optionally substituted phenylacetamide-containing prodrugs, 5-fluorocytosine and other 5-fluorouridine prodrugs which can be converted into the more active cytotoxic free drug. Examples of cytotoxic drugs that can be derivatized into a prodrug form for use in this invention include, but are not limited to, those chemotherapeutic agents described above.

A "growth inhibitory agent" when used herein refers to a compound or composition which inhibits growth and/or proliferation of a cell (e.g., a cell whose growth is dependent on PD-L1 expression) either *in vitro* or *in vivo*. Thus, the growth inhibitory agent may be one which significantly reduces the percentage of cells in S phase. Examples of growth inhibitory agents include agents that block cell cycle progression (at a place other than S phase), such as agents that induce G1 arrest and M-phase arrest. Classical M-phase blockers include the vincas (vincristine and vinblastine), taxanes, and topoisomerase II inhibitors such as the anthracycline antibiotic doxorubicin ((8S-cis)-10-[(3-amino-2,3,6-trideoxy-α-L-lyxo-hexapyranosyl)oxy]-7,8,9,10-tetrahydro-6,8,11-trihydroxy-8-(hydroxyacetyl)-1-methoxy-5,12-naphthacenedione), epirubicin, daunorubicin, etoposide, and bleomycin. Those agents that arrest G1 also spill over into S-phase arrest, for example, DNA alkylating agents such as tamoxifen, prednisone, dacarbazine, mechlorethamine, cisplatin, methotrexate, 5-fluorouracil, and ara-C. Further information can be found in "*The Molecular Basis of Cancer*," Mendelsohn and Israel, eds., Chapter 1, entitled "Cell cycle regulation, oncogenes, and antineoplastic drugs" by Murakami *et al.* (WB Saunders: Philadelphia, 1995),

especially p. 13. The taxanes (paclitaxel and docetaxel) are anticancer drugs both derived from the yew tree. Docetaxel (TAXOTERE®, Rhone-Poulenc Rorer), derived from the European yew, is a semisynthetic analogue of paclitaxel (TAXOL®, Bristol-Myers Squibb). Paclitaxel and docetaxel promote the assembly of microtubules from tubulin dimers and stabilize microtubules by preventing depolymerization, which results in the inhibition of mitosis in cells.

By "radiation therapy" is meant the use of directed gamma rays or beta rays to induce sufficient damage to a cell so as to limit its ability to function normally or to destroy the cell altogether. It will be appreciated that there will be many ways known in the art to determine the dosage and duration of treatment. Typical treatments are given as a one-time administration and typical dosages range from 10 to 200 units (Grays) per day.

As used herein, the terms "patient" or "subject" are used interchangeably and refer to any single animal, more preferably a mammal (including such non-human animals as, for example, dogs, cats, horses, rabbits, zoo animals, cows, pigs, sheep, and non-human primates) for which treatment is desired. In particular embodiments, the patient herein is a human.

As used herein, "administering" is meant a method of giving a dosage of a compound (e.g., an antagonist) or a pharmaceutical composition (e.g., a pharmaceutical composition including an antagonist) to a subject (e.g., a patient). Administering can be by any suitable means, including parenteral, intrapulmonary, and intranasal, and, if desired for local treatment, intralesional administration. Parenteral infusions include, for example, intramuscular, intravenous, intraarterial, intraperitoneal, or subcutaneous administration. Dosing can be by any suitable route, e.g., by injections, such as intravenous or subcutaneous injections, depending in part on whether the administration is brief or chronic. Various dosing schedules including but not limited to single or multiple administrations over various time-points, bolus administration, and pulse infusion are contemplated herein.

The term "concurrently" is used herein to refer to administration of two or more therapeutic agents, where at least part of the administration overlaps in time. Accordingly, concurrent administration includes a dosing regimen when the administration of one or more agent(s) continues after discontinuing the administration of one or more other agent(s).

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

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

A "sterile" formulation is aseptic or free from all living microorganisms and their spores.

An "article of manufacture" is any manufacture (e.g., a package or container) or kit comprising at least one reagent, e.g., a medicament for treatment of a disease or disorder (e.g., cancer), or a probe for specifically detecting a biomarker (e.g., PD-L1) described herein. In certain embodiments, the

manufacture or kit is promoted, distributed, or sold as a unit for performing the methods described herein.

The phrase "based on" when used herein means that the information about one or more biomarkers is used to inform a treatment decision, information provided on a package insert, or marketing/promotional guidance, etc.

III. Methods

A. Diagnostic Methods

Provided herein are methods for determining whether a patient suffering from a cancer (e.g., a non-small cell lung cancer) is likely to respond to treatment comprising a PD-L1 axis binding antagonist. Also provided herein are methods for predicting responsiveness of a patient suffering from a cancer (e.g., a non-small cell lung cancer) to treatment comprising a PD-L1 axis binding antagonist. Further provided herein are methods for selecting a therapy for a patient suffering from a cancer (e.g., a non-small cell lung cancer). Any of the preceding methods may be based on the expression level of a biomarker provided herein, for example, PD-L1 expression in a tumor sample, e.g., in tumor-infiltrating immune cells and/or in tumor cells. Any of the methods may further include administering to the patient a PD-L1 axis binding antagonist (for example, as described in Section C, "PD-L1 Axis Binding Antagonists" below) to the patient. Any of the methods may further include administering an effective amount of a second therapeutic agent to the patient.

The invention provides a method for determining whether a patient suffering from a non-small cell lung cancer is likely to respond to treatment comprising a PD-L1 axis binding antagonist, the method comprising: determining the expression level of PD-L1 in tumor cells in a tumor sample obtained from the patient, wherein a detectable expression level of PD-L1 in about 1% or more (e.g., about 1%, about 2%, about 3%, about 4%, about 5% or more, about 10% or more, about 15% or more, about 20% or more, about 25% or more, about 30% or more, about 35% or more, about 40% or more, about 50% or more, about 55% or more, about 60% or more, about 65% or more, about 70% or more, about 80% or more, about 85% or more, about 90% or more, about 95% or more, or about 99% or more) of the tumor cells in the tumor sample indicates that the patient is likely to respond to treatment comprising a PD-L1 axis binding antagonist. For example, in some instances, a detectable expression level of PD-L1 in 5% or more of the tumor cells in the tumor sample indicates that the patient is likely to respond to treatment comprising a PD-L1 axis binding antagonist. In other instances, a detectable expression level of PD-L1 in 10% or more of the tumor cells in the tumor sample indicates that the patient is likely to respond to treatment comprising a PD-L1 axis binding antagonist. In other instances, a detectable expression level of PD-L1 in 20% or more of the tumor cells in the tumor sample indicates that the patient is likely to respond to treatment comprising a PD-L1 axis binding antagonist. In other instances, a detectable expression level of PD-L1 in 30% or more of the tumor cells in the tumor sample indicates that the patient is likely to respond to treatment comprising a PD-L1 axis binding antagonist. In yet other instances, a detectable expression level of PD-L1 in 50% or more of the tumor cells in the tumor sample indicates that the patient is likely to respond to treatment comprising a PD-L1 axis binding antagonist.

The invention also provides a method for predicting responsiveness of a patient suffering from a non-small cell lung cancer to treatment comprising a PD-L1 axis binding antagonist, the method comprising: determining the expression level of PD-L1 in tumor cells in a tumor sample obtained from the patient, wherein a detectable expression level of PD-L1 in about 1% or more (e.g., about 1%, about 2%, about 3%, about 4%, about 5% or more, about 10% or more, about 15% or more, about 20% or more, about 25% or more, about 30% or more, about 35% or more, about 40% or more, about 50% or more, about 55% or more, about 60% or more, about 65% or more, about 70% or more, about 80% or more, about 85% or more, about 90% or more, about 95% or more, or about 99% or more) of the tumor cells in the tumor sample indicates that the patient is likely to respond to treatment comprising a PD-L1 axis binding antagonist. For example, in some instances, a detectable expression level of PD-L1 in 5% or more of the tumor cells in the tumor sample indicates that the patient is likely to respond to treatment comprising a PD-L1 axis binding antagonist. In other instances, a detectable expression level of PD-L1 in 10% or more of the tumor cells in the tumor sample indicates that the patient is likely to respond to treatment comprising a PD-L1 axis binding antagonist. In other instances, a detectable expression level of PD-L1 in 20% or more of the tumor cells in the tumor sample indicates that the patient is likely to respond to treatment comprising a PD-L1 axis binding antagonist. In other instances, a detectable expression level of PD-L1 in 30% or more of the tumor cells in the tumor sample indicates that the patient is likely to respond to treatment comprising a PD-L1 axis binding antagonist. In yet other instances, a detectable expression level of PD-L1 in 50% or more of the tumor cells in the tumor sample indicates that the patient is likely to respond to treatment comprising a PD-L1 axis binding antagonist.

In some embodiments of any of the preceding methods, a detectable expression level of PD-L1 in about 5% to about 99% (e.g., about 5% to about 99%, about 5% to about 90%, about 5% to about 85%, about 5% to about 80%, about 5% to about 75%, about 5% to about 70%, about 5% to about 65%, about 5% to about 60%, about 5% to about 55%, about 5% to about 50%, about 5% to about 45%, about 5% to about 40%, about 5% to about 35%, about 5% to about 30%, about 5% to about 25%, about 5% to about 20%, about 5% to about 15%, about 50% to 99%, about 50% to 95%, about 50% to about 90%, about 50% to about 85%, about 50% to about 80%, about 50% to about 75%, about 50% to about 70%, about 50% to about 65%, about 50% to about 60%, or about 50% to about 55%) of the tumor cells in the tumor sample indicates that the patient is likely to respond to treatment with a PD-L1 binding antagonist.

The invention further provides a method for selecting a therapy for a patient suffering from a non-small cell lung cancer, the method comprising: determining the expression level of PD-L1 in tumor cells in a tumor sample obtained from the patient, and selecting a therapy comprising a PD-L1 axis binding antagonist for the patient based on a detectable expression level of PD-L1 in about 1% or more (e.g., about 1%, about 2%, about 3%, about 4%, about 5% or more, about 10% or more, about 15% or more, about 20% or more, about 25% or more, about 30% or more, about 35% or more, about 40% or more, about 50% or more, about 55% or more, about 60% or more, about 65% or more, about 70% or more, about 80% or more, about 85% or more, about 90% or more, about 95% or more, or about 99% or more) of the tumor cells in the tumor sample. For example, in some instances, the method includes selecting a therapy comprising a PD-L1 axis binding antagonist for the patient based on a detectable expression level of PD-L1 in 5% or more of the tumor cells in the tumor sample. In some instances, the method

includes selecting a therapy comprising a PD-L1 axis binding antagonist for the patient based on a detectable expression level of PD-L1 in 10% or more of the tumor cells in the tumor sample. In some instances, the method includes selecting a therapy comprising a PD-L1 axis binding antagonist for the patient based on a detectable expression level of PD-L1 in 20% or more of the tumor cells in the tumor sample. In some instances, the method includes selecting a therapy comprising a PD-L1 axis binding antagonist for the patient based on a detectable expression level of PD-L1 in 30% or more of the tumor cells in the tumor sample. In some embodiments, the method includes selecting a therapy comprising a PD-L1 axis binding antagonist for the patient based on a detectable expression level of PD-L1 in 50% or more of the tumor cells in the tumor sample.

In any of the preceding methods, the method may further include determining the expression level of PD-L1 in tumor-infiltrating immune cells in the tumor sample obtained from the patient. In some embodiments, the tumor sample obtained from the patient has a detectable expression level of PD-L1 in tumor-infiltrating immune cells that comprise less than 10% (e.g., less than 10%, less than 9%, less than 8%, less than 7%, less than 6%, less than 5%, less than 4%, less than 3%, less than 2%, or less than 1%) of the tumor sample. For example, in some embodiments, the tumor sample obtained from the patient has a detectable expression level of PD-L1 in tumor-infiltrating immune cells that cover less than 10% of tumor area (e.g., less than 10% of tumor area, less than 9% of tumor area, less than 8% of tumor area, less than 7% of tumor area, less than 6% of tumor area, less than 5% of tumor area, less than 4% of tumor area, less than 3% of tumor area, less than 2% of tumor area, or less than 1% of tumor area) in a section of the tumor sample, for example, as determined by immunohistochemistry using an anti-PD-L1 antibody.

In any of the preceding methods, the tumor sample obtained from the patient may have desmoplasia. For example, in some instances, a tumor sample obtained from the patient may include a population of fibroblasts and/or myofibroblasts. In any of the methods embodiments, the tumor sample obtained from the patient may have a sclerotic reaction. In some instances, the tumor sample obtained from the patient may comprise a cell-poor and/or collagenized stroma. In any of the preceding methods, the tumor sample may comprise an increased expression level of collagen, STAT1, and/or MEK relative to a reference tumor sample.

The invention also provides a method for determining whether a patient suffering from a non-small cell lung cancer is likely to respond to treatment comprising a PD-L1 axis binding antagonist, the method comprising: determining the expression level of PD-L1 in tumor-infiltrating immune cells and in tumor cells in a tumor sample obtained from the patient, wherein a detectable expression level of PD-L1 in tumor-infiltrating immune cells that comprise about 1% or more (e.g., about 1% or more, about 2% or more, about 3% or more, about 4% or more, about 5% or more, about 6% or more, about 7% or more, about 8% or more, about 10% or more, about 11% or more, about 12% or more, about 13% or more, about 14% or more, about 15% or more, about 20% or more, about 25% or more, about 30% or more, about 35% or more, about 40% or more, about 50% or more, about 45% or more, or about 50% or more) of the tumor sample, and a detectable expression level of PD-L1 in less than 50% (e.g., less than 50%, less than 45%, less than 40%, less than 35%, less than 30%, less than 25%, less than 20%, less than 15%, less than 10%, or less than 5%) of the tumor cells in the tumor sample, indicates that the patient is

likely to respond to treatment comprising a PD-L1 axis binding antagonist. For example, in some embodiments, a detectable expression level of PD-L1 in tumor-infiltrating immune cells that comprise about 5% or more of the tumor sample, and a detectable expression level of PD-L1 in less than 50% of the tumor cells in the tumor sample, indicates that the patient is likely to respond to treatment comprising a PD-L1 axis binding antagonist. In other embodiments, a detectable expression level of PD-L1 in tumor-infiltrating immune cells that comprise about 10% or more of the tumor sample, and a detectable expression level of PD-L1 in less than 50% of the tumor cells in the tumor sample, indicates that the patient is likely to respond to treatment comprising a PD-L1 axis binding antagonist.

The invention further provides a method for predicting responsiveness of a patient suffering from a non-small cell lung cancer to treatment comprising a PD-L1 axis binding antagonist, the method comprising: determining the expression level of PD-L1 in tumor-infiltrating immune cells and in tumor cells in a tumor sample obtained from the patient, wherein a detectable expression level of PD-L1 in tumor-infiltrating immune cells that comprise 1% or more (e.g., about 1% or more, about 2% or more, about 3% or more, about 4% or more, about 5% or more, about 6% or more, about 7% or more, about 8% or more, about 10% or more, about 11% or more, about 12% or more, about 13% or more, about 14% or more, about 15% or more, about 20% or more, about 25% or more, about 30% or more, about 35% or more, about 40% or more, about 50% or more, about 45% or more, or about 50% or more) of the tumor sample, and a detectable expression level of PD-L1 in less than 50% of the tumor cells (e.g., less than 50%, less than 45%, less than 40%, less than 35%, less than 30%, less than 25%, less than 20%, less than 15%, less than 10%, or less than 5%) in the tumor sample, indicates that the patient is likely to respond to treatment comprising a PD-L1 axis binding antagonist. For example, in some embodiments, a detectable expression level of PD-L1 in tumor-infiltrating immune cells that comprise about 5% or more of the tumor sample, and a detectable expression level of PD-L1 in less than 50% of the tumor cells in the tumor sample, indicates that the patient is likely to respond to treatment comprising a PD-L1 axis binding antagonist. In other embodiments, a detectable expression level of PD-L1 in tumor-infiltrating immune cells that comprise about 10% or more of the tumor sample, and a detectable expression level of PD-L1 in less than 50% of the tumor cells in the tumor sample, indicates that the patient is likely to respond to treatment comprising a PD-L1 axis binding antagonist.

The invention yet also provides a method for selecting a therapy for a patient suffering from a non-small cell lung cancer, the method comprising: determining the expression level of PD-L1 in tumor-infiltrating immune cells and in tumor cells in a tumor sample obtained from the patient, and selecting a therapy comprising a PD-L1 axis binding antagonist for the patient based on a detectable expression level of PD-L1 in tumor-infiltrating immune cells that comprise 1% or more (e.g., about 1% or more, about 2% or more, about 3% or more, about 4% or more, about 5% or more, about 6% or more, about 7% or more, about 8% or more, about 10% or more, about 11% or more, about 12% or more, about 13% or more, about 14% or more, about 15% or more, about 20% or more, about 25% or more, about 30% or more, about 35% or more, about 40% or more, about 50% or more, about 45% or more, or about 50% or more) of the tumor sample, and a detectable expression level of PD-L1 in less than 50% (e.g., less than 50%, less than 45%, less than 40%, less than 35%, less than 30%, less than 25%, less than 20%, less than 15%, less than 10%, or less than 5%) of the tumor cells in the tumor sample. For example, in some

embodiments, the method includes selecting a therapy comprising a PD-L1 axis binding antagonist based on a detectable expression level of PD-L1 in tumor-infiltrating immune cells that comprise about 5% or more of the tumor sample, and a detectable expression level of PD-L1 in less than 50% of the tumor cells in the tumor sample. In other embodiments, the method includes selecting a therapy comprising a PD-L1 axis binding antagonist based on a detectable expression level of PD-L1 in tumor-infiltrating immune cells that comprise about 10% or more of the tumor sample, and a detectable expression level of PD-L1 in less than 50% of the tumor cells in the tumor sample.

In any of the preceding methods, the tumor-infiltrating immune cells may cover about 1% or more (e.g., about 1% or more, about 2% or more, about 3% or more, about 4% or more, about 5% or more, about 6% or more, about 7% or more, about 8% or more, about 10% or more, about 11% or more, about 12% or more, about 13% or more, about 14% or more, about 15% or more, about 20% or more, about 25% or more, about 30% or more, about 35% or more, about 40% or more, about 50% or more, about 45% or more, or about 50% or more) of the tumor area in a section of the tumor sample obtained from the patient. For example, in some instances, the tumor-infiltrating immune cells may cover about 1% or more of the tumor area in a section of the tumor sample. In some instances, the tumor-infiltrating immune cells may cover about 5% or more of the tumor area in a section of the tumor sample. In other instances, the tumor-infiltrating immune cells may cover about 10% or more of the tumor area in a section of the tumor sample. In some instances, the tumor-infiltrating immune cells may cover about 15% or more of the tumor area in a section of the tumor sample. In yet other instances, the tumor-infiltrating immune cells may cover about 20% or more of the tumor area in a section of the tumor sample. In further instances, the tumor-infiltrating immune cells may cover about 25% or more of the tumor area in a section of the tumor sample. In some instances, the tumor-infiltrating immune cells may cover about 30% or more of the tumor area in a section of the tumor sample. In some instances, the tumor-infiltrating immune cells may cover about 35% or more of the tumor area in a section of the tumor sample. In some instances, the tumor-infiltrating immune cells may cover about 40% or more of the tumor area in a section of the tumor sample. In some instances, the tumor-infiltrating immune cells may cover about 50% or more of the tumor area in a section of the tumor sample.

In any of the preceding methods, the tumor sample obtained from the patient may include an increased number of intra-epithelial and/or stromal immune cells relative to a reference tumor sample. In any of the preceding methods, the tumor sampel obtained from the patient may include an increased number of CD8+ T-cells relative to a reference tumor sample. In some instances, the tumor sample obtained from the patient has an increased expression level of one or more B-cell-related genes or natural killer (NK) cell-related genes relative to a reference tumor sample. In some instances, the one or more B-cell-related genes is selected from the group consisting of CD19, MS4A1, and CD79A. In some instances, the one or more NK cell-related genes is selected from the group consisting of *KLRB1*, *KLRC1*, *KLRC2*, *KLRC3*, *KLRD1*, *KLRF1*, *KLRG1*, *KLRK1*, *NCAM1*, *PRF1*, *NCR1*, *KIR2DL2*, *KIR2DL3*, *KIR2DL4*, *KIR2DS2*, *KIR3DL1*, *FCGR3A*, *MICA*, and *MICB*.

In some embodiments, the methods include determining the expression level of one or more additional biomarkers. In some embodiments, the additional biomarker is an immune-related marker. An immune -related marker refers to a marker that is expressed by immune cells, or by other cells (e.g.,

tumor cells, endothelial cells, fibroblasts, or other stromal cells). If expressed by cells other than immune cells, the marker may be involved in regulation of immune cell biology and function, including, for example, activation, priming, antigen recognition and presentation, cytokine and chemokine production, proliferation, migration, survival, or antibody production. In some embodiments, the immune-related marker is a T-cell-related marker. In some embodiments, the T-cell-related marker is selected from the group consisting of CD8A, IFN-γ, EOMES, Granzyme-A, CXCL9, and any combinations thereof. In some embodiments, the immune-related marker is selected from the group consisting of CX3CL1, CD45RO, IDOl, Galectin 9, MIC-A, MIC-B, CTLA-4, and any combinations thereof. In some embodiments, the additional biomarker is a NK cell-related gene. In some embodiments, an NK cell-related gene includes, but is not limited to, *KLRB1*, *KLRC1*, *KLRC2*, *KLRC3*, *KLRD1*, *KLRF1*, *KLRG1*, *KLRK1*, *NCAM1*, *PRF1*, *NCR1*, *KIR2DL2*, *KIR2DL3*, *KIR2DL4*, *KIR2DS2*, *KIR3DL1*, *FCGR3A*, *MICA*, *MICB*, and any combinations thereof, In some embodiments, the additional biomarker is a myeloid cell-related gene. In some embodiments, the myeloid cell-related gene includes, but is not limited to, *IL1B, IL8*, *CCL2,* and any combinations thereof. In some embodiments, the additional biomarker is a B cell-related gene. In some embodiments, the B cell-related gene includes, but is not limited to, *CD19*, *MS4A1*, *CD79A*, and any combinations thereof. In some embodiments, the additional biomarker is an effector T-cell (T_{eff})-related gene. In some embodiments, a T_{eff}-related gene includes, but is not limited to, *CD8A, GZMA, GZMB, IFNG, EOMES, PRF1, CXCL9, CXCL10, TBX21,* and any combinations thereof. In some embodiments, the Teff-related gene is *IFNG*, *GZMB*, or *CXCL9*. In some embodiments, the additional biomarker is a collagen gene. In some embodiments, the collagen gene is *COL6A1* or *COL6A2*.

In any of the preceding methods, the method may further include administering to the patient a therapeutically effective amount of a PD-L1 axis binding antagonist based on the expression level of PD-L1 in tumor cells or in tumor-infiltrating immune cells in the tumor sample. The PD-L1 axis binding antagonist may be any PD-L1 axis binding antagonist known in the art or described herein, for example, in Section C, "PD-L1 Axis Binding Antagonists" below.

For example, in some instances, the PD-L1 axis binding antagonist is selected from the group consisting of a PD-L1 binding antagonist, a PD-1 binding antagonist, and a PD-L2 binding antagonist. In some instances, the PD-L1 axis binding antagonist is a PD-L1 binding antagonist. In some instances, the PD-L1 binding antagonist inhibits the binding of PD-L1 to one or more of its ligand binding partners. In other instances, the PD-L1 binding antagonist inhibits the binding of PD-L1 to PD-1. In yet other instances, the PD-L1 binding antagonist inhibits the binding of PD-L1 to B7-1. In some instances, the PD-L1 binding antagonist inhibits the binding of PD-L1 to both PD-1 and B7-1. In some instances, the PD-L1 binding antagonist is an antibody. In some embodiments, the antibody is selected from the group consisting of: YW243.55.S70, MPDL3280A (atezolizumab), MDX-1105, MEDI4736 (durvalumab), and MSB0010718C (avelumab). In some embodiments, the antibody comprises a heavy chain comprising HVR-H1 sequence of SEQ ID NO:19, HVR-H2 sequence of SEQ ID NO:20, and HVR-H3 sequence of SEQ ID NO:21; and a light chain comprising HVR-L1 sequence of SEQ ID NO:22, HVR-L2 sequence of SEQ ID NO:23, and HVR-L3 sequence of SEQ ID NO:24. In some embodiments, the antibody comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:26 and a light chain variable region comprising the amino acid sequence of SEQ ID NO:4.

In some instances, the PD-L1 axis binding antagonist is a PD-1 binding antagonist. For example, in some instances, the PD-1 binding antagonist inhibits the binding of PD-1 to one or more of its ligand binding partners. In some instances, the PD-1 binding antagonist inhibits the binding of PD-1 to PD-L1. In other instances, the PD-1 binding antagonist inhibits the binding of PD-1 to PD-L2. In yet other instances, the PD-1 binding antagonist inhibits the binding of PD-1 to both PD-L1 and PD-L2. In some instances, the PD-1 binding antagonist is an antibody. In some instances, the antibody is selected from the group consisting of: MDX 1106 (nivolumab), MK-3475 (pembrolizumab), CT-011 (pidilizumab), MEDI-0680 (AMP-514), PDR001, REGN2810, and BGB-108. In some instances, the PD-1 binding antagonist is an Fc-fusion protein. For example, in some instances, the Fc-fusion protein is AMP-224.

In some instances, the method further includes administering to the patient an effective amount of a second therapeutic agent. In some instances, the second therapeutic agent is selected from the group consisting of a cytotoxic agent, a growth-inhibitory agent, a radiation therapy agent, an anti-angiogenic agent, and combinations thereof.

In any of the preceding instances, the non-small cell lung cancer may be a locally advanced or metastatic non-small cell lung cancer. In any of the preceding instances, the non-small cell lung cancer may be squamous NSCLC or non-squamous NSCLC.

Presence and/or expression levels/amount of a biomarker (e.g., PD-L1) can be determined qualitatively and/or quantitatively based on any suitable criterion known in the art, including but not limited to DNA, mRNA, cDNA, proteins, protein fragments and/or gene copy number.

In any of the preceding methods, the sample obtained from the patient is selected from the group consisting of tissue, whole blood, plasma, serum, and combinations thereof. In some embodiments, the sample is a tissue sample. In some embodiments, the tissue sample is a tumor sample. In some embodiments, the tumor sample comprises tumor cells, tumor-infiltrating immune cells, stromal cells, or any combinations thereof. In any of the preceding embodiments, the tumor sample may be a formalin-fixed and paraffin-embedded (FFPE) tumor sample, an archival tumor sample, a fresh tumor sample, or a frozen tumor sample.

The presence and/or expression level/amount of various biomarkers described herein in a sample can be analyzed by a number of methodologies, many of which are known in the art and understood by the skilled artisan, including, but not limited to, immunohistochemistry ("IHC"), Western blot analysis, immunoprecipitation, molecular binding assays, ELISA, ELIFA, fluorescence activated cell sorting ("FACS"), MassARRAY, proteomics, quantitative blood based assays (e.g., Serum ELISA), biochemical enzymatic activity assays, in situ hybridization, fluorescence in situ hybridization (FISH), Southern analysis, Northern analysis, whole genome sequencing, polymerase chain reaction (PCR) including quantitative real time PCR (qRT-PCR) and other amplification type detection methods, such as, for example, branched DNA, SISBA, TMA and the like, RNA-Seq, microarray analysis, gene expression profiling, and/or serial analysis of gene expression ("SAGE"), as well as any one of the wide variety of assays that can be performed by protein, gene, and/or tissue array analysis. Typical protocols for evaluating the status of genes and gene products are found, for example in Ausubel et al., eds., 1995, *Current Protocols In Molecular Biology*, Units 2 (Northern Blotting), 4 (Southern Blotting), 15

(Immunoblotting) and 18 (PCR Analysis). Multiplexed immunoassays such as those available from Rules Based Medicine or Meso Scale Discovery ("MSD") may also be used.

In any of the preceding methods, the presence and/or expression level/amount of a biomarker (e.g., PD-L1) is measured by determining protein expression levels of the biomarker. In certain embodiments, the method comprises contacting the biological sample with antibodies that specifically bind to a biomarker (e.g., anti-PD-L1 antibodies) described herein under conditions permissive for binding of the biomarker, and detecting whether a complex is formed between the antibodies and biomarker. Such method may be an *in vitro* or *in vivo* method. In some instances, an antibody is used to select subjects eligible for therapy with a PD-L1 axis binding antagonist, e.g., a biomarker for selection of individuals. Any method of measuring protein expression levels known in the art or provided herein may be used. For example, in some embodiments, a protein expression level of a biomarker (e.g., PD-L1) is determined using a method selected from the group consisting of flow cytometry (e.g., fluorescence-activated cell sorting (FACS™)), Western blot, enzyme-linked immunosorbent assay (ELISA), immunoprecipitation, immunohistochemistry (IHC), immunofluorescence, radioimmunoassay, dot blotting, immunodetection methods, HPLC, surface plasmon resonance, optical spectroscopy, mass spectrometry, and HPLC. In some embodiments, the protein expression level of the biomarker (e.g., PD-L1) is determined in tumor-infiltrating immune cells. In some embodiments, the protein expression level of the biomarker (e.g., PD-L1) is determined in tumor cells. In some embodiments, the protein expression level of the biomarker (e.g., PD-L1) is determined in tumor-infiltrating immune cells and in tumor cells.

In certain embodiments, the presence and/or expression level/amount of a biomarker protein (e.g., PD-L1) in a sample is examined using IHC and staining protocols. IHC staining of tissue sections has been shown to be a reliable method of determining or detecting the presence of proteins in a sample. In some embodiments of any of the methods, assays and/or kits, the biomarker is PD-L1. In one embodiment, expression level of biomarker is determined using a method comprising: (a) performing IHC analysis of a sample (such as a tumor sample obtained from a patient) with an antibody; and (b) determining expression level of a biomarker in the sample. In some embodiments, IHC staining intensity is determined relative to a reference. In some embodiments, the reference is a reference value. In some embodiments, the reference is a reference sample (e.g., a control cell line staining sample, a tissue sample from non-cancerous patient, or a PD-L1-negative tumor sample).

IHC may be performed in combination with additional techniques such as morphological staining and/or *in situ* hybridization (e.g., FISH). Two general methods of IHC are available; direct and indirect assays. According to the first assay, binding of antibody to the target antigen is determined directly. This direct assay uses a labeled reagent, such as a fluorescent tag or an enzyme-labeled primary antibody, which can be visualized without further antibody interaction. In a typical indirect assay, unconjugated primary antibody binds to the antigen and then a labeled secondary antibody binds to the primary antibody. Where the secondary antibody is conjugated to an enzymatic label, a chromogenic or fluorogenic substrate is added to provide visualization of the antigen. Signal amplification occurs because several secondary antibodies may react with different epitopes on the primary antibody.

The primary and/or secondary antibody used for IHC typically will be labeled with a detectable moiety. Numerous labels are available which can be generally grouped into the following categories: (a)

radioisotopes, such as ^{35}S, ^{14}C, ^{125}I, ^{3}H, and ^{131}I; (b) colloidal gold particles; (c) fluorescent labels including, but are not limited to, rare earth chelates (europium chelates), Texas Red, rhodamine, fluorescein, dansyl, lissamine, umbelliferone, phycocrytherin, phycocyanin, or commercially-available fluorophores such as SPECTRUM ORANGE7 and SPECTRUM GREEN7 and/or derivatives of any one or more of the above; (d) various enzyme-substrate labels are available and U.S. Patent No. 4,275,149 provides a review of some of these. Examples of enzymatic labels include luciferases (e.g., firefly luciferase and bacterial luciferase; see, e.g., U.S. Patent No. 4,737,456), luciferin, 2,3-dihydrophthalazinediones, malate dehydrogenase, urease, peroxidase such as horseradish peroxidase (HRPO), alkaline phosphatase, β-galactosidase, glucoamylase, lysozyme, saccharide oxidases (e.g., glucose oxidase, galactose oxidase, and glucose-6-phosphate dehydrogenase), heterocyclic oxidases (such as uricase and xanthine oxidase), lactoperoxidase, microperoxidase, and the like.

Examples of enzyme-substrate combinations include, for example, horseradish peroxidase (HRPO) with hydrogen peroxidase as a substrate; alkaline phosphatase (AP) with para-Nitrophenyl phosphate as chromogenic substrate; and β-D-galactosidase (β-D-Gal) with a chromogenic substrate (e.g., p-nitrophenyl-β-D-galactosidase) or fluorogenic substrate (e.g., 4-methylumbelliferyl-β-D-galactosidase). For a general review of these, see, for example, U.S. Patent Nos. 4,275,149 and 4,318,980.

Specimens may be prepared, for example, manually, or using an automated staining instrument (e.g., a Ventana BenchMark XT or Benchmark ULTRA instrument; see, e.g., Example 1 below). Specimens thus prepared may be mounted and coverslipped. Slide evaluation is then determined, for example, using a microscope, and staining intensity criteria, routinely used in the art, may be employed. In one embodiment, it is to be understood that when cells and/or tissue from a tumor is examined using IHC, staining is generally determined or assessed in tumor cell(s) and/or tissue (as opposed to stromal or surrounding tissue that may be present in the sample). In some embodiments, it is understood that when cells and/or tissue from a tumor is examined using IHC, staining includes determining or assessing in tumor-infiltrating immune cells, including intratumoral or peritumoral immune cells. In some embodiments, the presence of a biomarker (e.g., PD-L1) is detected by IHC in >0% of the sample, in at least 1% of the sample, in at least 5% of the sample, in at least 10% of the sample, in at least 15% of the sample, in at least 15% of the sample, in at least 20% of the sample, in at least 25% of the sample, in at least 30% of the sample, in at least 35% of the sample, in at least 40% of the sample, in at least 45% of the sample, in at least 50% of the sample, in at least 55% of the sample, in at least 60% of the sample, in at least 65% of the sample, in at least 70% of the sample, in at least 75% of the sample, in at least 80% of the sample, in at least 85% of the sample, in at least 90% of the sample, in at least 95% of the sample, or more. Samples may be scored using any of the criteria described herein (see, e.g., Table 2 and 3), for example, by a pathologist or automated image analysis.

In some embodiments of any of the methods, PD-L1 is detected by immunohistochemistry using an anti-PD-L1 diagnostic antibody (i.e., primary antibody). In some embodiments, the PD-L1 diagnostic antibody specifically binds human PD-L1. In some embodiments, the PD-L1 diagnostic antibody is a non-human antibody. In some embodiments, the PD-L1 diagnostic antibody is a rat, mouse, or rabbit antibody. In some embodiments, the PD-L1 diagnostic antibody is a rabbit antibody. In some

embodiments, the PD-L1 diagnostic antibody is a monoclonal antibody. In some embodiments, the PD-L1 diagnostic antibody is directly labeled. In other embodiments, the PD-L1 diagnostic antibody is indirectly labeled.

In some embodiments of any of the preceding methods, the expression level of PD-L1 is detected in tumor cells, tumor-infiltrating immune cells, or combinations thereof using IHC. Tumor-infiltrating immune cells include, but are not limited to, intratumoral immune cells, peritumoral immune cells or any combinations thereof, and other tumor stroma cells (e.g., fibroblasts). Such tumor infiltrating immune cells may be T lymphocytes (such as CD8+ T lymphocytes and/or CD4+ T lymphocytes), B lymphocytes, or other bone marrow-lineage cells including granulocytes (neutrophils, eosinophils, basophils), monocytes, macrophages, dendritic cells (e.g., interdigitating dendritic cells), histiocytes, and natural killer cells. In some embodiments, the staining for PD-L1 is detected as membrane staining, cytoplasmic staining and combinations thereof. In other embodiments, the absence of PD-L1 is detected as absent or no staining in the sample.

In any of the preceding methods, the expression level of a biomarker (e.g., PD-L1) may be a nucleic acid expression level. In some embodiments, the nucleic acid expression level is determined using qPCR, rtPCR, RNA-seq, multiplex qPCR or RT-qPCR, microarray analysis, SAGE, MassARRAY technique, or *in situ* hybridization (e.g., FISH). In some embodiments the expression level of a biomarker (e.g., PD-L1) is determined in tumor cells, tumor infiltrating immune cells, stromal cells, or combinations thereof. In some embodiments, the expression level of a biomarker (e.g., PD-L1) is determined in tumor cells. In some embodiments, the expression level of a biomarker (e.g., PD-L1) is determined in tumor-infiltrating immune cells.

Methods for the evaluation of mRNAs in cells are well known and include, for example, hybridization assays using complementary DNA probes (such as in situ hybridization using labeled riboprobes specific for the one or more genes, Northern blot and related techniques) and various nucleic acid amplification assays (such as RT-PCR using complementary primers specific for one or more of the genes, and other amplification type detection methods, such as, for example, branched DNA, SISBA, TMA and the like). In addition, such methods can include one or more steps that allow one to determine the levels of target mRNA in a biological sample (e.g., by simultaneously examining the levels a comparative control mRNA sequence of a "housekeeping" gene such as an actin family member). Optionally, the sequence of the amplified target cDNA can be determined. Optional methods include protocols which examine or detect mRNAs, such as target mRNAs, in a tissue or cell sample by microarray technologies. Using nucleic acid microarrays, test and control mRNA samples from test and control tissue samples are reverse transcribed and labeled to generate cDNA probes. The probes are then hybridized to an array of nucleic acids immobilized on a solid support. The array is configured such that the sequence and position of each member of the array is known. For example, a selection of genes whose expression correlates with increased or reduced clinical benefit of treatment comprising a PD-L1 axis binding antagonist may be arrayed on a solid support. Hybridization of a labeled probe with a particular array member indicates that the sample from which the probe was derived expresses that gene.

In certain embodiments, the presence and/or expression levels/amount of a biomarker in a first sample is increased or elevated as compared to presence/absence and/or expression levels/amount in a

second sample. In certain embodiments, the presence/absence and/or expression levels/amount of a biomarker in a first sample is decreased or reduced as compared to presence and/or expression levels/amount in a second sample. In certain embodiments, the second sample is a reference sample, reference cell, reference tissue, control sample, control cell, or control tissue. Additional disclosures for determining the presence/absence and/or expression levels/amount of a gene are described herein.

In certain embodiments, a reference sample, reference cell, reference tissue, control sample, control cell, or control tissue is a single sample or combined multiple samples from the same subject or individual that are obtained at one or more different time points than when the test sample is obtained. For example, a reference sample, reference cell, reference tissue, control sample, control cell, or control tissue is obtained at an earlier time point from the same subject or individual than when the test sample is obtained. Such reference sample, reference cell, reference tissue, control sample, control cell, or control tissue may be useful if the reference sample is obtained during initial diagnosis of cancer and the test sample is later obtained when the cancer becomes metastatic.

In certain embodiments, a reference sample, reference cell, reference tissue, control sample, control cell, or control tissue is a combined multiple samples from one or more healthy individuals who are not the patient. In certain embodiments, a reference sample, reference cell, reference tissue, control sample, control cell, or control tissue is a combined multiple samples from one or more individuals with a disease or disorder (e.g., cancer) who are not the subject or individual. In certain embodiments, a reference sample, reference cell, reference tissue, control sample, control cell, or control tissue is pooled RNA samples from normal tissues or pooled plasma or serum samples from one or more individuals who are not the patient. In certain embodiments, a reference sample, reference cell, reference tissue, control sample, control cell, or control tissue is pooled RNA samples from tumor tissues or pooled plasma or serum samples from one or more individuals with a disease or disorder (e.g., cancer) who are not the patient.

In some embodiments of any of the methods, elevated or increased expression refers to an overall increase of about any of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or greater, in the level of biomarker (e.g., protein or nucleic acid (e.g., gene or mRNA)), detected by standard art-known methods such as those described herein, as compared to a reference sample, reference cell, reference tissue, control sample, control cell, or control tissue. In certain embodiments, the elevated expression refers to the increase in expression level/amount of a biomarker in the sample wherein the increase is at least about any of 1.5X, 1.75X, 2X, 3X, 4X, 5X, 6X, 7X, 8X, 9X, 10X, 25X, 50X, 75X, or 100X the expression level/amount of the respective biomarker in a reference sample, reference cell, reference tissue, control sample, control cell, or control tissue. In some embodiments, elevated expression refers to an overall increase of greater than about 1.5 fold, about 1.75 fold, about 2 fold, about 2.25 fold, about 2.5 fold, about 2.75 fold, about 3.0 fold, or about 3.25 fold as compared to a reference sample, reference cell, reference tissue, control sample, control cell, control tissue, or internal control (e.g., housekeeping gene).

In some embodiments of any of the methods, reduced expression refers to an overall reduction of about any of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or greater, in the level of biomarker (e.g., protein or nucleic acid (e.g., gene or mRNA)), detected by standard art

known methods such as those described herein, as compared to a reference sample, reference cell, reference tissue, control sample, control cell, or control tissue. In certain embodiments, reduced expression refers to the decrease in expression level/amount of a biomarker in the sample wherein the decrease is at least about any of 0.9X, 0.8X, 0.7X, 0.6X, 0.5X, 0.4X, 0.3X, 0.2X, 0.1X, 0.05X, or 0.01X the expression level/amount of the respective biomarker in a reference sample, reference cell, reference tissue, control sample, control cell, or control tissue.

B. Therapeutic Methods

The present invention provides methods for treating a patient suffering from a cancer (e.g., a non-small cell lung cancer). In some instances, the methods of the invention include administering to the patient an anti-cancer therapy that includes a PD-L1 axis binding antagonist. Any of the PD-L1 axis binding antagonists described herein (see, for example, Section C, "PD-L1 Axis Binding Antagonists" below) or known in the art may used in the methods. In some instances, the methods involve determining the presence and/or expression level of a biomarker described herein in a sample obtained from a patient and administering an anti-cancer therapy to the patient based on the presence and/or expression level of the biomarker in the sample, for example, using any of the methods described herein (for example, those described in Section A, "Diagnostic Methods," or in the Examples below) or known in the art. In some embodiments, the biomarker is PD-L1.

The invention provides a method of treating a patient suffering from a non-small cell lung cancer, the method comprising administering to the patient a therapeutically effective amount of a PD-L1 axis binding antagonist, wherein a tumor sample obtained from the patient has been determined to have a detectable expression level of PD-L1 in 1% or more (e.g., about 1%, about 2%, about 3%, about 4%, about 5% or more, about 10% or more, about 15% or more, about 20% or more, about 25% or more, about 30% or more, about 35% or more, about 40% or more, about 50% or more, about 55% or more, about 60% or more, about 65% or more, about 70% or more, about 80% or more, about 85% or more, about 90% or more, about 95% or more, or about 99% or more) of the tumor cells in the tumor sample.

For example, in some embodiments, the tumor sample obtained from the patient has been determined to have a detectable expression level of PD-L1 in 5% or more of the tumor cells in the tumor sample. In other embodiments, the tumor sample obtained from the patient has been determined to have a detectable expression level of PD-L1 in 10% or more of the tumor cells in the tumor sample. In other embodiments, the tumor sample obtained from the patient has been determined to have a detectable expression level of PD-L1 in 15% or more of the tumor cells in the tumor sample. In other embodiments, the tumor sample obtained from the patient has been determined to have a detectable expression level of PD-L1 in 20% or more of the tumor cells in the tumor sample. In other embodiments, the tumor sample obtained from the patient has been determined to have a detectable expression level of PD-L1 in 30% or more of the tumor cells in the tumor sample. In yet other embodiments, the tumor sample obtained from the patient has been determined to have a detectable expression level of PD-L1 in 50% or more of the tumor cells in the tumor sample. In some embodiments, the tumor sample obtained from the patient has a detectable expression level of PD-L1 in tumor-infiltrating immune cells that comprise less than 10% (e.g., less than 10%, less than 9%, less than 8%, less than 7%, less than 6%,

less than 5%, less than 4%, less than 3%, less than 2%, or less than 1%) of the tumor sample. For example, in some embodiments, the tumor sample obtained from the patient has a detectable expression level of PD-L1 in tumor-infiltrating immune cells that cover less than 10% of tumor area (e.g., less than 10% of tumor area, less than 9% of tumor area, less than 8% of tumor area, less than 7% of tumor area, less than 6% of tumor area, less than 5% of tumor area, less than 4% of tumor area, less than 3% of tumor area, less than 2% of tumor area, or less than 1% of tumor area) in a section of the tumor sample obtained from the patient, for example, as determined by immunohistochemistry using an anti-PD-L1 antibody. In some embodiments, the tumor sample obtained from the patient does not have a detectable expression level of PD-L1 in tumor-infiltrating immune cells.

The invention also provides a method of treating a patient suffering from a non-small cell lung cancer, the method comprising administering to the patient a therapeutically effective amount of a PD-L1 axis binding antagonist, wherein a tumor sample obtained from the patient has been determined to have a detectable expression level of PD-L1 in tumor-infiltrating immune cells that comprise 1% or more (e.g., about 1% or more, about 2% or more, about 3% or more, about 4% or more, about 5% or more, about 6% or more, about 7% or more, about 8% or more, about 10% or more, about 11% or more, about 12% or more, about 13% or more, about 14% or more, about 15% or more, about 20% or more, about 25% or more, about 30% or more, about 35% or more, about 40% or more, about 50% or more, about 45% or more, or about 50% or more) of the tumor sample, and a detectable expression level of PD-L1 in less than 50% (e.g., less than 50%, less than 45%, less than 40%, less than 35%, less than 30%, less than 25%, less than 20%, less than 15%, less than 10%, or less than 5%) of the tumor cells in the tumor sample.

In any of the preceding methods, the tumor-infiltrating immune cells may cover about 1% or more (e.g., about 1% or more, about 2% or more, about 3% or more, about 4% or more, about 5% or more, about 6% or more, about 7% or more, about 8% or more, about 10% or more, about 11% or more, about 12% or more, about 13% or more, about 14% or more, about 15% or more, about 20% or more, about 25% or more, about 30% or more, about 35% or more, about 40% or more, about 50% or more, about 45% or more, or about 50% or more) of the tumor area in a section of the tumor sample obtained from the patient. For example, in some instances, the tumor-infiltrating immune cells may cover about 1% or more of the tumor area in a section of the tumor sample. In some instances, the tumor-infiltrating immune cells may cover about 5% or more of the tumor area in a section of the tumor sample. In other instances, the tumor-infiltrating immune cells may cover about 10% or more of the tumor area in a section of the tumor sample. In some instances, the tumor-infiltrating immune cells may cover about 15% or more of the tumor area in a section of the tumor sample. In yet other instances, the tumor-infiltrating immune cells may cover about 20% or more of the tumor area in a section of the tumor sample. In further instances, the tumor-infiltrating immune cells may cover about 25% or more of the tumor area in a section of the tumor sample. In some instances, the tumor-infiltrating immune cells may cover about 30% or more of the tumor area in a section of the tumor sample. In some instances, the tumor-infiltrating immune cells may cover about 35% or more of the tumor area in a section of the tumor sample. In some instances, the tumor-infiltrating immune cells may cover about 40% or more of the tumor area in a section of the tumor

sample. In some instances, the tumor-infiltrating immune cells may cover about 50% or more of the tumor area in a section of the tumor sample.

In any of the preceding methods, the PD-L1 axis binding antagonist may be any PD-L1 axis binding antagonist known in the art or described herein, for example, in Section C, "PD-L1 Axis Binding Antagonists" below.

For example, in some instances, the PD-L1 axis binding antagonist is selected from the group consisting of a PD-L1 binding antagonist, a PD-1 binding antagonist, and a PD-L2 binding antagonist. In some instances, the PD-L1 axis binding antagonist is a PD-L1 binding antagonist. In some instances, the PD-L1 binding antagonist inhibits the binding of PD-L1 to one or more of its ligand binding partners. In other instances, the PD-L1 binding antagonist inhibits the binding of PD-L1 to PD-1. In yet other instances, the PD-L1 binding antagonist inhibits the binding of PD-L1 to B7-1. In some instances, the PD-L1 binding antagonist inhibits the binding of PD-L1 to both PD-1 and B7-1. In some instances, the PD-L1 binding antagonist is an antibody. In some embodiments, the antibody is selected from the group consisting of: YW243.55.S70, MPDL3280A (atezolizumab), MDX-1105, MEDI4736 (durvalumab), and MSB0010718C (avelumab). In some embodiments, the antibody comprises a heavy chain comprising HVR-H1 sequence of SEQ ID NO:19, HVR-H2 sequence of SEQ ID NO:20, and HVR-H3 sequence of SEQ ID NO:21; and a light chain comprising HVR-L1 sequence of SEQ ID NO:22, HVR-L2 sequence of SEQ ID NO:23, and HVR-L3 sequence of SEQ ID NO:24. In some embodiments, the antibody comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:26 and a light chain variable region comprising the amino acid sequence of SEQ ID NO:4.

In some instances, the PD-L1 axis binding antagonist is a PD-1 binding antagonist. For example, in some instances, the PD-1 binding antagonist inhibits the binding of PD-1 to one or more of its ligand binding partners. In some instances, the PD-1 binding antagonist inhibits the binding of PD-1 to PD-L1. In other instances, the PD-1 binding antagonist inhibits the binding of PD-1 to PD-L2. In yet other instances, the PD-1 binding antagonist inhibits the binding of PD-1 to both PD-L1 and PD-L2. In some instances, the PD-1 binding antagonist is an antibody. In some instances, the antibody is selected from the group consisting of: MDX 1106 (nivolumab), MK-3475 (pembrolizumab), CT-011 (pidilizumab), MEDI-0680 (AMP-514), PDR001, REGN2810, and BGB-108. In some instances, the PD-1 binding antagonist is an Fc-fusion protein. For example, in some instances, the Fc-fusion protein is AMP-224.

In some instances, the method further includes administering to the patient an effective amount of a second therapeutic agent. In some instances, the second therapeutic agent is selected from the group consisting of a cytotoxic agent, a growth-inhibitory agent, a radiation therapy agent, an anti-angiogenic agent, and combinations thereof.

In any of the preceding instances, the non-small cell lung cancer may be a locally advanced or metastatic non-small cell lung cancer. In any of the preceding instances, the non-small cell lung cancer may be squamous NSCLC or non-squamous NSCLC.

In a further aspect, the invention provides for the use of a PD-L1 axis binding antagonist in the manufacture or preparation of a medicament. In one embodiment, the medicament is for treatment of a cancer. In a further embodiment, the medicament is for use in a method of treating a cancer comprising administering to a patient suffering from a cancer (e.g., NSCLC) an effective amount of the medicament.

In one such embodiment, the method further comprises administering to the individual an effective amount of at least one additional therapeutic agent, e.g., as described below.

The compositions utilized in the methods described herein (e.g., PD-L1 axis binding antagonists) can be administered by any suitable method, including, for example, intravenously, intramuscularly, subcutaneously, intradermally, percutaneously, intraarterially, intraperitoneally, intralesionally, intracranially, intraarticularly, intraprostatically, intrapleurally, intratracheally, intrathecally, intranasally, intravaginally, intrarectally, topically, intratumorally, peritoneally, subconjunctivally, intravesicularly, mucosally, intrapericardially, intraumbilically, intraocularly, intraorbitally, orally, topically, transdermally, intravitreally (e.g., by intravitreal injection), by eye drop, by inhalation, by injection, by implantation, by infusion, by continuous infusion, by localized perfusion bathing target cells directly, by catheter, by lavage, in cremes, or in lipid compositions. The compositions utilized in the methods described herein can also be administered systemically or locally. The method of administration can vary depending on various factors (e.g., the compound or composition being administered and the severity of the condition, disease, or disorder being treated). In some embodiments, the PD-L1 axis binding antagonist is administered intravenously, intramuscularly, subcutaneously, topically, orally, transdermally, intraperitoneally, intraorbitally, by implantation, by inhalation, intrathecally, intraventricularly, or intranasally. Dosing can be by any suitable route, e.g., by injections, such as intravenous or subcutaneous injections, depending in part on whether the administration is brief or chronic. Various dosing schedules including but not limited to single or multiple administrations over various time-points, bolus administration, and pulse infusion are contemplated herein.

PD-L1 axis binding antagonists (e.g., an antibody, binding polypeptide, and/or small molecule) described herein (any any additional therapeutic agent) may be formulated, dosed, and administered in a fashion consistent with good medical practice. Factors for consideration in this context include the particular disorder being treated, the particular mammal being treated, the clinical condition of the individual patient, the cause of the disorder, the site of delivery of the agent, the method of administration, the scheduling of administration, and other factors known to medical practitioners. The PD-L1 axis binding antagonist need not be, but is optionally formulated with and/or administered concurrently with one or more agents currently used to prevent or treat the disorder in question. The effective amount of such other agents depends on the amount of the PD-L1 axis binding antagonist present in the formulation, the type of disorder or treatment, and other factors discussed above. These are generally used in the same dosages and with administration routes as described herein, or about from 1 to 99% of the dosages described herein, or in any dosage and by any route that is empirically/clinically determined to be appropriate.

For the prevention or treatment of a cancer (e.g., a non-small cell lung cancer), the appropriate dosage of a PD-L1 axis binding antagonist described herein (when used alone or in combination with one or more other additional therapeutic agents) will depend on the type of disease to be treated, the severity and course of the disease, whether the PD-L1 axis binding antagonist is administered for preventive or therapeutic purposes, previous therapy, the patient's clinical history and response to the PD-L1 axis binding antagonist, and the discretion of the attending physician. The PD-L1 axis binding antagonist is suitably administered to the patient at one time or over a series of treatments. One typical daily dosage

might range from about 1 μg/kg to 100 mg/kg or more, depending on the factors mentioned above. For repeated administrations over several days or longer, depending on the condition, the treatment would generally be sustained until a desired suppression of disease symptoms occurs. Such doses may be administered intermittently, e.g., every week or every three weeks (e.g., such that the patient receives, for example, from about two to about twenty, or e.g., about six doses of the PD-L1 axis binding antagonist). An initial higher loading dose, followed by one or more lower doses may be administered. However, other dosage regimens may be useful. The progress of this therapy is easily monitored by conventional techniques and assays.

For example, as a general proposition, the therapeutically effective amount of a PD-L1 axis binding antagonist antibody administered to human will be in the range of about 0.01 to about 50 mg/kg of patient body weight, whether by one or more administrations. In some embodiments, the antibody used is about 0.01 mg/kg to about 45 mg/kg, about 0.01 mg/kg to about 40 mg/kg, about 0.01 mg/kg to about 35 mg/kg, about 0.01 mg/kg to about 30 mg/kg, about 0.01 mg/kg to about 25 mg/kg, about 0.01 mg/kg to about 20 mg/kg, about 0.01 mg/kg to about 15 mg/kg, about 0.01 mg/kg to about 10 mg/kg, about 0.01 mg/kg to about 5 mg/kg, or about 0.01 mg/kg to about 1 mg/kg administered daily, weekly, every two weeks, every three weeks, or monthly, for example. In some embodiments, the antibody is administered at 15 mg/kg. However, other dosage regimens may be useful. In one embodiment, an anti-PD-L1 antibody described herein is administered to a human at a dose of about 100 mg, about 200 mg, about 300 mg, about 400 mg, about 500 mg, about 600 mg, about 700 mg, about 800 mg, about 900 mg, about 1000 mg, about 1100 mg, about 1200 mg, about 1300 mg, about 1400 mg, about 1500 mg, about 1600 mg, about 1700 mg, or about 1800 mg on day 1 of 21-day cycles (every three weeks, q3w). In some embodiments, anti-PD-L1 antibody MPDL3280A is administered at 1200 mg intravenously every three weeks (q3w). The dose may be administered as a single dose or as multiple doses (e.g., 2 or 3 doses), such as infusions. The dose of the antibody administered in a combination treatment may be reduced as compared to a single treatment. The progress of this therapy is easily monitored by conventional techniques.

In some embodiments, the methods further involve administering to the patient an effective amount of a second therapeutic agent. In some embodiments, the second therapeutic agent is selected from the group consisting of a cytotoxic agent, a chemotherapeutic agent, a growth-inhibitory agent, a radiation therapy agent, an anti-angiogenic agent, and combinations thereof. In some embodiments, a PD-L1 axis binding antagonist may be administered in conjunction with a chemotherapy or chemotherapeutic agent. In some embodiments, a PD-L1 axis binding antagonist may be administered in conjunction with a radiation therapy agent. In some embodiments, a PD-L1 axis binding antagonist may be administered in conjunction with a targeted therapy or targeted therapeutic agent. In some embodiments, a PD-L1 axis binding antagonist may be administered in conjunction with an immunotherapy or immunotherapeutic agent, for example a monoclonal antibody. In some embodiments, the second therapeutic agent is an agonist directed against an activating co-stimulatory molecule. In some embodiments, the second therapeutic agent is an antagonist directed against an inhibitory co-stimulatory molecule.

Such combination therapies noted above encompass combined administration (where two or more therapeutic agents are included in the same or separate formulations), and separate administration, in which case, administration of a PD-L1 axis binding antagonist can occur prior to, simultaneously, and/or following, administration of the additional therapeutic agent or agents. In one embodiment, administration of PD-L1 axis binding antagonist and administration of an additional therapeutic agent occur within about one month, or within about one, two or three weeks, or within about one, two, three, four, five, or six days, of each other.

Without wishing to be bound to theory, it is thought that enhancing T-cell stimulation, by promoting an activating co-stimulatory molecule or by inhibiting a negative co-stimulatory molecule, may

promote tumor cell death thereby treating or delaying progression of cancer. In some embodiments, a PD-L1 axis binding antagonist may be administered in conjunction with an agonist directed against an activating co-stimulatory molecule. In some embodiments, an activating co-stimulatory molecule may include CD40, CD226, CD28, OX40, GITR, CD137, CD27, HVEM, or CD127. In some embodiments, the agonist directed against an activating co-stimulatory molecule is an agonist antibody that binds to CD40, CD226, CD28, OX40, GITR, CD137, CD27, HVEM, or CD127. In some embodiments, a PD-L1 axis binding antagonist may be administered in conjunction with an antagonist directed against an inhibitory co-stimulatory molecule. In some embodiments, an inhibitory co-stimulatory molecule may include CTLA-4 (also known as CD152), TIM-3, BTLA, VISTA, LAG-3, B7-H3, B7-H4, IDO, TIGIT, MICA/B, or arginase. In some embodiments, the antagonist directed against an inhibitory co-stimulatory molecule is an antagonist antibody that binds to CTLA-4, TIM-3, BTLA, VISTA, LAG-3, B7-H3, B7-H4, IDO, TIGIT, MICA/B, or arginase.

In some embodiments, a PD-L1 axis binding antagonist may be administered in conjunction with an antagonist directed against CTLA-4 (also known as CD152), e.g., a blocking antibody. In some embodiments, a PD-L1 axis binding antagonist may be administered in conjunction with ipilimumab (also known as MDX-010, MDX-101, or YERVOY®). In some embodiments, a PD-L1 axis binding antagonist may be administered in conjunction with tremelimumab (also known as ticilimumab or CP-675,206). In some embodiments, a PD-L1 axis binding antagonist may be administered in conjunction with an antagonist directed against B7-H3 (also known as CD276), e.g., a blocking antibody. In some embodiments, a PD-L1 axis binding antagonist may be administered in conjunction with MGA271. In some embodiments, a PD-L1 axis binding antagonist may be administered in conjunction with an antagonist directed against a TGF-beta, e.g., metelimumab (also known as CAT-192), fresolimumab (also known as GC1008), or LY2157299.

In some embodiments, a PD-L1 axis binding antagonist may be administered in conjunction with a treatment comprising adoptive transfer of a T-cell (e.g., a cytotoxic T-cell or CTL) expressing a chimeric antigen receptor (CAR). In some embodiments, a PD-L1 axis binding antagonist may be administered in conjunction with a treatment comprising adoptive transfer of a T-cell comprising a dominant-negative TGF beta receptor, e.g., a dominant-negative TGF beta type II receptor. In some embodiments, a PD-L1 axis binding antagonist may be administered in conjunction with a treatment comprising a HERCREEM protocol (see, e.g., ClinicalTrials.gov Identifier NCT00889954).

In some embodiments, a PD-L1 axis binding antagonist may be administered in conjunction with an agonist directed against CD137 (also known as TNFRSF9, 4-1BB, or ILA), e.g., an activating antibody. In some embodiments, a PD-L1 axis binding antagonist may be administered in conjunction with urelumab (also known as BMS-663513). In some embodiments, a PD-L1 axis binding antagonist may be administered in conjunction with an agonist directed against CD40, e.g., an activating antibody. In some embodiments, a PD-L1 axis binding antagonist may be administered in conjunction with CP-870893. In some embodiments, a PD-L1 axis binding antagonist may be administered in conjunction with an agonist directed against OX40 (also known as CD134), e.g., an activating antibody. In some embodiments, a PD-L1 axis binding antagonist may be administered in conjunction with an anti-OX40 antibody (e.g., AgonOX). In some embodiments, a PD-L1 axis binding antagonist may be administered in conjunction

with an agonist directed against CD27, e.g., an activating antibody. In some embodiments, a PD-L1 axis binding antagonist may be administered in conjunction with CDX-1127. In some embodiments, a PD-L1 axis binding antagonist may be administered in conjunction with an antagonist directed against indoleamine-2,3-dioxygenase (IDO). In some embodiments, with the IDO antagonist is 1-methyl-D-tryptophan (also known as 1-D-MT).

In some embodiments, a PD-L1 axis binding antagonist may be administered in conjunction with an antibody-drug conjugate. In some embodiments, the antibody-drug conjugate comprises mertansine or monomethyl auristatin E (MMAE). In some embodiments, a PD-L1 axis binding antagonist may be administered in conjunction with and anti-NaPi2b antibody-MMAE conjugate (also known as DNIB0600A or RG7599). In some embodiments, a PD-L1 axis binding antagonist may be administered in conjunction with trastuzumab emtansine (also known as T-DM1, ado-trastuzumab emtansine, or KADCYLA®, Genentech). In some embodiments, a PD-L1 axis binding antagonist may be administered in conjunction with DMUC5754A. In some embodiments, a PD-L1 axis binding antagonist may be administered in conjunction with an antibody-drug conjugate targeting the endothelin B receptor (EDNBR), e.g., an antibody directed against EDNBR conjugated with MMAE.

In some embodiments, a PD-L1 axis binding antagonist may be administered in conjunction with an anti-angiogenesis agent. In some embodiments, a PD-L1 axis binding antagonist may be administered in conjunction with an antibody directed against a VEGF, *e.g.,* VEGF-A. In some embodiments, a PD-L1 axis binding antagonist may be administered in conjunction with bevacizumab (also known as AVASTIN®, Genentech). In some embodiments, a PD-L1 axis binding antagonist may be administered in conjunction with an antibody directed against angiopoietin 2 (also known as Ang2). In some embodiments, a PD-L1 axis binding antagonist may be administered in conjunction with MEDI3617.

In some embodiments, a PD-L1 axis binding antagonist may be administered in conjunction with an antineoplastic agent. In some embodiments, a PD-L1 axis binding antagonist may be administered in conjunction with an agent targeting CSF-1R (also known as M-CSFR or CD115). In some embodiments, a PD-L1 axis binding antagonist may be administered in conjunction with anti-CSF-1R (also known as IMC-CS4). In some embodiments, a PD-L1 axis binding antagonist may be administered in conjunction with an interferon, for example interferon alpha or interferon gamma. In some embodiments, a PD-L1 axis binding antagonist may be administered in conjunction with Roferon-A (also known as recombinant Interferon alpha-2a). In some embodiments, a PD-L1 axis binding antagonist may be administered in conjunction with GM-CSF (also known as recombinant human granulocyte macrophage colony stimulating factor, rhu GM-CSF, sargramostim, or LEUKINE®). In some embodiments, a PD-L1 axis binding antagonist may be administered in conjunction with IL-2 (also known as aldesleukin or PROLEUKIN®). In some embodiments, a PD-L1 axis binding antagonist may be administered in conjunction with IL-12. In some embodiments, a PD-L1 axis binding antagonist may be administered in conjunction with an antibody targeting CD20. In some embodiments, the antibody targeting CD20 is obinutuzumab (also known as GA101 or GAZYVA®) or rituximab. In some embodiments, a PD-L1 axis binding antagonist may be administered in conjunction with an antibody targeting GITR. In some embodiments, the antibody targeting GITR is TRX518.

In some embodiments, a PD-L1 axis binding antagonist may be administered in conjunction with a cancer vaccine. In some embodiments, the cancer vaccine is a peptide cancer vaccine, which in some embodiments is a personalized peptide vaccine. In some embodiments the peptide cancer vaccine is a multivalent long peptide, a multi-peptide, a peptide cocktail, a hybrid peptide, or a peptide-pulsed dendritic cell vaccine (see, e.g., Yamada et al., *Cancer Sci.* 104:14-21, 2013). In some embodiments, a PD-L1 axis binding antagonist may be administered in conjunction with an adjuvant. In some embodiments, a PD-L1 axis binding antagonist may be administered in conjunction with a treatment comprising a TLR agonist, e.g., Poly-ICLC (also known as HILTONOL®), LPS, MPL, or CpG ODN. In some embodiments, a PD-L1 axis binding antagonist may be administered in conjunction with tumor necrosis factor (TNF) alpha. In some embodiments, a PD-L1 axis binding antagonist may be administered in conjunction with IL-1. In some embodiments, a PD-L1 axis binding antagonist may be administered in conjunction with HMGB1. In some embodiments, a PD-L1 axis binding antagonist may be administered in conjunction with an IL-10 antagonist. In some embodiments, a PD-L1 axis binding antagonist may be administered in conjunction with an IL-4 antagonist. In some embodiments, a PD-L1 axis binding antagonist may be administered in conjunction with an IL-13 antagonist. In some embodiments, a PD-L1 axis binding antagonist may be administered in conjunction with an HVEM antagonist. In some embodiments, a PD-L1 axis binding antagonist may be administered in conjunction with an ICOS agonist, e.g., by administration of ICOS-L, or an agonistic antibody directed against ICOS. In some embodiments, a PD-L1 axis binding antagonist may be administered in conjunction with a treatment targeting CX3CL1. In some embodiments, a PD-L1 axis binding antagonist may be administered in conjunction with a treatment targeting CXCL9. In some embodiments, a PD-L1 axis binding antagonist may be administered in conjunction with a treatment targeting CXCL10. In some embodiments, a PD-L1 axis binding antagonist may be administered in conjunction with a treatment targeting CCL5. In some embodiments, a PD-L1

axis binding antagonist may be administered in conjunction with an LFA-1 or ICAM1 agonist. In some embodiments, a PD-L1 axis binding antagonist may be administered in conjunction with a Selectin agonist.

In some embodiments, a PD-L1 axis binding antagonist may be administered in conjunction with a targeted therapy. In some embodiments, a PD-L1 axis binding antagonist may be administered in conjunction with an inhibitor of B-Raf. In some embodiments, a PD-L1 axis binding antagonist may be administered in conjunction with vemurafenib (also known as ZELBORAF®). In some embodiments, a PD-L1 axis binding antagonist may be administered in conjunction with dabrafenib (also known as TAFINLAR®). In some embodiments, a PD-L1 axis binding antagonist may be administered in conjunction with erlotinib (also known as TARCEVA®). In some embodiments, a PD-L1 axis binding antagonist may be administered in conjunction with an inhibitor of a MEK, such as MEK1 (also known as MAP2K1) or MEK2 (also known as MAP2K2). In some embodiments, a PD-L1 axis binding antagonist may be administered in conjunction with cobimetinib (also known as GDC-0973 or XL-518). In some embodiments, a PD-L1 axis binding antagonist may be administered in conjunction with trametinib (also known as MEKINIST®). In some embodiments, a PD-L1 axis binding antagonist may be administered in conjunction with an inhibitor of K-Ras. In some embodiments, a PD-L1 axis binding antagonist may be administered in conjunction with an inhibitor of c-Met. In some embodiments, a PD-L1 axis binding antagonist may be administered in conjunction with onartuzumab (also known as MetMAb). In some embodiments, a PD-L1 axis binding antagonist may be administered in conjunction with an inhibitor of Alk. In some embodiments, a PD-L1 axis binding antagonist may be administered in conjunction with AF802 (also known as CH5424802 or alectinib). In some embodiments, a PD-L1 axis binding antagonist may be administered in conjunction with an inhibitor of a phosphatidylinositol 3-kinase (PI3K). In some embodiments, a PD-L1 axis binding antagonist may be administered in conjunction with BKM120. In some embodiments, a PD-L1 axis binding antagonist may be administered in conjunction with idelalisib (also known as GS-1101 or CAL-101). In some embodiments, a PD-L1 axis binding antagonist may be administered in conjunction with perifosine (also known as KRX-0401). In some embodiments, a PD-L1 axis binding antagonist may be administered in conjunction with an inhibitor of an Akt. In some embodiments, a PD-L1 axis binding antagonist may be administered in conjunction with MK2206. In some embodiments, a PD-L1 axis binding antagonist may be administered in conjunction with GSK690693. In some embodiments, a PD-L1 axis binding antagonist may be administered in conjunction with GDC-0941. In some embodiments, a PD-L1 axis binding antagonist may be administered in conjunction with an inhibitor of mTOR. In some embodiments, a PD-L1 axis binding antagonist may be administered in conjunction with sirolimus (also known as rapamycin). In some embodiments, a PD-L1 axis binding antagonist may be administered in conjunction with temsirolimus (also known as CCI-779 or Torisel®). In some embodiments, a PD-L1 axis binding antagonist may be administered in conjunction with everolimus (also known as RAD001). In some embodiments, a PD-L1 axis binding antagonist may be administered in conjunction with ridaforolimus (also known as AP-23573, MK-8669, or deforolimus). In some embodiments, a PD-L1 axis binding antagonist may be administered in conjunction with OSI-027. In some embodiments, a PD-L1 axis binding antagonist may be administered in conjunction with AZD8055. In some embodiments, a PD-L1 axis binding antagonist may be administered in conjunction

with INK128. In some embodiments, a PD-L1 axis binding antagonist may be administered in conjunction with a dual PI3K/mTOR inhibitor. In some embodiments, a PD-L1 axis binding antagonist may be administered in conjunction with XL765. In some embodiments, a PD-L1 axis binding antagonist may be administered in conjunction with GDC-0980. In some embodiments, a PD-L1 axis binding antagonist may be administered in conjunction with BEZ235 (also known as NVP-BEZ235). In some embodiments, a PD-L1 axis binding antagonist may be administered in conjunction with BGT226. In some embodiments, a PD-L1 axis binding antagonist may be administered in conjunction with GSK2126458. In some embodiments, a PD-L1 axis binding antagonist may be administered in conjunction with PF-04691502. In some embodiments, a PD-L1 axis binding antagonist may be administered in conjunction with PF-05212384 (also known as PKI-587).

C. PD-L1 Axis Binding Antagonists for Use in the Methods of the Invention

Provided herein are methods for treating or delaying progression of a cancer (e.g., a non-small cell lung cancer) in a patient comprising administering to the patient a therapeutically effective amount of a PD-L1 axis binding antagonist. Provided herein are methods for determining whether a patient suffering from a cancer (e.g., a non-small cell lung cancer) is likely to respond to treatment comprising a PD-L1 axis binding antagonist. Provided herein are methods for predicting responsiveness of a patient suffering from a cancer (e.g., a non-small cell lung cancer) to treatment comprising a PD-L1 axis binding antagonist. Provided herein are methods for selecting a therapy for a patient suffering from a cancer (e.g., a non-small cell lung cancer). Any of the preceding methods may be based on the expression level of a biomarker provided herein, for example, PD-L1 expression in a tumor sample, e.g., in tumor-infiltrating immune cells and/or in tumor cells.

For example, a PD-L1 axis binding antagonist includes a PD-1 binding antagonist, a PD-L1 binding antagonist, and a PD-L2 binding antagonist. PD-1 (programmed death 1) is also referred to in the art as "programmed cell death 1," "PDCD1," "CD279," and "SLEB2." An exemplary human PD-1 is shown in UniProtKB/Swiss-Prot Accession No. Q15116. PD-L1 (programmed death ligand 1) is also referred to in the art as "programmed cell death 1 ligand 1," "PDCD1LG1," "CD274," "B7-H," and "PDL1." An exemplary human PD-L1 is shown in UniProtKB/Swiss-Prot Accession No.Q9NZQ7.1. PD-L2 (programmed death ligand 2) is also referred to in the art as "programmed cell death 1 ligand 2," "PDCD1LG2," "CD273," "B7-DC," "Btdc," and "PDL2." An exemplary human PD-L2 is shown in UniProtKB/Swiss-Prot Accession No. Q9BQ51. In some embodiments, PD-1, PD-L1, and PD-L2 are human PD-1, PD-L1 and PD-L2.

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 ligands. 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 ligand binding partners. In a specific aspect, the PD-L2 binding ligand partner is PD-1. The antagonist may be an antibody, an antigen binding fragment thereof, an immunoadhesin, a fusion protein, or oligopeptide.

In some embodiments, the PD-1 binding antagonist is an anti-PD-1 antibody (e.g., a human antibody, a humanized antibody, or a chimeric antibody), for example, as described below. In some embodiments, the anti-PD-1 antibody is selected from the group consisting of MDX-1106 (nivolumab), MK-3475 (pembrolizumab), CT-011 (pidilizumab), MEDI-0680 (AMP-514), PDR001, REGN2810, and BGB-108. MDX-1106, also known as MDX- 1106-04, ONO-4538, BMS-936558, or nivolumab, is an anti-PD-1 antibody described in WO2006/121168. MK-3475, also known as pembrolizumab or lambrolizumab, is an anti-PD-1 antibody described in WO 2009/114335. CT-011, also known as hBAT, hBAT-1 or pidilizumab, is an anti-PD-1 antibody described in WO 2009/101611. 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. AMP-224, also known as B7-DCIg, is a PD-L2-Fc fusion soluble receptor described in WO 2010/027827 and WO 2011/066342.

In some embodiments, the anti-PD-1 antibody is MDX-1106. Alternative names for "MDX-1106" include MDX-1106-04, ONO-4538, BMS-936558, and nivolumab. In some embodiments, the anti-PD-1 antibody is nivolumab (CAS Registry Number: 946414-94-4). In a still further embodiment, provided is an isolated anti-PD-1 antibody comprising a heavy chain variable region comprising the heavy chain variable region amino acid sequence from SEQ ID NO:1 and/or a light chain variable region comprising the light chain variable region amino acid sequence from SEQ ID NO:2. In a still further embodiment, provided is an isolated 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 heavy chain sequence:

QVQLVESGGGVVQPGRSLRLDCKASGITFSNSGMHWVRQAPGKGLEWVAVIWYDGSKRYYADSVKGR
FTISRDNSKNTLFLQMNSLRAEDTAVYYCATNDDYWGQGTLVTVSSASTKGPSVFPLAPCSRSTSESTAA
LGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTK
VDKRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGV
EVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLP
PSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEG
NVFSCSVMHEALHNHYTQKSLSLSLGK (SEQ ID NO:1), and

(b) the light chain sequences 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 light chain sequence:

EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYDASNRATGIPARFSGSGSGTD
FTLTISSLEPEDFAVYYCQQSSNWPRTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYP
REAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFN
RGEC (SEQ ID NO:2).

In some embodiments, the PD-L1 axis binding antagonist is a PD-L2 binding antagonist. In some embodiments, the PD-L2 binding antagonist is an anti-PD-L2 antibody (e.g., a human antibody, a

humanized antibody, or a chimeric antibody). In some embodiments, the PD-L2 binding antagonist is an immunoadhesin.

In some embodiments, the PD-L1 binding antagonist is an anti-PD-L1 antibody, for example, as described below. 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 antibody is selected from the group consisting of YW243.55.S70, MPDL3280A (atezolizumab), MDX-1105, MEDI4736 (durvalumab), and MSB0010718C (avelumab). Antibody YW243.55.S70 is an anti-PD-L1 described in WO 2010/077634. MDX-1105, also known as BMS-936559, is an anti-PD-L1 antibody described in WO2007/005874. MEDI4736 (durvalumab) is an anti-PD-L1 monoclonal antibody described in WO2011/066389 and US2013/034559. Examples of anti-PD-L1 antibodies useful for the methods of this invention, and methods for making thereof are described in PCT patent application WO 2010/077634, WO 2007/005874, WO 2011/066389, U.S. Pat. No. 8,217,149, and US 2013/034559, which are incorporated herein by reference.

Anti-PD-L1 antibodies described in WO 2010/077634 A1 and US 8,217,149 may be used in the methods described herein. In some embodiments, the anti-PD-L1 antibody comprises a heavy chain variable region sequence of SEQ ID NO:3 and/or a light chain variable region sequence of SEQ ID NO:4. In a still further embodiment, provided is an isolated anti-PD-L1 antibody comprising a heavy chain variable region and/or a light chain variable region 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 heavy chain sequence:
EVQLVESGGGLVQPGGSLRLSCAASGFTFSDSWIHWVRQAPGKGLEWVAWISPYGGSTYYADSVKGRF TISADTSKNTAYLQMNSLRAEDTAVYYCARRHWPGGFDYWGQGTLVTVSA (SEQ ID NO:3), and

(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 light chain sequence:
DIQMTQSPSSLSASVGDRVTITCRASQDVSTAVAWYQQKPGKAPKLLIYSASFLYSGVPSRFSGSGSGTD FTLTISSLQPEDFATYYCQQYLYHPATFGQGTKVEIKR (SEQ ID NO:4).

In one embodiment, the anti-PD-L1 antibody comprises a heavy chain variable region comprising an HVR-H1, HVR-H2 and HVR-H3 sequence, wherein:

(a) the HVR-H1 sequence is $GFTFSX_1SWIH$ (SEQ ID NO:5);

(b) the HVR-H2 sequence is $AWIX_2PYGGSX_3YYADSVKG$ (SEQ ID NO:6);

(c) the HVR-H3 sequence is RHWPGGFDY (SEQ ID NO:7);

further wherein: X_1 is D or G; X_2 is S or L; X_3 is T or S. In one specific aspect, X_1 is D; X_2 is S and X_3 is T. In another aspect, the polypeptide further comprises variable region heavy chain framework sequences juxtaposed between the HVRs according to the formula: (FR-H1)-(HVR-H1)-(FR-H2)-(HVR-H2)-(FR-H3)-(HVR-H3)-(FR-H4). In yet another aspect, the framework sequences are derived from

human consensus framework sequences. In a further aspect, the framework sequences are VH subgroup III consensus framework. In a still further aspect, at least one of the framework sequences is the following:

FR-H1 is EVQLVESGGGLVQPGGSLRLSCAAS (SEQ ID NO:8)

FR-H2 is WVRQAPGKGLEWV (SEQ ID NO:9)

FR-H3 is RFTISADTSKNTAYLQMNSLRAEDTAVYYCAR (SEQ ID NO:10)

FR-H4 is WGQGTLVTVSA (SEQ ID NO:11).

In a still further aspect, the heavy chain polypeptide is further combined with a variable region light chain comprising an HVR-L1, HVR-L2 and HVR-L3, wherein:

(a) the HVR-L1 sequence is $RASQX_4X_5X_6TX_7X_8A$ (SEQ ID NO:12);

(b) the HVR-L2 sequence is $SASX_9LX_{10}S$, (SEQ ID NO:13);

(c) the HVR-L3 sequence is $QQX_{11}X_{12}X_{13}X_{14}PX_{15}T$ (SEQ ID NO:14);

wherein: X_4 is D or V; X_5 is V or I; X_6 is S or N; X_7 is A or F; X_8 is V or L; X_9 is F or T; X_{10} is Y or A; X_{11} is Y, G, F, or S; X_{12} is L, Y, F or W; X_{13} is Y, N, A, T, G, F or I; X_{14} is H, V, P, T or I; X_{15} is A, W, R, P or T. In a still further aspect, X_4 is D; X_5 is V; X_6 is S; X_7 is A; X_8 is V; X_9 is F; X_{10} is Y; X_{11} is Y; X_{12} is L; X_{13} is Y; X_{14} is H; X_{15} is A.

In a still further aspect, the light chain further comprises variable region light chain framework sequences juxtaposed between the HVRs according to the formula: (FR-L1)-(HVR-L1)-(FR-L2)-(HVR-L2)-(FR-L3)-(HVR-L3)-(FR-L4). In a still further aspect, the framework sequences are derived from human consensus framework sequences. In a still further aspect, the framework sequences are VL kappa I consensus framework. In a still further aspect, at least one of the framework sequence is the following:

FR-L1 is DIQMTQSPSSLSASVGDRVTITC (SEQ ID NO:15)

FR-L2 is WYQQKPGKAPKLLIY (SEQ ID NO:16)

FR-L3 is GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC (SEQ ID NO:17)

FR-L4 is FGQGTKVEIKR (SEQ ID NO:18).

In another embodiment, provided is an isolated anti-PD-L1 antibody or antigen binding fragment comprising a heavy chain and a light chain variable region sequence, wherein:

(a) the heavy chain comprises an HVR-H1, HVR-H2 and HVR-H3, wherein further:

(i) the HVR-H1 sequence is $GFTFSX_1SWIH$; (SEQ ID NO:5)

(ii) the HVR-H2 sequence is $AWIX_2PYGGSX_3YYADSVKG$ (SEQ ID NO:6)

(iii) the HVR-H3 sequence is RHWPGGFDY, and (SEQ ID NO:7)

(b) the light chain comprises an HVR-L1, HVR-L2 and HVR-L3, wherein further:

(i) the HVR-L1 sequence is $RASQX_4X_5X_6TX_7X_8A$ (SEQ ID NO:12)

(ii) the HVR-L2 sequence is $SASX_9LX_{10}S$; and (SEQ ID NO:13)

(iii) the HVR-L3 sequence is $QQX_{11}X_{12}X_{13}X_{14}PX_{15}T$; (SEQ ID NO:14)

wherein: X_1 is D or G; X_2 is S or L; X_3 is T or S; X_4 is D or V; X_5 is V or I; X_6 is S or N; X_7 is A or F; X_8 is V or L; X_9 is F or T; X_{10} is Y or A; X_{11} is Y, G, F, or S; X_{12} is L, Y, F or W; X_{13} is Y, N, A, T, G, F or I; X_{14} is H, V, P, T or I; X_{15} is A, W, R, P or T. In a specific aspect, X_1 is D; X_2 is S and X_3 is T. In another aspect, X_4 is D; X_5 is V; X_6 is S; X_7 is A; X_8 is V; X_9 is F; X_{10} is Y; X_{11} is Y; X_{12} is L; X_{13} is Y; X_{14} is H; X_{15} is A. In yet

another aspect, X_1 is D; X_2 is S and X_3 is T, X_4 is D; X_5 is V; X_6 is S; X_7 is A; X_8 is V; X_9 is F; X_{10} is Y; X_{11} is Y; X_{12} is L; X_{13} is Y; X_{14} is H and X_{15} is A.

In a further aspect, the heavy chain variable region comprises one or more framework sequences juxtaposed between the HVRs as: (FR-H1)-(HVR-H1)-(FR-H2)-(HVR-H2)-(FR-H3)-(HVR-H3)-(FR-H4), and the light chain variable regions comprises one or more framework sequences juxtaposed between the HVRs as: (FR-L1)-(HVR-L1)-(FR-L2)-(HVR-L2)-(FR-L3)-(HVR-L3)-(FR-L4). In a still further aspect, the framework sequences are derived from human consensus framework sequences. In a still further aspect, the heavy chain framework sequences are derived from a Kabat subgroup I, II, or III sequence. In a still further aspect, the heavy chain framework sequence is a VH subgroup III consensus framework. In a still further aspect, one or more of the heavy chain framework sequences are set forth as SEQ ID NOs:8, 9, 10 and 11. In a still further aspect, the light chain framework sequences are derived from a Kabat kappa I, II, II or IV subgroup sequence. In a still further aspect, the light chain framework sequences are VL kappa I consensus framework. In a still further aspect, one or more of the light chain framework sequences are set forth as SEQ ID NOs:15, 16, 17 and 18.

In a still further specific aspect, the 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, 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 if 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 an "effector-less Fc mutation" or aglycosylation. In still a further embodiment, the effector-less Fc mutation is an N297A or D265A/N297A substitution in the constant region.

In yet another embodiment, provided is an anti-PD-L1 antibody comprising a heavy chain and a light chain variable region sequence, wherein:

(a) the heavy chain further comprises an HVR-H1, HVR-H2 and an HVR-H3 sequence having at least 85% sequence identity to GFTFSDSWIH (SEQ ID NO:19), AWISPYGGSTYYADSVKG (SEQ ID NO:20) and RHWPGGFDY (SEQ ID NO:21), respectively, or

(b) the light chain further comprises an HVR-L1, HVR-L2 and an HVR-L3 sequence having at least 85% sequence identity to RASQDVSTAVA (SEQ ID NO:22), SASFLYS (SEQ ID NO:23) and QQYLYHPAT (SEQ ID NO:24), respectively.

In a specific aspect, the sequence identity is 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100%.

In another aspect, the heavy chain variable region comprises one or more framework sequences juxtaposed between the HVRs as: (FR-H1)-(HVR-H1)-(FR-H2)-(HVR-H2)-(FR-H3)-(HVR-H3)-(FR-H4), and the light chain variable regions comprises one or more framework sequences juxtaposed between the HVRs as: (FR-L1)-(HVR-L1)-(FR-L2)-(HVR-L2)-(FR-L3)-(HVR-L3)-(FR-L4). In yet another aspect, the framework sequences are derived from human consensus framework sequences. In a still further aspect, the heavy chain framework sequences are derived from a Kabat subgroup I, II, or III sequence. In a still further aspect, the heavy chain framework sequence is a VH subgroup III consensus framework. In a still

further aspect, one or more of the heavy chain framework sequences are set forth as SEQ ID NOs:8, 9, 10 and 11. In a still further aspect, the light chain framework sequences are derived from a Kabat kappa I, II, II or IV subgroup sequence. In a still further aspect, the light chain framework sequences are VL kappa I consensus framework. In a still further aspect, one or more of the light chain framework sequences are set forth as SEQ ID NOs:15, 16, 17 and 18.

In a still further specific aspect, the 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, 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 if 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 an "effector-less Fc mutation" or aglycosylation. In still a further embodiment, the effector-less Fc mutation is an N297A or D265A/N297A substitution in the constant region.

In another further embodiment, provided is an isolated anti-PD-L1 antibody comprising a heavy chain and a light chain variable region sequence, wherein:

(a) the heavy chain sequence has at least 85% sequence identity to the heavy chain sequence:
EVQLVESGGGLVQPGGSLRLSCAASGFTFSDSWIHWVRQAPGKGLEWVAWISPYGGSTYYADSVKGRF TISADTSKNTAYLQMNSLRAEDTAVYYCARRHWPGGFDYWGQGTLVTVSS (SEQ ID NO:25), and/or

(b) the light chain sequences has at least 85% sequence identity to the light chain sequence:
DIQMTQSPSSLSASVGDRVTITCRASQDVSTAVAWYQQKPGKAPKLLIYSASFLYSGVPSRFSGSGSGTD FTLTISSLQPEDFATYYCQQYLYHPATFGQGTKVEIKR (SEQ ID NO:4).

In a specific aspect, the sequence identity is 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100%. In another aspect, the heavy chain variable region comprises one or more framework sequences juxtaposed between the HVRs as: (FR-H1)-(HVR-H1)-(FR-H2)-(HVR-H2)-(FR-H3)-(HVR-H3)-(FR-H4), and the light chain variable regions comprises one or more framework sequences juxtaposed between the HVRs as: (FR-L1)-(HVR-L1)-(FR-L2)-(HVR-L2)-(FR-L3)-(HVR-L3)-(FR-L4). In yet another aspect, the framework sequences are derived from human consensus framework sequences. In a further aspect, the heavy chain framework sequences are derived from a Kabat subgroup I, II, or III sequence. In a still further aspect, the heavy chain framework sequence is a VH subgroup III consensus framework. In a still further aspect, one or more of the heavy chain framework sequences are set forth as SEQ ID NOs:8, 9, 10 and WGQGTLVTVSS (SEQ ID NO:27).

In a still further aspect, the light chain framework sequences are derived from a Kabat kappa I, II, II or IV subgroup sequence. In a still further aspect, the light chain framework sequences are VL kappa I consensus framework. In a still further aspect, one or more of the light chain framework sequences are set forth as SEQ ID NOs:15, 16, 17 and 18.

In a still further specific aspect, the 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, 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 if 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 still a further embodiment, the effector-less Fc mutation is an N297A or D265A/N297A substitution in the constant region.

In a further aspect, the heavy chain variable region comprises one or more framework sequences juxtaposed between the HVRs as: (FR-H1)-(HVR-H1)-(FR-H2)-(HVR-H2)-(FR-H3)-(HVR-H3)-(FR-H4), and the light chain variable regions comprises one or more framework sequences juxtaposed between the HVRs as: (FR-L1)-(HVR-L1)-(FR-L2)-(HVR-L2)-(FR-L3)-(HVR-L3)-(FR-L4). In a still further aspect, the framework sequences are derived from human consensus framework sequences. In a still further aspect, the heavy chain framework sequences are derived from a Kabat subgroup I, II, or III sequence. In a still further aspect, the heavy chain framework sequence is a VH subgroup III consensus framework. In a still further aspect, one or more of the heavy chain framework sequences is the following:

FR-H1	EVQLVESGGGLVQPGGSLRLSCAASGFTFS	(SEQ ID NO:29)
FR-H2	WVRQAPGKGLEWVA	(SEQ ID NO:30)
FR-H3	RFTISADTSKNTAYLQMNSLRAEDTAVYYCAR	(SEQ ID NO:10)
FR-H4	WGQGTLVTVSS	(SEQ ID NO:27).

In a still further aspect, the light chain framework sequences are derived from a Kabat kappa I, II, II or IV subgroup sequence. In a still further aspect, the light chain framework sequences are VL kappa I consensus framework. In a still further aspect, one or more of the light chain framework sequences is the following:

FR-L1	DIQMTQSPSSLSASVGDRVTITC	(SEQ ID NO:15)
FR-L2	WYQQKPGKAPKLLIY	(SEQ ID NO:16)
FR-L3	GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC	(SEQ ID NO:17)
FR-L4	FGQGTKVEIK	(SEQ ID NO:28).

In a still further specific aspect, the 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, 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 if 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 an "effector-less Fc mutation" or aglycosylation. In still a further embodiment, the effector-less Fc mutation is an N297A or D265A/N297A substitution in the constant region.

In yet another embodiment, provided is an anti-PD-L1 antibody comprising a heavy chain and a light chain variable region sequence, wherein:

(c) the heavy chain further comprises an HVR-H1, HVR-H2 and an HVR-H3 sequence having at least 85% sequence identity to GFTFSDSWIH (SEQ ID NO:19), AWISPYGGSTYYADSVKG (SEQ ID NO:20) and RHWPGGFDY (SEQ ID NO:21), respectively, and/or

(d) the light chain further comprises an HVR-L1, HVR-L2 and an HVR-L3 sequence having at least 85% sequence identity to RASQDVSTAVA (SEQ ID NO:22), SASFLYS (SEQ ID NO:23) and QQYLYHPAT (SEQ ID NO:24), respectively.

In a specific aspect, the sequence identity is 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100%.

In another aspect, the heavy chain variable region comprises one or more framework sequences juxtaposed between the HVRs as: (FR-H1)-(HVR-H1)-(FR-H2)-(HVR-H2)-(FR-H3)-(HVR-H3)-(FR-H4), and the light chain variable regions comprises one or more framework sequences juxtaposed between the HVRs as: (FR-L1)-(HVR-L1)-(FR-L2)-(HVR-L2)-(FR-L3)-(HVR-L3)-(FR-L4). In yet another aspect, the framework sequences are derived from human consensus framework sequences. In a still further aspect, the heavy chain framework sequences are derived from a Kabat subgroup I, II, or III sequence. In a still further aspect, the heavy chain framework sequence is a VH subgroup III consensus framework. In a still further aspect, one or more of the heavy chain framework sequences are set forth as SEQ ID NOs:8, 9, 10 and WGQGTLVTVSSASTK (SEQ ID NO:31).

In a still further aspect, the light chain framework sequences are derived from a Kabat kappa I, II, II or IV subgroup sequence. In a still further aspect, the light chain framework sequences are VL kappa I consensus framework. In a still further aspect, one or more of the light chain framework sequences are set forth as SEQ ID NOs:15, 16, 17 and 18. In a still further specific aspect, the 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, 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 if 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 an "effector-less Fc mutation" or aglycosylation. In still a further embodiment, the effector-less Fc mutation is an N297A or D265A/N297A substitution in the constant region.

In a still further embodiment, provided is an isolated anti-PD-L1 antibody comprising a heavy chain and a light chain variable region sequence, wherein:

(a) the heavy chain sequence has at least 85% sequence identity to the heavy chain sequence:
EVQLVESGGGLVQPGGSLRLSCAASGFTFSDSWIHWVRQAPGKGLEWVAWISPYGGSTYYADSVKGRF
TISADTSKNTAYLQMNSLRAEDTAVYYCARRHWPGGFDYWGQGTLVTVSSASTK (SEQ ID NO:26), or

(b) the light chain sequences has at least 85% sequence identity to the light chain sequence:
DIQMTQSPSSLSASVGDRVTITCRASQDVSTAVAWYQQKPGKAPKLLIYSASFLYSGVPSRFSGSGSGTD
FTLTISSLQPEDFATYYCQQYLYHPATFGQGTKVEIKR (SEQ ID NO:4).

In some embodiments, provided is an isolated anti-PD-L1 antibody comprising a heavy chain and a light chain variable region sequence, wherein the light chain variable region sequence has at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, 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 amino acid sequence of SEQ ID NO:4. In some embodiments, provided is an

isolated anti-PD-L1 antibody comprising a heavy chain and a light chain variable region sequence, wherein the heavy chain variable region sequence has at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, 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 amino acid sequence of SEQ ID NO:26. In some embodiments, provided is an isolated anti-PD-L1 antibody comprising a heavy chain and a light chain variable region sequence, wherein the light chain variable region sequence has at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, 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 amino acid sequence of SEQ ID NO:4 and the heavy chain variable region sequence has at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, 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 amino acid sequence of SEQ ID NO:26. In some embodiments, one, two, three, four or five amino acid residues at the N-terminal of the heavy and/or light chain may be deleted, substituted or modified.

In a still further embodiment, provided is an isolated anti-PD-L1 antibody comprising a heavy chain and a light chain sequence, wherein:

(a) the heavy chain sequence has at least 85% sequence identity to the heavy chain sequence:

EVQLVESGGGLVQPGGSLRLSCAASGFTFSDSWIHWVRQAPGKGLEWVAWISPYGGSTYYADSVKGRF
TISADTSKNTAYLQMNSLRAEDTAVYYCARRHWPGGFDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTS
GGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKP
SNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
WYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE
PQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKS
RWQQGNVFSCSVMHEALHNHYTQKSLSLSPG (SEQ ID NO:32), and/or

(b) the light chain sequences has at least 85% sequence identity to the light chain sequence:

DIQMTQSPSSLSASVGDRVTITCRASQDVSTAVAWYQQKPGKAPKLLIYSASFLYSGVPSRFSGSGSGTD
FTLTISSLQPEDFATYYCQQYLYHPATFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYP
REAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFN
RGEC (SEQ ID NO:33).

In some embodiments, provided is an isolated anti-PD-L1 antibody comprising a heavy chain and a light chain sequence, wherein the light chain sequence has at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, 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%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO:33. In some embodiments, provided is an isolated anti-PD-L1 antibody comprising a heavy chain and a light chain sequence, wherein the heavy chain sequence has at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, 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%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO:32. In some embodiments, provided is an isolated anti-PD-L1 antibody comprising a heavy chain and a light chain sequence, wherein the light chain

sequence has at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, 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%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO:33 and the heavy chain sequence has at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, 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%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO:32.

In some embodiments, the isolated anti-PD-L1 antibody is aglycosylated. Glycosylation of antibodies is typically either N-linked or O-linked. N-linked refers to the attachment of the carbohydrate moiety to the side chain of an asparagine residue. The tripeptide sequences asparagine-X-serine and asparagine-X-threonine, where X is any amino acid except proline, are the recognition sequences for enzymatic attachment of the carbohydrate moiety to the asparagine side chain. Thus, the presence of either of these tripeptide sequences in a polypeptide creates a potential glycosylation site. O-linked glycosylation refers to the attachment of one of the sugars N-aceylgalactosamine, galactose, or xylose to a hydroxyamino acid, most commonly serine or threonine, although 5-hydroxyproline or 5-hydroxylysine may also be used. Removal of glycosylation sites form an antibody is conveniently accomplished by altering the amino acid sequence such that one of the above-described tripeptide sequences (for N-linked glycosylation sites) is removed. The alteration may be made by substitution of an asparagine, serine or threonine residue within the glycosylation site another amino acid residue (e.g., glycine, alanine or a conservative substitution).

In any of the embodiments herein, the isolated anti-PD-L1 antibody can bind to a human PD-L1, for example a human PD-L1 as shown in UniProtKB/Swiss-Prot Accession No.Q9NZQ7.1, or a variant thereof.

In a still further embodiment, provided is an isolated nucleic acid 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 antibodies. In a still further specific aspect, the vector is in 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) cell.

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 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.

It is expressly contemplated that such PD-L1 axis binding antagonist antibodies (e.g., anti-PD-L1 antibodies, anti-PD-1 antibodies, and anti-PD-L2 antibodies), or other antibodies described herein (e.g., anti-PD-L1 antibodies for detection of PD-L1 expression levels) for use in any of the embodiments enumerated above may have any of the features, singly or in combination, described in Sections 1-7 below.

1. Antibody Affinity

In certain embodiments, an antibody provided herein (e.g., an anti-PD-L1 antibody or an anti-PD-1 antibody) has a dissociation constant (Kd) of ≤ 1μM, ≤ 100 nM, ≤ 10 nM, ≤ 1 nM, ≤ 0.1 nM, ≤ 0.01 nM, or ≤ 0.001 nM (e.g., 10^{-8} M or less, e.g., from 10^{-8} M to 10^{-13} M, e.g., from 10^{-9} M to 10^{-13} M).

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

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

in a spectrometer, such as a stop-flow equipped spectrophometer (Aviv Instruments) or a 8000-series SLM-AMINCO ™ spectrophotometer (ThermoSpectronic) with a stirred cuvette.

2. Antibody Fragments

In certain embodiments, an antibody (e.g., an anti-PD-L1 antibody or an anti-PD-1 antibody) provided herein is an antibody fragment. Antibody fragments include, but are not limited to, Fab, Fab', Fab'-SH, $F(ab')_2$, Fv, and scFv fragments, and other fragments described below. For a review of certain antibody fragments, see Hudson et al. *Nat. Med.* 9:129-134 (2003). For a review of scFv fragments, see, e.g., Pluckthün, in *The Pharmacology of Monoclonal Antibodies*, vol. 113, Rosenburg and Moore eds., (Springer-Verlag, New York), pp. 269-315 (1994); see also WO 93/16185; and U.S. Patent Nos. 5,571,894 and 5,587,458. For discussion of Fab and $F(ab')_2$ fragments comprising salvage receptor binding epitope residues and having increased *in vivo* half-life, see U.S. Patent No. 5,869,046.

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

Single-domain antibodies are antibody fragments comprising all or a portion of the heavy chain variable domain or all or a portion of the light chain variable domain of an antibody. In certain embodiments, a single-domain antibody is a human single-domain antibody (Domantis, Inc., Waltham, MA; see, e.g., U.S. Patent No. 6,248,516 B1).

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

3. Chimeric and Humanized Antibodies

In certain embodiments, an antibody (e.g., an anti-PD-L1 antibody or an anti-PD-1 antibody) provided herein is a chimeric antibody. Certain chimeric antibodies are described, e.g., in U.S. Patent No. 4,816,567; and Morrison et al. *Proc. Natl. Acad. Sci. USA*, 81:6851-6855 (1984)). In one example, a chimeric antibody comprises a non-human variable region (e.g., a variable region derived from a mouse, rat, hamster, rabbit, or non-human primate, such as a monkey) and a human constant region. In a further example, a chimeric antibody is a "class switched" antibody in which the class or subclass has been changed from that of the parent antibody. Chimeric antibodies include antigen-binding fragments thereof.

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

antibody (e.g., the antibody from which the HVR residues are derived), e.g., to restore or improve antibody specificity or affinity.

Humanized antibodies and methods of making them are reviewed, e.g., in Almagro and Fransson, *Front. Biosci.* 13:1619-1633 (2008), and are further described, e.g., in Riechmann et al., *Nature* 332:323-329 (1988); Queen et al., *Proc. Natl. Acad. Sci. USA* 86:10029-10033 (1989); US Patent Nos. 5, 821,337, 7,527,791, 6,982,321, and 7,087,409; Kashmiri *et al.*, *Methods* 36:25-34 (2005) (describing specificity determining region (SDR) grafting); Padlan, *Mol. Immunol.* 28:489-498 (1991) (describing "resurfacing"); Dall'Acqua et al., *Methods* 36:43-60 (2005) (describing "FR shuffling"); and Osbourn et al., *Methods* 36:61-68 (2005) and Klimka et al., *Br. J. Cancer*, 83:252-260 (2000) (describing the "guided selection" approach to FR shuffling).

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

4. Human Antibodies

In certain embodiments, an antibody (e.g., an anti-PD-L1 antibody or an anti-PD-1 antibody) provided herein is a human antibody. Human antibodies can be produced using various techniques known in the art. Human antibodies are described generally in van Dijk and van de Winkel, *Curr. Opin. Pharmacol.* 5: 368-74 (2001) and Lonberg, *Curr. Opin. Immunol.* 20:450-459 (2008).

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

Human antibodies can also be made by hybridoma-based methods. Human myeloma and mouse-human heteromyeloma cell lines for the production of human monoclonal antibodies have been described. (See, e.g., Kozbor *J. Immunol.*, 133: 3001 (1984); Brodeur et al., *Monoclonal Antibody Production Techniques and Applications*, pp. 51-63 (Marcel Dekker, Inc., New York, 1987); and Boerner

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

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

5. Library-Derived Antibodies

Antibodies of the invention (e.g., anti-PD-L1 antibodies and anti-PD-1 antibodies) may be isolated by screening combinatorial libraries for antibodies with the desired activity or activities. For example, a variety of methods are known in the art for generating phage display libraries and screening such libraries for antibodies possessing the desired binding characteristics. Such methods are reviewed, e.g., in Hoogenboom et al. in *Methods in Molecular Biology* 178:1-37 (O'Brien et al., ed., Human Press, Totowa, NJ, 2001) and further described, e.g., in the McCafferty et al., *Nature* 348:552-554; Clackson et al., *Nature* 352: 624-628 (1991); Marks et al., *J. Mol. Biol.* 222: 581-597 (1992); Marks and Bradbury, in *Methods in Molecular Biology* 248:161-175 (Lo, ed., Human Press, Totowa, NJ, 2003); Sidhu et al., *J. Mol. Biol.* 338(2): 299-310 (2004); Lee et al., *J. Mol. Biol.* 340(5): 1073-1093 (2004); Fellouse, *Proc. Natl. Acad. Sci. USA* 101(34): 12467-12472 (2004); and Lee et al., *J. Immunol. Methods* 284(1-2): 119-132(2004).

In certain phage display methods, repertoires of VH and VL genes are separately cloned by polymerase chain reaction (PCR) and recombined randomly in phage libraries, which can then be screened for antigen-binding phage as described in Winter et al., *Ann. Rev. Immunol.*, 12: 433-455 (1994). Phage typically display antibody fragments, either as single-chain Fv (scFv) fragments or as Fab fragments. Libraries from immunized sources provide high-affinity antibodies to the immunogen without the requirement of constructing hybridomas. Alternatively, the naive repertoire can be cloned (e.g., from human) to provide a single source of antibodies to a wide range of non-self and also self antigens without any immunization as described by Griffiths et al., *EMBO J,* 12: 725-734 (1993). Finally, naive libraries can also be made synthetically by cloning unrearranged V-gene segments from stem cells, and using PCR primers containing random sequence to encode the highly variable CDR3 regions and to accomplish rearrangement *in vitro*, as described by Hoogenboom and Winter, *J. Mol. Biol.*, 227: 381-388 (1992). Patent publications describing human antibody phage libraries include, for example: US Patent No. 5,750,373, and US Patent Publication Nos. 2005/0079574, 2005/0119455, 2005/0266000, 2007/0117126, 2007/0160598, 2007/0237764, 2007/0292936, and 2009/0002360.

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

6. Multispecific Antibodies

In any one of the above aspects, an antibody (e.g., an anti-PD-L1 antibody or an anti-PD-1 antibody) provided herein may be a multispecific antibody, for example, a bispecific antibody. Multispecific antibodies are monoclonal antibodies that have binding specificities for at least two different sites. In certain embodiments, an antibody provided herein is a multispecific antibody, e.g., a bispecific antibody. In certain embodiments, one of the binding specificities is for PD-L1 and the other is for any other antigen. In certain embodiments, bispecific antibodies may bind to two different epitopes of PD-L1. Bispecific antibodies may also be used to localize cytotoxic agents to cells which express PD-L1. Bispecific antibodies can be prepared as full length antibodies or antibody fragments.

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

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

The antibody or fragment herein also includes a "Dual Acting FAb" or "DAF" comprising an antigen binding site that binds to PD-L1 as well as another, different antigen.

7. Antibody Variants

In certain embodiments, amino acid sequence variants of the antibodies of the invention (e.g., anti-PD-L1 antibodies and anti-PD-1 antibodies) are contemplated. For example, it may be desirable to improve the binding affinity and/or other biological properties of the antibody. Amino acid sequence variants of an antibody may be prepared by introducing appropriate modifications into the nucleotide sequence encoding the antibody, or by peptide synthesis. Such modifications include, for example, deletions from, and/or insertions into and/or substitutions of residues within the amino acid sequences of the antibody. Any combination of deletion, insertion, and substitution can be made to arrive at the final construct, provided that the final construct possesses the desired characteristics, for example, antigen-binding.

I. Substitution, Insertion, and Deletion Variants

In certain embodiments, antibody variants having one or more amino acid substitutions are provided. Sites of interest for substitutional mutagenesis include the HVRs and FRs. Conservative

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

Table 1. Exemplary and Preferred Amino Acid Substitutions

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

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

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

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

(3) acidic: Asp, Glu;

(4) basic: His, Lys, Arg;

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

(6) aromatic: Trp, Tyr, Phe.

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

One type of substitutional variant involves substituting one or more hypervariable region residues of a parent antibody (e.g., a humanized or human antibody). Generally, the resulting variant(s) selected for further study will have modifications (e.g., improvements) in certain biological properties (e.g., increased affinity and/or reduced immunogenicity) relative to the parent antibody and/or will have substantially retained certain biological properties of the parent antibody. An exemplary substitutional variant is an affinity matured antibody, which may be conveniently generated, for example, using phage display-based affinity maturation techniques such as those described herein. Briefly, one or more HVR residues are mutated and the variant antibodies displayed on phage and screened for a particular biological activity (e.g., binding affinity).

Alterations (e.g., substitutions) may be made in HVRs, e.g., to improve antibody affinity. Such alterations may be made in HVR "hotspots," i.e., residues encoded by codons that undergo mutation at high frequency during the somatic maturation process (see, e.g., Chowdhury, *Methods Mol. Biol.* 207:179-196 (2008)), and/or residues that contact antigen, with the resulting variant VH or VL being tested for binding affinity. Affinity maturation by constructing and reselecting from secondary libraries has been described, e.g., in Hoogenboom et al. in *Methods in Molecular Biology* 178:1-37 (O'Brien et al., ed., Human Press, Totowa, NJ, (2001)). In some embodiments of affinity maturation, diversity is introduced into the variable genes chosen for maturation by any of a variety of methods (e.g., error-prone PCR, chain shuffling, or oligonucleotide-directed mutagenesis). A secondary library is then created. The library is then screened to identify any antibody variants with the desired affinity. Another method to introduce diversity involves HVR-directed approaches, in which several HVR residues (e.g., 4-6 residues at a time) are randomized. HVR residues involved in antigen binding may be specifically identified, e.g., using alanine scanning mutagenesis or modeling. CDR-H3 and CDR-L3 in particular are often targeted.

In certain embodiments, substitutions, insertions, or deletions may occur within one or more HVRs so long as such alterations do not substantially reduce the ability of the antibody to bind antigen. For example, conservative alterations (e.g., conservative substitutions as provided herein) that do not substantially reduce binding affinity may be made in HVRs. Such alterations may, for example, be outside of antigen-contacting residues in the HVRs. In certain embodiments of the variant VH and VL sequences provided above, each HVR either is unaltered, or contains no more than one, two or three amino acid substitutions.

A useful method for identification of residues or regions of an antibody that may be targeted for mutagenesis is called "alanine scanning mutagenesis" as described by Cunningham and Wells (1989) *Science*, 244:1081-1085. In this method, a residue or group of target residues (e.g., charged residues such as Arg, Asp, His, Lys, and Glu) are identified and replaced by a neutral or negatively charged amino acid (e.g., alanine or polyalanine) to determine whether the interaction of the antibody with antigen is affected. Further substitutions may be introduced at the amino acid locations demonstrating functional sensitivity to the initial substitutions. Alternatively, or additionally, a crystal structure of an antigen-antibody complex to identify contact points between the antibody and antigen. Such contact residues and

neighboring residues may be targeted or eliminated as candidates for substitution. Variants may be screened to determine whether they contain the desired properties.

Amino acid sequence insertions include amino- and/or carboxyl-terminal fusions ranging in length from one residue to polypeptides containing a hundred or more residues, as well as intrasequence insertions of single or multiple amino acid residues. Examples of terminal insertions include an antibody with an N-terminal methionyl residue. Other insertional variants of the antibody molecule include the fusion to the N- or C-terminus of the antibody to an enzyme (e.g., for ADEPT) or a polypeptide which increases the serum half-life of the antibody.

II. Glycosylation variants

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

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

In one embodiment, antibody variants are provided having a carbohydrate structure that lacks fucose attached (directly or indirectly) to an Fc region. For example, the amount of fucose in such antibody may be from 1% to 80%, from 1% to 65%, from 5% to 65% or from 20% to 40%. The amount of fucose is determined by calculating the average amount of fucose within the sugar chain at Asn297, relative to the sum of all glycostructures attached to Asn 297 (e. g. complex, hybrid and high mannose structures) as measured by MALDI-TOF mass spectrometry, as described in WO 2008/077546, for example. Asn297 refers to the asparagine residue located at about position 297 in the Fc region (EU numbering of Fc region residues); however, Asn297 may also be located about ± 3 amino acids upstream or downstream of position 297, i.e., between positions 294 and 300, due to minor sequence variations in antibodies. Such fucosylation variants may have improved ADCC function. See, for example, U.S. Patent Publication Nos. US 2003/0157108; US 2004/0093621. Examples of publications related to "defucosylated" or "fucose-deficient" antibody variants include: US 2003/0157108; WO 2000/61739; WO 2001/29246; US 2003/0115614; US 2002/0164328; US 2004/0093621; US 2004/0132140; US 2004/0110704; US 2004/0110282; US 2004/0109865; WO 2003/085119; WO 2003/084570; WO 2005/035586; WO 2005/035778; WO2005/053742; WO2002/031140; Okazaki et al. *J. Mol. Biol.* 336:1239-1249 (2004); Yamane-Ohnuki et al. *Biotech. Bioeng.* 87: 614 (2004). Examples of cell lines capable of producing defucosylated antibodies include Lec13 CHO cells deficient in protein fucosylation (Ripka et al. *Arch. Biochem. Biophys.* 249:533-545 (1986); U.S. Pat. Appl. No. US 2003/0157108 A1; and

WO 2004/056312 A1, Adams et al., especially at Example 11), and knockout cell lines, such as alpha-1,6-fucosyltransferase gene, *FUT8,* knockout CHO cells (see, e.g., Yamane-Ohnuki et al. *Biotech. Bioeng.* 87: 614 (2004); Kanda, Y. et al., *Biotechnol. Bioeng.*, 94(4):680-688 (2006); and WO2003/085107).

Antibody variants are further provided with bisected oligosaccharides, for example, in which a biantennary oligosaccharide attached to the Fc region of the antibody is bisected by GlcNAc. Such antibody variants may have reduced fucosylation and/or improved ADCC function. Examples of such antibody variants are described, e.g., in WO 2003/011878; US Patent No. 6,602,684; and US 2005/0123546. Antibody variants with at least one galactose residue in the oligosaccharide attached to the Fc region are also provided. Such antibody variants may have improved CDC function. Such antibody variants are described, e.g., in WO 1997/30087; WO 1998/58964; and WO 1999/22764.

III. Fc region variants

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

In certain embodiments, the invention contemplates an antibody variant that possesses some but not all effector functions, which make it a desirable candidate for applications in which the half life of the antibody *in vivo* is important yet certain effector functions (such as complement and ADCC) are unnecessary or deleterious. *In vitro* and/or *in vivo* cytotoxicity assays can be conducted to confirm the reduction/depletion of CDC and/or ADCC activities. For example, Fc receptor (FcR) binding assays can be conducted to ensure that the antibody lacks FcγR binding (hence likely lacking ADCC activity), but retains FcRn binding ability. The primary cells for mediating ADCC, NK cells, express FcγRIII only, whereas monocytes express FcγRI, FcγRII and FcγRIII. FcR expression on hematopoietic cells is summarized in Table 3 on page 464 of Ravetch and Kinet, *Annu. Rev. Immunol.* 9:457-492 (1991). Non-limiting examples of *in vitro* assays to assess ADCC activity of a molecule of interest is described in U.S. Patent No. 5,500,362 (see, e.g., Hellstrom, I. et al. *Proc. Natl. Acad. Sci. USA* 83:7059-7063 (1986)) and Hellstrom, I et al., *Proc. Natl. Acad. Sci. USA* 82:1499-1502 (1985); 5,821,337 (see Bruggemann, M. et al., *J. Exp. Med.* 166:1351-1361 (1987)). Alternatively, non-radioactive assays methods may be employed (see, for example, ACTI™ non-radioactive cytotoxicity assay for flow cytometry (CellTechnology, Inc. Mountain View, CA; and CYTOTOX 96® non-radioactive cytotoxicity assay (Promega, Madison, WI). Useful effector cells for such assays include peripheral blood mononuclear cells (PBMC) and Natural Killer (NK) cells. Alternatively, or additionally, ADCC activity of the molecule of interest may be assessed *in vivo*, e.g., in a animal model such as that disclosed in Clynes et al. *Proc. Natl. Acad. Sci. USA* 95:652-656 (1998). C1q binding assays may also be carried out to confirm that the antibody is unable to bind C1q and hence lacks CDC activity. See, e.g., C1q and C3c binding ELISA in WO 2006/029879 and WO 2005/100402. To assess complement activation, a CDC assay may be performed (see, e.g., Gazzano-Santoro et al., *J. Immunol. Methods* 202:163 (1996); Cragg et al., *Blood.* 101:1045-1052 (2003); and Cragg et al., *Blood.* 103:2738-2743 (2004)). FcRn binding and *in vivo* clearance/half life determinations can also be performed using methods known in the art (see, e.g., Petkova et al. *Int'l. Immunol.* 18(12):1759-1769 (2006)).

Antibodies with reduced effector function include those with substitution of one or more of Fc region residues 238, 265, 269, 270, 297, 327 and 329 (U.S. Patent Nos. 6,737,056 and 8,219,149). Such Fc mutants include Fc mutants with substitutions at two or more of amino acid positions 265, 269, 270, 297 and 327, including the so-called "DANA" Fc mutant with substitution of residues 265 and 297 to alanine (US Patent No. 7,332,581 and 8,219,149).

Certain antibody variants with improved or diminished binding to FcRs are described. (See, e.g., U.S. Patent No. 6,737,056; WO 2004/056312, and Shields et al., *J. Biol. Chem.* 9(2): 6591-6604 (2001).)

In certain embodiments, an antibody variant comprises an Fc region with one or more amino acid substitutions which improve ADCC, e.g., substitutions at positions 298, 333, and/or 334 of the Fc region (EU numbering of residues).

In some embodiments, alterations are made in the Fc region that result in altered (i.e., either improved or diminished) C1q binding and/or Complement Dependent Cytotoxicity (CDC), e.g., as described in US Patent No. 6,194,551, WO 99/51642, and Idusogie et al. *J. Immunol.* 164: 4178-4184 (2000).

Antibodies with increased half lives and improved binding to the neonatal Fc receptor (FcRn), which is responsible for the transfer of maternal IgGs to the fetus (Guyer et al., *J. Immunol.* 117:587 (1976) and Kim et al., *J. Immunol.* 24:249 (1994)), are described in US2005/0014934A1 (Hinton et al.). Those antibodies comprise an Fc region with one or more substitutions therein which improve binding of the Fc region to FcRn. Such Fc variants include those with substitutions at one or more of Fc region residues: 238, 256, 265, 272, 286, 303, 305, 307, 311, 312, 317, 340, 356, 360, 362, 376, 378, 380, 382, 413, 424 or 434, e.g., substitution of Fc region residue 434 (US Patent No. 7,371,826).

See also Duncan & Winter, *Nature* 322:738-40 (1988); U.S. Patent No. 5,648,260; U.S. Patent No. 5,624,821; and WO 94/29351 concerning other examples of Fc region variants.

IV. Cysteine engineered antibody variants

In certain embodiments, it may be desirable to create cysteine engineered antibodies, e.g., "thioMAbs," in which one or more residues of an antibody are substituted with cysteine residues. In particular embodiments, the substituted residues occur at accessible sites of the antibody. By substituting those residues with cysteine, reactive thiol groups are thereby positioned at accessible sites of the antibody and may be used to conjugate the antibody to other moieties, such as drug moieties or linker-drug moieties, to create an immunoconjugate, as described further herein. In certain embodiments, any one or more of the following residues may be substituted with cysteine: V205 (Kabat numbering) of the light chain; A118 (EU numbering) of the heavy chain; and S400 (EU numbering) of the heavy chain Fc region. Cysteine engineered antibodies may be generated as described, e.g., in U.S. Patent No. 7,521,541.

V. Antibody derivatives

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

be determined based on considerations including, but not limited to, the particular properties or functions of the antibody to be improved, whether the antibody derivative will be used in a therapy under defined conditions, etc.

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

VI. Immunoconjugates

The invention also provides immunoconjugates comprising an antibody herein (e.g., an anti-PD-L1 antibody or an anti-PD-1 antibody) conjugated to one or more cytotoxic agents, such as chemotherapeutic agents or drugs, growth inhibitory agents, toxins (e.g., protein toxins, enzymatically active toxins of bacterial, fungal, plant, or animal origin, or fragments thereof), or radioactive isotopes.

In one embodiment, an immunoconjugate is an antibody-drug conjugate (ADC) in which an antibody is conjugated to one or more drugs, including but not limited to a maytansinoid (see U.S. Patent Nos. 5,208,020, 5,416,064 and European Patent EP 0 425 235 B1); an auristatin such as monomethylauristatin drug moieties DE and DF (MMAE and MMAF) (see U.S. Patent Nos. 5,635,483 and 5,780,588, and 7,498,298); a dolastatin; a calicheamicin or derivative thereof (see U.S. Patent Nos. 5,712,374, 5,714,586, 5,739,116, 5,767,285, 5,770,701, 5,770,710, 5,773,001, and 5,877,296; Hinman et al., *Cancer Res.* 53:3336-3342 (1993); and Lode et al., *Cancer Res.* 58:2925-2928 (1998)); an anthracycline such as daunomycin or doxorubicin (see Kratz et al., *Current Med. Chem.* 13:477-523 (2006); Jeffrey et al., *Bioorganic & Med. Chem. Letters* 16:358-362 (2006); Torgov et al., *Bioconj. Chem.* 16:717-721 (2005); Nagy et al., *Proc. Natl. Acad. Sci. USA* 97:829-834 (2000); Dubowchik et al., *Bioorg. & Med. Chem. Letters* 12:1529-1532 (2002); King et al., *J. Med. Chem.* 45:4336-4343 (2002); and U.S. Patent No. 6,630,579); methotrexate; vindesine; a taxane such as docetaxel, paclitaxel, larotaxel, tesetaxel, and ortataxel; a trichothecene; and CC1065.

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

In another embodiment, an immunoconjugate comprises an antibody as described herein conjugated to a radioactive atom to form a radioconjugate. A variety of radioactive isotopes are available for the production of radioconjugates. Examples include At^{211}, I^{131}, I^{125}, Y^{90}, Re^{186}, Re^{188}, Sm^{153}, Bi^{212}, P^{32}, Pb^{212} and radioactive isotopes of Lu. When the radioconjugate is used for detection, it may comprise a

radioactive atom for scintigraphic studies, for example tc99m or I123, or a spin label for nuclear magnetic resonance (NMR) imaging (also known as magnetic resonance imaging, mri), such as iodine-123 again, iodine-131, indium-111, fluorine-19, carbon-13, nitrogen-15, oxygen-17, gadolinium, manganese or iron. Conjugates of an antibody and cytotoxic agent may be made using a variety of bifunctional protein coupling agents such as N-succinimidyl-3-(2-pyridyldithio) propionate (SPDP), succinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate (SMCC), iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCl), active esters (such as disuccinimidyl suberate), aldehydes (such as glutaraldehyde), bis-azido compounds (such as bis (p-azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as toluene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene). For example, a ricin immunotoxin can be prepared as described in Vitetta et al., *Science* 238:1098 (1987). Carbon-14-labeled 1-isothiocyanatobenzyl-3-methyldiethylene triaminepentaacetic acid (MX-DTPA) is an exemplary chelating agent for conjugation of radionucleotide to the antibody. See WO94/11026. The linker may be a "cleavable linker" facilitating release of a cytotoxic drug in the cell. For example, an acid-labile linker, peptidase-sensitive linker, photolabile linker, dimethyl linker or disulfide-containing linker (Chari et al., *Cancer Res.* 52:127-131 (1992); U.S. Patent No. 5,208,020) may be used.

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

V. Pharmaceutical Formulations

Therapeutic formulations of the PD-L1 axis binding antagonists used in accordance with the present invention (e.g., an anti-PD-L1 antibody (e.g., MPDL3280A)) are prepared for storage by mixing the antagonist having the desired degree of purity with optional pharmaceutically acceptable carriers, excipients, or stabilizers in the form of lyophilized formulations or aqueous solutions. For general information concerning formulations, see, e.g., Gilman et al. (eds.) *The Pharmacological Bases of Therapeutics*, 8th Ed., Pergamon Press, 1990; A. Gennaro (ed.), *Remington's Pharmaceutical Sciences,* 18th Edition, Mack Publishing Co., Pennsylvania, 1990; Avis et al. (eds.) *Pharmaceutical Dosage Forms: Parenteral Medications* Dekker, New York, 1993; Lieberman et al. (eds.) *Pharmaceutical Dosage Forms: Tablets* Dekker, New York, 1990; Lieberman et al. (eds.), *Pharmaceutical Dosage Forms: Disperse Systems* Dekker, New York, 1990; and Walters (ed.) *Dermatological and Transdermal Formulations* (Drugs and the Pharmaceutical Sciences), Vol 119, Marcel Dekker, 2002.

Acceptable carriers, excipients, or stabilizers are non-toxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (e.g., Zn-protein complexes); and/or non-ionic surfactants such as TWEEN™, PLURONICS™, or polyethylene glycol (PEG).

The formulation herein may also contain more than one active compound, preferably those with complementary activities that do not adversely affect each other. The type and effective amounts of such medicaments depend, for example, on the amount and type of antagonist present in the formulation, and clinical parameters of the subjects.

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

Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semi-permeable matrices of solid hydrophobic polymers containing the antagonist, which matrices are in the form of shaped articles, e.g., films, or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (for example, poly(2-hydroxyethyl-methacrylate), or poly(vinylalcohol)), polylactides (U.S. Pat. No. 3,773,919), copolymers of L-glutamic acid and γ ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such

as the LUPRON DEPOT™ (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D-(-)-3-hydroxybutyric acid.

The formulations to be used for *in vivo* administration must be sterile. This is readily accomplished by filtration through sterile filtration membranes.

It is to be understood that any of the above articles of manufacture may include an immunoconjugate described herein in place of or in addition to a PD-L1 axis binding antagonist.

VI. Diagnostic Kits and Articles of Manufacture

Provided herein are diagnostic kits comprising one or more reagents for determining the presence of a biomarker (e.g., PD-L1 expression levels, for instance, in tumor cells and/or tumor-infiltrating immune cells) in a sample from an individual or patient with a disease or disorder (e.g., cancer, including non-small cell lung cancer). In some instances, the presence of the biomarker in the sample indicates a higher likelihood of efficacy when the individual is treated with a PD-L1 axis binding antagonist. In some instances, the absence of the biomarker in the sample indicates a lower likelihood of efficacy when the individual with the disease is treated with the PD-L1 axis binding antagonist. Optionally, the kit may further include instructions to use the kit to select a medicament (e.g., a PD-L1 axis binding antagonist, such as an anti-PD-L1 antibody such as MPDL3280A) for treating the disease or disorder if the individual expresses the biomarker in the sample. In another instance, the instructions are to use the kit to select a medicament other than PD-L1 axis binding antagonist if the individual does not express the biomarker in the sample.

Provided herein are also articles of manufacture including, packaged together, a PD-L1 axis binding antagonist (e.g., an anti- PD-L1 antibody) in a pharmaceutically acceptable carrier and a package insert indicating that the PD-L1 axis binding antagonist (e.g., anti-PD-L1 antibody) is for treating a patient with a disease or disorder (e.g., cancer) based on expression of a biomarker. Treatment methods include any of the treatment methods disclosed herein. Further provided are the invention concerns a method for manufacturing an article of manufacture comprising combining in a package a pharmaceutical composition comprising a PD-L1 axis binding antagonist (e.g., an anti-PD-L1 antibody) and a package insert indicating that the pharmaceutical composition is for treating a patient with a disease or disorder based on expression of a biomarker (e.g., PD-L1 expression levels, for instance, in tumor cells and/or tumor-infiltrating immune cells).

The article of manufacture may include, for example, a container and a label or package insert on or associated with the container. Suitable containers include, for example, bottles, vials, syringes, and the like. The container may be formed from a variety of materials such as glass or plastic. The container holds or contains a composition comprising the cancer medicament as the active agent and may have a sterile access port (e.g., the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle).

The article of manufacture may further include a second container comprising a pharmaceutically-acceptable diluent buffer, such as bacteriostatic water for injection (BWFI), phosphate-buffered saline, Ringer's solution, and/or dextrose solution. The article of manufacture may further include other materials

desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, and syringes.

The article of manufacture of the present invention also includes information, for example in the form of a package insert, indicating that the composition is used for treating cancer based on expression level of the biomarker(s) herein. The insert or label may take any form, such as paper or on electronic media such as a magnetically recorded medium (e.g., floppy disk), a CD-ROM, a Universal Serial Bus (USB) flash drive, and the like. The label or insert may also include other information concerning the pharmaceutical compositions and dosage forms in the kit or article of manufacture.

EXAMPLES

The following examples are provided to illustrate, but not to limit the presently claimed invention.

Example 1: Immunohistochemical (IHC) analysis of PD-L1 expression in tumor samples

Immunohistochemistry (IHC): Formalin-fixed, paraffin-embedded tissue sections were deparaffinized prior to antigen retrieval, blocking and incubation with primary anti-PD-L1 antibody. Following incubation with secondary antibody and enzymatic color development, sections were counterstained and dehydrated in series of alcohols and xylenes before coverslipping.

The following protocol was used for IHC. The Ventana Benchmark XT or Benchmark Ultra system was used to perform PD-L1 IHC staining using the following reagents and materials:

Primary antibody: anti- PD-L1 Rabbit Monoclonal Primary Antibody

Specimen Type: Formalin-fixed paraffin embedded (FFPE) section of tumor samples

Epitope Recovery Conditions: Cell Conditioning, standard 1 (CC1, Ventana, cat # 950-124)

Primary Antibody Conditions: 1/100, 6.5 µg/ml for 16 minutes at 36°C

Diluent: Antibody dilution buffer (Tris-buffered saline containing carrier protein and BRIJ™-35)

Negative control: Naive Rabbit IgG at 6.5 µg/ml (Cell Signaling) or diluent alone

Detection: Optiview or ultraView Universal DAB Detection kit (Ventana), and amplification kit (if applicable) were used according to manufacturer's instructions (Ventana).

Counterstain: Ventana Hematoxylin II (cat # 790-2208)/ with Bluing reagent (Cat # 760-2037) (4 minutes and 4 minutes, respectively)

The Ventana Benchmark Protocol was as follows:

1. paraffin (Selected)
2. Deparaffinization (Selected)
3. Cell Conditioning (Selected)
4. Conditioner #1 (Selected)
5. Standard CC1 (Selected)
6. Ab Incubation Temperatures (Selected)
7. 36C Ab Inc. (Selected)
8. Titration (Selected)

9. Auto-dispense (Primary Antibody), and Incubate for (16 minutes)
10. Countstain (Selected)
11. Apply One Drop of (Hematoxylin II) (Countstain), Apply Coverslip, and Incubate for (4 minutes)
12. Post Counterstain (Selected)
13. Apply One Drop of (BLUING REAGENT) (Post Countstain), Apply Coverslip, and Incubate for (4 minutes)
14. Wash slides in soap water to remove oil
15. Rinse slides with water
16. Dehydrate slides through 95% Ethanol, 100% Ethanol to xylene (Leica autostainer program #9)
17. Cover slip.

Example 2: PD-L1 expression in tumor-infiltrating immune cells and in tumor cells are independent predictors for response to treatment with an anti-PD-L1 antibody in non-small cell lung cancer

The correlation between PD-L1 expression in various cell types present within tumors with response to treatment with PD-L1 axis binding antagonists was evaluated.

PD-L1 expression in tumor samples prior to treatment was assessed using immunohistochemistry (IHC) as described in Example 1 in several ongoing clinical trials for treatment of cancer patients with MPDL3280A that include cohorts of NSCLC patients, including a phase Ia clinical trial and two phase II clinical trials. Tumor samples were scored for PD-L1 positivity in tumor-infiltrating immune cells and in tumor cells according to both of the criteria for diagnostic assessment shown in Table 2 and Table 3, respectively. The ongoing Phase Ia trial is in patients with advanced solid tumors and hematologic malignancies to evaluate the safety and tolerability of an anti-PD-L1 antibody (MPDL3280A) administered by intravenous infusion every 3 weeks at a dose of 1200 mg. The study contains a large cohort of NSCLC patients (n= 88). Both ongoing phase II studies (referred to herein as “phase II-1” and “phase II-2”) include locally advanced and metastatic NSCLC patients who have been administered MPDL3280A at the same dosing regimen as the phase Ia clinical trial (1200 mg IV every 3 weeks).

Table 2: Tumor-infiltrating immune cell (IC) IHC diagnostic criteria

PD-L1 Diagnostic Assessment	IC Score
Absence of any discernible PD-L1 staining OR Presence of discernible PD-L1 staining of any intensity in tumor-infiltrating immune cells covering <1% of tumor area occupied by tumor cells, associated intratumoral stroma, and contiguous peri-tumoral desmoplastic stroma	IC0
Presence of discernible PD-L1 staining of any intensity in tumor-infiltrating immune cells covering ≥1% to <5% of tumor area occupied by tumor cells, associated intratumoral stroma, and contiguous peri-tumoral desmoplastic stroma	IC1
Presence of discernible PD-L1 staining of any intensity in tumor-infiltrating immune cells covering ≥5% to <10% of tumor area occupied by tumor cells, associated intratumoral stroma, and	IC2

contiguous peri-tumoral desmoplastic stroma	
Presence of discernible PD-L1 staining of any intensity in tumor-infiltrating immune cells covering ≥10% of tumor area occupied by tumor cells, associated intratumoral stroma, and contiguous peri-tumoral desmoplastic stroma	IC3

Table 3: Tumor cell (TC) IHC diagnostic criteria

PD-L1 Diagnostic Assessment	TC Score
Absence of any discernible PD-L1 staining OR Presence of discernible PD-L1 staining of any intensity in <1% of tumor cells	TC0
Presence of discernible PD-L1 staining of any intensity in ≥1% to <5% of tumor cells	TC1
Presence of discernible PD-L1 staining of any intensity in ≥5% to <50% of tumor cells	TC2
Presence of discernible PD-L1 staining of any intensity in ≥50% of tumor cells	TC3

PD-L1 was broadly expressed in many human cancers, including NSCLC, bladder cancer, renal cell carcinoma, melanoma, head and neck squamous cell carcinoma (HNSCC), gastric cancer, pancreatic cancer, triple-negative breast cancer, and prostate cancer, as determined by IHC using an anti-PD-L1 diagnostic antibody, as described in Example 1 (Figure 1A). NSCLC specimens from both pre-treatment tumor trials from across MPDL3280A trials (n=498) and a non-trial cohort (n=706) were evaluated for PD-L1 expression in tumor cells and in tumor-infiltrating immune cells using the anti-PD-L1 IHC assay, as described in Example 1. The specimens were scored as TC0-3 and IC0-3 based on increasing PD-L1 expression in tumor cells and in tumor-infiltrating immune cells, respectively. TC3 or IC3, TC2/3 or IC2/3, and TC1/2/3 or IC1/2/3 tumors represented approximately 20%, 40%, and 65% of NSCLC specimens, respectively.

Distinct subpopulations of NSCLC patients were identified on the basis of PD-L1 expression in tumor-infiltrating immune cells and tumor cells in patient tumor samples (Figures 1B-1D; Figures 2A-2B). TC3 tumors and IC3 tumors represented two distinct sub-populations; there was a less than 1% overlap of tumor samples which scored as TC3 and which scored as IC3 using the criteria described in Tables 2 and 3. TC3 or IC3 tumor samples represented a significant fraction of NSCLC tumors (see, e.g., Figures 2A-2B and Figure 11).

The expression patterns of PD-L1 in tumor-infiltrating immune cells and in tumor cells in tumor samples obtained from NSCLC patients enrolled in the clinical trials described above was correlated with the patients' response to treatment. Across each clinical trial, PD-L1 expression in tumor-infiltrating immune cells and in tumor cells independently predicted outcome to MPDL3280 treatment in NSCLC patients (Figures 3A-3B). For example, patients whose tumor samples had an IC3 PD-L1 IHC score had a 44% overall response rate (ORR) in the phase Ia clinical trial, and patients whose tumor samples had a

TC3 PD-L1 IHC score had a 50% ORR (Figure 3A). In contrast, NSCLC patients whose tumor samples were not scored as TC3 or IC3 had a 14% ORR.

Similar results were observed in both phase II clinical trials. In the phase II-1 clinical trial, mRECIST (Figure 3B) and RECIST v1.1 (Figure 3C) were used to determine efficacy results. The study included 3 cohorts of patients: cohort 1 included patients with 1L NSCLC; cohort 2 included patients with ≥2L NSCLC with no brain metastases; and cohort 3 included patients with ≥2L NSCLC with treated brain metastases. In particular, patients in cohorts 2 and 3 whose tumor samples were scored as TC3 or IC3 showed an increased ORR and 6-month progression-free survival (PFS) compared to the entire patient population following treatment with atezolizumab (MPDL3280A) (Figures 3B-3C).

In the phase II-2 clinical trial, IC and TC IHC scoring was predictive for improved overall survival, progression-free survival, and overall response rate of NSCLC patients treated with atezolizumab (MPDL3280A) (Figures 3D-3F). PD-L1 expression in tumor cells and in tumor-infiltrating immune cells was associated with improved overall survival following treatment with MPDL3280A compared to patients whose tumor samples were TC0 and IC0 (Figure 3D). Patients whose tumor samples were scored as TC3 or IC3 showed increased overall survival following MPDL3280A treatment compared to patients whose tumor samples scored as TC0 and IC0 (Figure 3D). The lower cut-off of TC2/3 or IC2/3 was also predictive of overall survival (Figure 3D).

PD-L1 expression in tumor cells and in tumor-infiltrating immune cells was associated with improved progression-free survival following treatment with MPDL3280A compared to patients whose tumor samples were TC0 and IC0 (Figure 3E). Patients whose tumor samples were scored as TC3 or IC3 showed improved progression-free survival following MPDL3280A treatment compared to patients whose tumor samples scored as TC0 and IC0 (Figure 3E).

PD-L1 expression in tumor cells and in tumor-infiltrating immune cells was also associated with an increased ORR in the phase II-2 study (Figure 3F). Patients whose tumor samples had a TC3 or IC3 PD-L1 IHC score had a 38% ORR following treatment with atezolizumab (MPDL3280A), compared to a 13% ORR following treatment with docetaxel (Figure 3F). The ORR of patients with IC3 or TC3 tumors treated with MPDL3280A was also higher than patients whose tumor samples were scored as TC0 and IC0 (Figure 3F).

Therefore, PD-L1 expression in tumor-infiltrating immune cells and PD-L1 expression in tumor cells represent independent predictive biomarkers for response to treatment with PD-L1 axis binding antagonists, including anti-PD-L1 antibodies such as MPDL3280A. PD-L1 expression in tumor-infiltrating immune cells and in tumor cells was associated with OS, PFS, and ORR in NSCLC patients treated with MPDL3280A. For example, based on these results, a patient could be selected for treatment with a PD-L1 axis binding antagonist based on PD-L1 expression in tumor-infiltrating immune cells and/or tumor cells. In particular, patients with a TC3 or an IC3 IHC classification are predicted to respond to treatment with a PD-L1 axis binding antagonist.

Example 3: Tumors characterized by PD-L1-positive tumor-infiltrating immune cells and tumors characterized by PD-L1-positive tumor cells represent biologically distinct sub-populations of NSCLC

To understand how PD-L1 expression both in tumor-infiltrating immune cells and in tumor cells were predictive of response to treatment with PD-L1 axis binding antagonists such as the anti-PD-L1 antibody MPDL3280A, the IC3 and TC3 sub-populations were studied by histopathological and gene expression analyses.

The presence of tumor-infiltrating immune cells was necessary but not sufficient to reflect PD-L1 positivity in the IC IHC diagnostic criteria (Figure 4). Immune cell infiltrate was observed in patients whose tumors were classified as TC3 based on PD-L1 IHC, although to a lower extent as compared to tumors that were classified as IC3 based on PD-L1 IHC (Figure 5A). The immune cell infiltrate present in TC3 patients, although present, was largely PD-L1-negative (Figure 5A). The TC3 sub-population could further be divided into populations that showed high levels of *CD68* mRNA expression levels or low levels of *CD68* mRNA expression levels (Figure 5B). Expression of *CD8* mRNA was increased in both TC3 and IC3 tumor samples as compared to PD-L1-negative tumor samples (Figure 5C).

NSCLC tumor samples with a TC3 IHC classification showed distinct histopathological features compared to tumor samples with an IC3 IHC classification (Figures 6A-6B). Tumor samples were analyzed using hematoxylin and eosin (H&E) staining. TC3 tumors exhibited a desmoplastic and sclerotic tumor microenvironment with low intra-epithelial and stromal tumor-infiltrating immune cells, while IC3 tumors demonstrated a high frequency of immune cell infiltrates within the tumor, stroma, and tumor/stroma interface, with minimal to no sclerotic reaction (Figures 6A-6B). When present, immune cell infiltrates were primarily located in the surrounding stroma. Consistent with the histopathology, TC3 tumors showed an increased expression level of collagen genes (*COL6A1* and *COL6A2*) compared to PD-L1-negative tumors and IC3 tumors (Figures 7 18A, and 18B). Tumors that were TC0 and IC0 (approximately 35% of NSCLC samples) showed little to no evidence of immune cell infiltration or activation, consistent with immunologic ignorance.

Gene expression analysis using a custom NanoString immune panel comprising 798 custom-annotated immune-related genes was performed to determine the expression patterns of immune signatures in NSCLC tumor samples across the IC and TC subtypes (Figure 8). NSCLC tumor samples with an IC3 IHC classification had increased mRNA expression levels of *CD8* and *CXCL9* (Figures 9A-9B). NSCLC tumor samples with an IC3 IHC classification also had increased expression of B-cell gene signatures (Figure 12A) as well as natural killer (NK) cell gene signatures (Figure 12B). The B-cell gene signatures included the genes *CD19*, *MS4A1*, and *CD79A.* The NK cell gene signatures included *KLRB1*, *KLRC1*, *KLRC2*, *KLRC3*, *KLRD1*, *KLRF1*, *KLRG1*, *KLRK1*, *NCAM1*, *PRF1*, *NCR1*, *KIR2DL2*, *KIR2DL3*, *KIR2DL4*, *KIR2DS2*, *KIR3DL1*, *FCGR3A*, *MICA*, and *MICB*, Therefore, IC3 tumor samples are characterized by an increased expression level of a set of biomarkers that includes *CD8*, *CXCL9*, B-cell genes, and NK cell genes.

NSCLC tumor samples with a TC3 IHC classification had increased expression levels of STAT1 and MEK compared to PD-L1-negative tumors and IC3 tumors (Figures 10A-10B). JAK1 expression

levels were determined to be higher in PD-L1-positive tumors as compared to PD-L1-negative tumors (Figure 10C). However, despite active STAT signaling, some tumor cell lines do not upregulate PD-L1 in response to interferon gamma (IFNγ) (Figure 10D). Therefore, TC3 NSCLC tumor samples are characterized by an increased expression level of STAT, MEK, and JAK1.

NSCLC tumor samples with a TC3 IHC score had significantly increased expression levels of PD-L1 compared to IC3 tumor samples and TC0 and IC0 tumor samples as assessed by RNA sequencing (Figure 17A). Expression of PD-L2 was consistent between TC3 and IC3 tumor sample populations as assessed by RNA sequencing (Figure 17B).

The NanoString analysis also showed that PD-L1-positive tumor samples (including TC3 and IC3) had elevated expression levels of genes in the T_{eff} gene signature, which included *CD8A, GZMA, GZMB, IFNG, EOMES, PRF1, CXCL9, CXCL10,* and *TBX21,* compared to PD-L1-negative tumor samples (Figure 13A). RNA sequencing showed that IC3 tumors in particular were characterized by high expression of T_{eff} signature markers (Figure 13D). RNA sequencing confirmed that IC3 tumors had increased expression of *IFNG, GZMB,* and *CXCL9* (Figures 13E-13F). Without being bound by theory, these results suggested that PD-L1 expression in IC is regulated by adaptive interferon gamma (IFNG)-mediated mechanism(s), reflecting pre-existing immunity. The expression level of the MDSC gene signature (which included *ITGAM*, *CD33*, *ARG1*, *NOS1*, *CD68*, *CD163*, *LAIR1*, and *IL34*) was also determined (Figure 13B). The expression level of the Th2 gene signature (which included *IL13*, *IL4*, *GATA3*, *IL5*, *CXCR4*, *CCR3*, and *CCR4*) was also determined (Figure 13C).

Based on these data, NSCLC can be classified into distinct molecular and histopathological subsets that define sensitivity to treatment with PD-L1 targeted therapy. While expression of PD-L1 in tumor-infiltrating immune cells and/or immune cells may confer sensitivity to MPDL3280A, the immunologic context and response to treatment may differ.

Example 4: Surrogate biomarkers for predicting response of NSCLC patients to PD-L1 axis binding antagonists

Circulating pharmacodynamic and predictive biomarkers for treatment with PD-L1 axis binding antagonists (e.g, anti-PD-L1 antibodies such as MPDL3280A) were evaluated.

The interferon gamma (IFNγ) associated markers interleukin-18 and ITAC showed increased expression levels following treatment of NSCLC patients with MPDL3280A (Figures 14A-14B). Therefore, these proteins represent pharmacodynamic biomarkers for response to PD-L1 axis binding antagonists.

The NanoString immune panel comprising 798 custom-annotated immune-related genes was used to assess whether expression levels of biomarkers in peripheral blood mononuclear cells (PBMCs) was associated with treatment with PD-L1 axis binding antagonists (e.g, anti-PD-L1 antibodies such as MPDL3280A). The patient population for this study included NSCLC patients, as well as urothelial bladder cancer (UBC), melanoma, breast cancer, and renal cell carcinoma (RCC) patients being treated with MPDL3280A. The association between baseline PD-L1, and PD-1 mRNA expression in PBMCs with response to MDP3280A treatment in NSCLC patients was evaluated (Figures 15A-15C). Baseline mRNA expression levels of NK cell (Figure 16A) and myeloid cell (Figure 16B) gene signatures in PBMCs were

associated with response of NSCLC patients to treatment with MPDL3280A. The NK cell gene signature included the genes *KLRB1*, *KLRC1*, *KLRC2*, *KLRC3*, *KLRD1*, *KLRF1*, *KLRG1*, *KLRK1*, *NCAM1*, *PRF1*, *NCR1*, *KIR2DL2*, *KIR2DL3*, *KIR2DL4*, *KIR2DS2*, *KIR3DL1*, *FCGR3A*, *MICA*, and *MICB*, The myeloid cell gene signatures included the genes *IL1B, IL8*, and *CCL2*. Increased expression levels of immuno-suppressive myeloid cell gene signatures were associated with progression, while increased expression levels of cytotoxic NK cells were associated with response to MPDL3280A.

Therefore, the genes in the NK cell gene signature represent biomarkers predictive of the response of NSCLC patients to treatment with a PD-L1 axis binding antagonist, with an increased expression level indicating an increased likelihood of response to treatment. The genes in the myeloid cell gene signature represent biomarkers predictive of the response of NSCLC patients to treatment with a PD-L1 axis binding antagonist, with an increased expression level indicating a decreased likelihood of response to treatment.

Other Embodiments

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

CLAIMS

What is claimed is:

1. A method of treating a patient suffering from a non-small cell lung cancer, the method comprising administering to the patient a therapeutically effective amount of a PD-L1 axis binding antagonist, wherein a tumor sample obtained from the patient has been determined to have a detectable expression level of PD-L1 in 5% or more of the tumor cells in the tumor sample.

2. The method of claim 1, wherein the tumor sample obtained from the patient has been determined to have a detectable expression level of PD-L1 in 10% or more of the tumor cells in the tumor sample.

3. The method of claim 2, wherein the tumor sample obtained from the patient has been determined to have a detectable expression level of PD-L1 in 20% or more of the tumor cells in the tumor sample.

4. The method of claim 3, wherein the tumor sample obtained from the patient has been determined to have a detectable expression level of PD-L1 in 50% or more of the tumor cells in the tumor sample.

5. The method of any one of claims 1-4, wherein the tumor sample obtained from the patient has a detectable expression level of PD-L1 in tumor-infiltrating immune cells that comprise less than 10% of the sample.

6. A method of treating a patient suffering from a non-small cell lung cancer, the method comprising administering to the patient a therapeutically effective amount of a PD-L1 axis binding antagonist, wherein a tumor sample obtained from the patient has been determined to have a detectable expression level of PD-L1 in tumor-infiltrating immune cells that comprise 5% or more of the tumor sample, and a detectable expression level of PD-L1 in less than 50% of the tumor cells in the tumor sample.

7. The method of claim 6, wherein the tumor sample obtained from the patient has been determined to have a detectable expression level of PD-L1 in tumor-infiltrating immune cells that comprise 10% or more of the tumor sample.

8. A method for determining whether a patient suffering from a non-small cell lung cancer is likely to respond to treatment comprising a PD-L1 axis binding antagonist, the method comprising:

determining the expression level of PD-L1 in tumor cells in a tumor sample obtained from the patient,

wherein a detectable expression level of PD-L1 in 5% or more of the tumor cells in the tumor sample indicates that the patient is likely to respond to treatment comprising a PD-L1 axis binding antagonist.

9. A method for determining whether a patient suffering from a non-small cell lung cancer is likely to respond to treatment comprising a PD-L1 axis binding antagonist, the method comprising:

determining the expression level of PD-L1 in tumor-infiltrating immune cells and in tumor cells in a tumor sample obtained from the patient,

wherein a detectable expression level of PD-L1 in tumor-infiltrating immune cells that comprise 5% or more of the tumor sample, and a detectable expression level of PD-L1 in less than 50% of the tumor cells in the tumor sample, indicates that the patient is likely to respond to treatment comprising a PD-L1 axis binding antagonist.

10. A method for predicting responsiveness of a patient suffering from a non-small cell lung cancer to treatment comprising a PD-L1 axis binding antagonist, the method comprising:

determining the expression level of PD-L1 in tumor cells in a tumor sample obtained from the patient,

wherein a detectable expression level of PD-L1 in 5% or more of the tumor cells in the tumor sample indicates that the patient is likely to respond to treatment comprising a PD-L1 axis binding antagonist.

11. A method for predicting responsiveness of a patient suffering from a non-small cell lung cancer to treatment comprising a PD-L1 axis binding antagonist, the method comprising:

determining the expression level of PD-L1 in tumor-infiltrating immune cells and in tumor cells in a tumor sample obtained from the patient,

wherein a detectable expression level of PD-L1 in tumor-infiltrating immune cells that comprise 5% or more of the tumor sample, and a detectable expression level of PD-L1 in less than 50% of the tumor cells in the tumor sample, indicates that the patient is likely to respond to treatment comprising a PD-L1 axis binding antagonist.

12. The method of claim 8 or 10, wherein a detectable expression level of PD-L1 in 10% or more of the tumor cells in the tumor sample indicates that the patient is likely to respond to treatment comprising a PD-L1 axis binding antagonist.

13. The method of claim 12, wherein a detectable expression level of PD-L1 in 20% or more of the tumor cells in the tumor sample indicates that the patient is likely to respond to treatment comprising a PD-L1 axis binding antagonist.

14. The method of claim 13, wherein a detectable expression level of PD-L1 in 50% or more of the tumor cells in the tumor sample indicates that the patient is likely to respond to treatment comprising a PD-L1 axis binding antagonist.

15. The method of claim 8 or 10, wherein the method further comprises determining the expression level of PD-L1 in tumor-infiltrating immune cells in the tumor sample obtained from the patient.

16. The method of claim 15, wherein the tumor sample obtained from the patient has a detectable expression level of PD-L1 in tumor-infiltrating immune cells that comprise less than 10% of the sample.

17. The method of claim 9 or 11, wherein the tumor sample obtained from the patient has been determined to have a detectable expression level of PD-L1 in tumor-infiltrating immune cells that comprise 10% or more of the tumor sample.

18. A method for selecting a therapy for a patient suffering from a non-small cell lung cancer, the method comprising:

determining the expression level of PD-L1 in tumor cells in a tumor sample obtained from the patient, and

selecting a therapy comprising a PD-L1 axis binding antagonist for the patient based on a detectable expression level of PD-L1 in 5% or more of the tumor cells in the tumor sample.

19. The method of claim 18, wherein the method comprises selecting a therapy comprising a PD-L1 axis binding antagonist for the patient based on a detectable expression level of PD-L1 in 10% or more of the tumor cells in the tumor sample.

20. The method of claim 19, wherein the method comprises selecting a therapy comprising a PD-L1 axis binding antagonist for the patient based on a detectable expression level of PD-L1 in 20% or more of the tumor cells in the tumor sample.

21. The method of claim 20, wherein the method comprises selecting a therapy comprising a PD-L1 axis binding antagonist for the patient based on a detectable expression level of PD-L1 in 50% or more of the tumor cells in the tumor sample.

22. The method of any one of claims 18-21, wherein the method further comprises determining the expression level of PD-L1 in tumor-infiltrating immune cells in the tumor sample obtained from the patient.

23. The method of claim 22, wherein the tumor sample obtained from the patient has a detectable expression level of PD-L1 in tumor-infiltrating immune cells that comprise less than 10% of the sample.

24. A method for selecting a therapy for a patient suffering from a non-small cell lung cancer, the method comprising:

determining the expression level of PD-L1 in tumor-infiltrating immune cells and in tumor cells in a tumor sample obtained from the patient, and

selecting a therapy comprising a PD-L1 axis binding antagonist for the patient based on a detectable expression level of PD-L1 in tumor-infiltrating immune cells that comprise 5% or more of the tumor sample, and a detectable expression level of PD-L1 in less than 50% of the tumor cells in the tumor sample.

25. The method of claim 24, wherein the expression level of PD-L1 in tumor-infiltrating immune cells is determined to be detectable in tumor-infiltrating cells that comprise at least 10% of the tumor sample.

26. The method of any one of claims 6, 7, 9, 11, 17, 24, and 25, wherein the tumor sample obtained from the patient comprises an increased number of intra-epithelial and/or stromal immune cells relative to a reference tumor sample.

27. The method of any one of claims 6, 7, 9, 11, 17, and 24-26, wherein the tumor sample obtained from the patient comprises an increased number of CD8+ T-cells relative to a reference tumor sample.

28. The method of any one of claims 6, 7, 9, 11, 17, and 24-27, wherein the tumor sample obtained from the patient has an increased expression level of one or more B-cell-related genes or natural killer (NK) cell-related genes relative to a reference tumor sample.

29. The method of claim 28, wherein the one or more B-cell-related genes is selected from the group consisting of *CD19*, *MS4A1*, and *CD79A*.

30. The method of claim 28, wherein the one or more NK cell-related genes is selected from the group consisting of *KLRB1*, *KLRC1*, *KLRC2*, *KLRC3*, *KLRD1*, *KLRF1*, *KLRG1*, *KLRK1*, *NCAM1*, *PRF1*, *NCR1*, *KIR2DL2*, *KIR2DL3*, *KIR2DL4*, *KIR2DS2*, *KIR3DL1*, *FCGR3A*, *MICA*, and *MICB*.

31. The method of any one of claims 1-5, 8, 10, 12-16, and 18-23, wherein the tumor sample obtained from the patient comprises a population of fibroblasts and/or myofibroblasts.

32. The method of any one of claims 1-5, 8, 10, 12-16, 18-23, and 31, wherein the tumor sample obtained from the patient comprises a cell-poor and/or collagenized stroma.

33. The method of any one of claims 1-5, 8, 10, 12-16, 18-23, 31, and 32, wherein the tumor sample has an increased expression level of collagen, STAT1, or MEK relative to a reference tumor sample.

34. The method of any one of claims 8-33, further comprising administering to the patient a therapeutically effective amount of a PD-L1 axis binding antagonist based on the expression level of PD-L1 in tumor cells or in tumor-infiltrating immune cells in the tumor sample.

35. The method of any one of claims 1-34, wherein the PD-L1 axis binding antagonist is selected from the group consisting of a PD-L1 binding antagonist, a PD-1 binding antagonist, and a PD-L2 binding antagonist.

36. The method of claim 35, wherein the PD-L1 axis binding antagonist is a PD-L1 binding antagonist.

37. The method of claim 36, wherein the PD-L1 binding antagonist inhibits the binding of PD-L1 to one or more of its ligand binding partners.

38. The method of claim 37, wherein the PD-L1 binding antagonist inhibits the binding of PD-L1 to PD-1.

39. The method of claim 37, wherein the PD-L1 binding antagonist inhibits the binding of PD-L1 to B7-1.

40. The method of any one of claims 37-39, wherein the PD-L1 binding antagonist inhibits the binding of PD-L1 to both PD-1 and B7-1.

41. The method of any one of claims 36-40, wherein the PD-L1 binding antagonist is an antibody.

42. The method of claim 41, wherein the antibody is selected from the group consisting of: YW243.55.S70, MPDL3280A (atezolizumab), MDX-1105, MEDI4736 (durvalumab), and MSB0010718C (avelumab).

43. The method of claim 41, wherein the antibody comprises a heavy chain comprising HVR-H1 sequence of SEQ ID NO:19, HVR-H2 sequence of SEQ ID NO:20, and HVR-H3 sequence of SEQ ID

NO:21; and a light chain comprising HVR-L1 sequence of SEQ ID NO:22, HVR-L2 sequence of SEQ ID NO:23, and HVR-L3 sequence of SEQ ID NO:24.

44. The method of claim 41, wherein the antibody comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:26 and a light chain variable region comprising the amino acid sequence of SEQ ID NO:4.

45. The method of claim 35, wherein the PD-L1 axis binding antagonist is a PD-1 binding antagonist.

46. The method of claim 45, wherein the PD-1 binding antagonist inhibits the binding of PD-1 to one or more of its ligand binding partners.

47. The method of claim 46, wherein the PD-1 binding antagonist inhibits the binding of PD-1 to PD-L1.

48. The method of claim 46, wherein the PD-1 binding antagonist inhibits the binding of PD-1 to PD-L2.

49. The method of any one of claims 46-48, wherein the PD-1 binding antagonist inhibits the binding of PD-1 to both PD-L1 and PD-L2.

50. The method of any one of claims 45-49, wherein the PD-1 binding antagonist is an antibody.

51. The method of claim 50, wherein the antibody is selected from the group consisting of: MDX-1106 (nivolumab), MK-3475 (pembrolizumab), CT-011 (pidilizumab), MEDI-0680 (AMP-514), PDR001, REGN2810, and BGB-108.

52. The method of any one of claims 45-49, wherein the PD-1 binding antagonist is an Fc-fusion protein.

53. The method of claim 52, wherein the Fc-fusion protein is AMP-224.

54. The method of any one of claims 1-7 or 34-53, further comprising administering to the patient an effective amount of a second therapeutic agent.

55. The method of claim 54, wherein the second therapeutic agent is selected from the group consisting of a cytotoxic agent, a growth-inhibitory agent, a radiation therapy agent, an anti-angiogenic agent, and combinations thereof.

56. The method of any one of claims 1-55, wherein the non-small cell lung cancer is a locally advanced or metastatic non-small cell lung cancer.

57. The method of any one of claims 1-56, wherein the tumor sample is a formalin-fixed and paraffin-embedded (FFPE) tumor sample, an archival tumor sample, a fresh tumor sample, or a frozen tumor sample.

58. The method of any one of claims 1-57, wherein the expression level of PD-L1 is a protein expression level.

59. The method of claim 58, wherein the protein expression level of PD-L1 is determined using a method selected from the group consisting of immunohistochemistry (IHC), immunofluorescence, flow cytometry, and Western blot.

60. The method of claim 59, wherein the protein expression level of PD-L1 is determined using IHC.

61. The method of claim 59 or 60, wherein the protein expression level of PD-L1 is detected using an anti-PD-L1 antibody.

62. The method of any one of claims 1-57, wherein the expression level of PD-L1 is an mRNA expression level.

63. The method of claim 62, wherein the mRNA expression level of PD-L1 is determined using a method selected from the group consisting of quantitative polymerase chain reaction (qPCR), reverse transcription qPCR (RT-qPCR), RNA sequencing, microarray analysis, in situ hybridization, and serial analysis of gene expression (SAGE).

64. A PD-L1 axis binding antagonist for use in treating a patient suffering from a non-small cell lung cancer, wherein a tumor sample obtained from the patient has been determined to have a detectable expression level of PD-L1 in 5% or more of the tumor cells in the tumor sample.

65. Use of an effective amount of a PD-L1 axis binding antagonist in the manufacture of a medicament for use in treating a patient suffering from a non-small cell lung cancer, wherein a tumor sample obtained from the patient has been determined to have a detectable expression level of PD-L1 in 5% or more of the tumor cells in the tumor sample.

66. A composition comprising an effective amount of a PD-L1 axis binding antagonist for use in a method of treating a patient suffering from a non-small cell lung cancer, wherein a tumor sample obtained

from the patient has been determined to have a detectable expression level of PD-L1 in 5% or more of the tumor cells in the tumor sample.

67. A PD-L1 axis binding antagonist for use in treating a patient suffering from a non-small cell lung cancer, wherein a tumor sample obtained from the patient has been determined to have a detectable expression level of PD-L1 in tumor-infiltrating immune cells that comprise 5% or more of the tumor sample, and a detectable expression level of PD-L1 in less than 50% of the tumor cells in the tumor sample.

68. Use of an effective amount of a PD-L1 axis binding antagonist in the manufacture of a medicament for use in treating a patient suffering from a non-small cell lung cancer, wherein a tumor sample obtained from the patient has been determined to have a detectable expression level of PD-L1 in tumor-infiltrating immune cells that comprise 5% or more of the tumor sample, and a detectable expression level of PD-L1 in less than 50% of the tumor cells in the tumor sample.

69. A composition comprising an effective amount of a PD-L1 axis binding antagonist for use in a method of treating a patient suffering from a non-small cell lung cancer, wherein a tumor sample obtained from the patient has been determined to have a detectable expression level of PD-L1 in tumor-infiltrating immune cells that comprise 5% or more of the tumor sample, and a detectable expression level of PD-L1 in less than 50% of the tumor cells in the tumor sample.

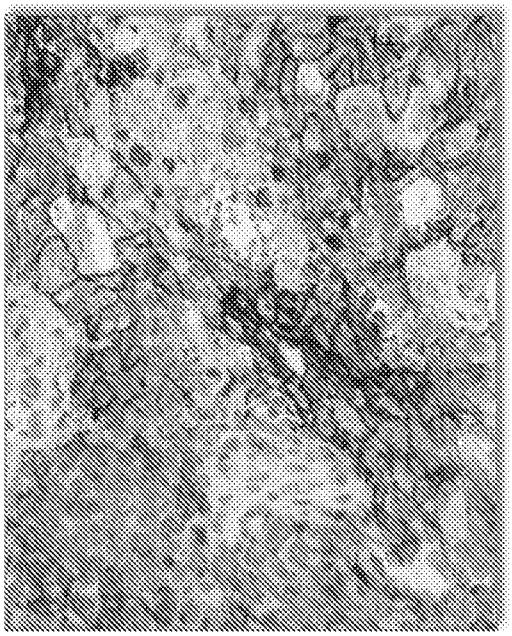
Figure 1A

Indication	N	~% of tumors with PD-L1 in IC (IC2/3, IC>=5%)	~% of tumors with PD-L1 in TC (TC2/3, TC>=5%)
NSCLC	184	26	24
Bladder	205	27	11
RCC	88	25	10
Melanoma	59	36	5
HNSCC	101	28	19
Gastric	141	18	5
Pancreatic	83	12	4
TNBC	317	22	4
Prostate	29	0	3

Figures 1B-1D

1B

1C



1D

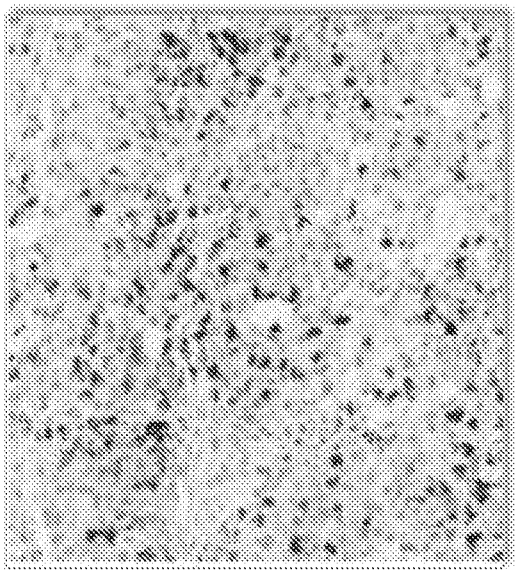


Figure 2A

N=287 Phase II	TC0	TC1	TC2	TC3	TOTAL
IC0	32%	5%	4%	2%	43%
IC1	21%	5%	6%	5%	37%
IC2	6%	2%	2%	2%	12%
IC3	3%	1%	1%	<1%	6%
TOTAL	62%	13%	13%	10%	

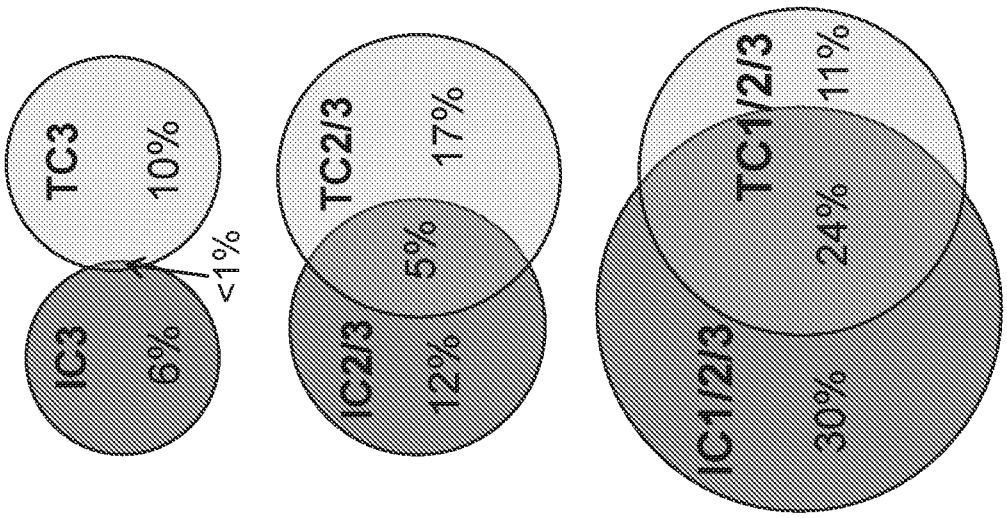


Figure 2B

Figure 3A


PD-L1 IHC Score
Best Overall Confirmed Response % (95% CI)
TC3 (n=9)
44% (14%, 79%)
IC3 (n=12)
50% (21%, 79%)
TC3 or IC3 (n=20)
45% (23%, 68%)
All Treated Patients (n=88)
21% (13%, 30%)

Figure 3B

	Cohort 1 (1L)		Cohort 2 (≥ 2L no brain mets)[a]		Cohort 3 (≥ 2L treated brain mets)	
Confirmed ORR, % (95% CI)						
	n		n		n	
All	31	29 (14–48)	92	17 (10–27)	13	23 (5–54)
TC3 or IC3	7	29 (4–71)	38	26 (13–43)	8	25 (3–65)
DOR, range (months)[b]						
All	9	2.3–9.0 (median: 9.0)	16	1.4+–17.3+	3	5.6+–9.9+
TC3 or IC3	2	2.8–4.2+	10	1.4+–17.3+	2	5.6+–9.9+
6-month PFS, % (95% CI)						
All	31	43 (25–61)	93	39 (29–49)	13	45 (17–73)
TC3 or IC3	7	29 (0–62)	38	50 (33–66)	8	50 (15–85)

Figure 3C

	Cohort 1 (1L)		Cohort 2 (≥ 2L no brain mets)[a]		Cohort 3 (≥ 2L treated brain mets)	
Confirmed ORR, % (95% CI)						
	n		n		n	
All	31	26 (12–45)	92	16 (9–25)	13	23 (5–54)
TC3 or IC3	7	29 (4–71)	38	24 (11–40)	8	25 (3–65)
DOR, range (months)[b]						
All	8	2.3–8.4+	15	4.1+–17.3+	3	4.2–9.9+
TC3 or IC3	2	2.9–4.2+	9	4.1+–17.3+	2	5.6+–9.9+
6-month PFS, % (95% CI)						
All	31	34 (17–50)	93	32 (23–42)	13	15 (0–35)
TC3 or IC3	7	29 (0–62)	38	42 (26–59)	8	25 (0–55)

Figure 3D

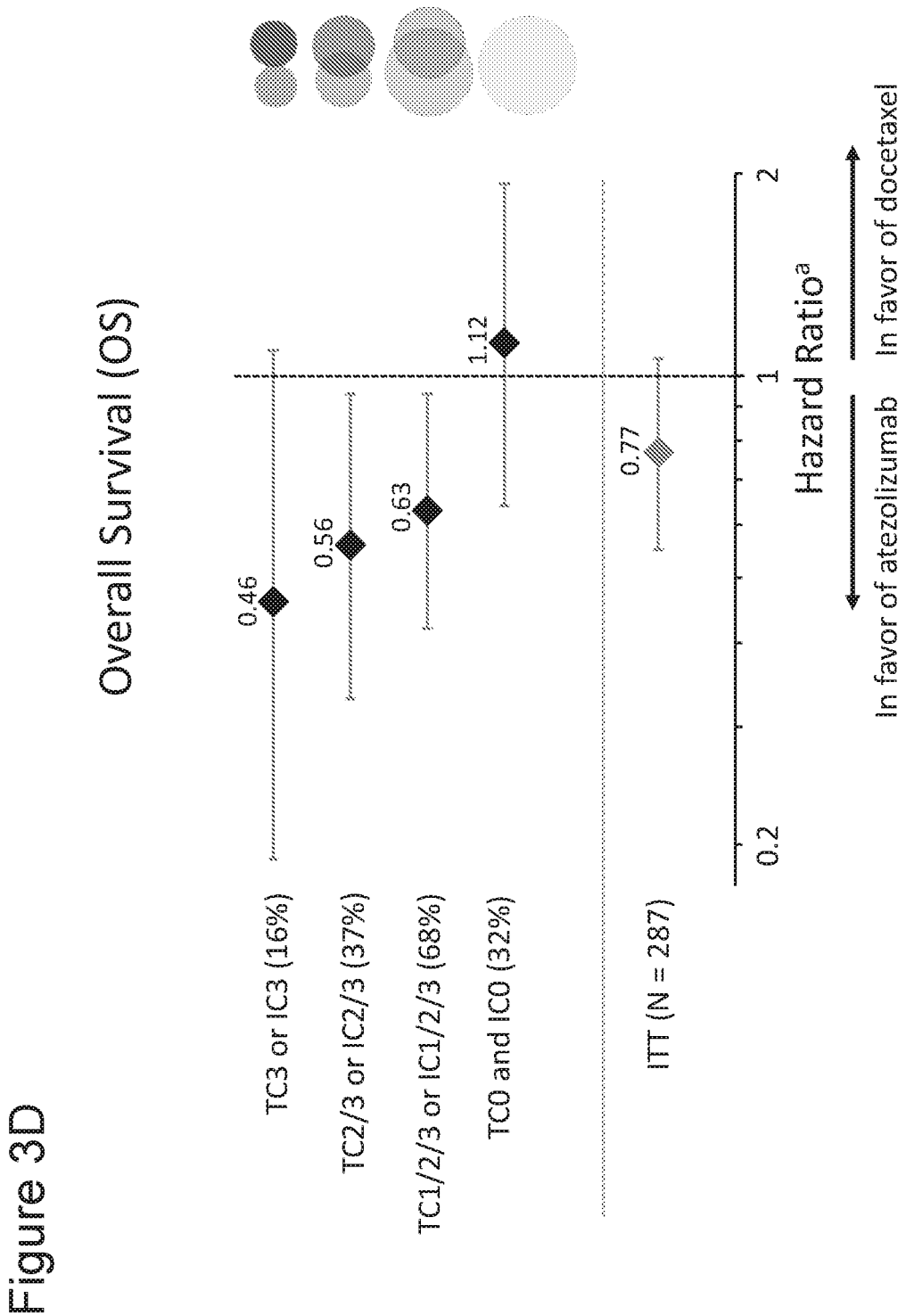
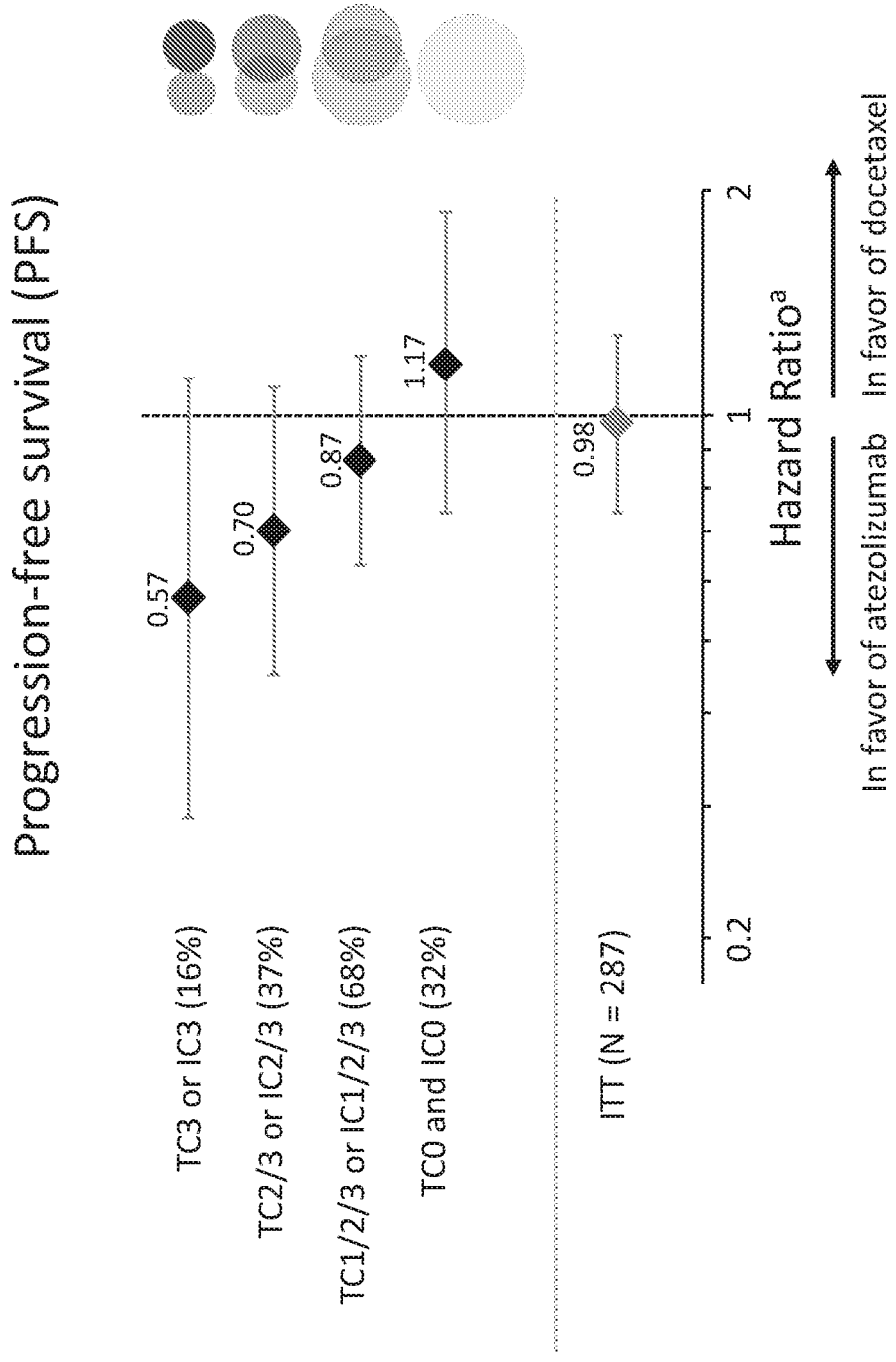

Overall Survival (OS)
0.46
0.56
0.63
1.12
0.77
TC3 or IC3 (16%)
TC2/3 or IC2/3 (37%)
TC1/2/3 or IC1/2/3 (68%)
TC0 and IC0 (32%)
ITT (N = 287)
0.2
1
2
Hazard Ratio[a]
In favor of atezolizumab
In favor of docetaxel

Figure 3E


Progression-free survival (PFS)
TC3 or IC3 (16%)
TC2/3 or IC2/3 (37%)
TC1/2/3 or IC1/2/3 (68%)
TC0 and IC0 (32%)
ITT (N = 287)
0.57
0.70
0.87
1.17
0.98
0.2
1
2
Hazard Ratio[a]
In favor of atezolizumab
In favor of docetaxel

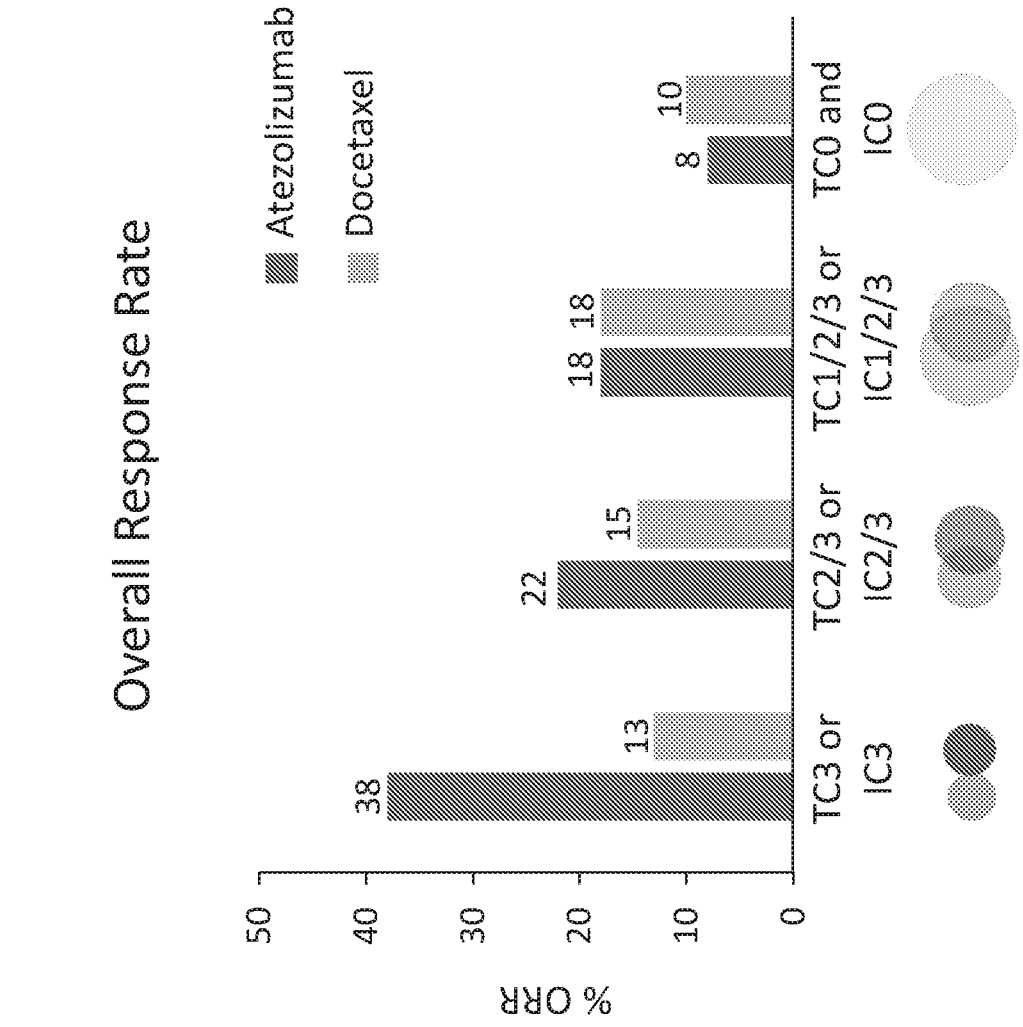

Overall Response Rate
Atezolizumab
Docetaxel
% ORR
50
40
30
20
10
0
38
13
22
15
18
18
8
10
TC3 or IC3
TC2/3 or IC2/3
TC1/2/3 or IC1/2/3
TC0 and IC0

Figure 3F

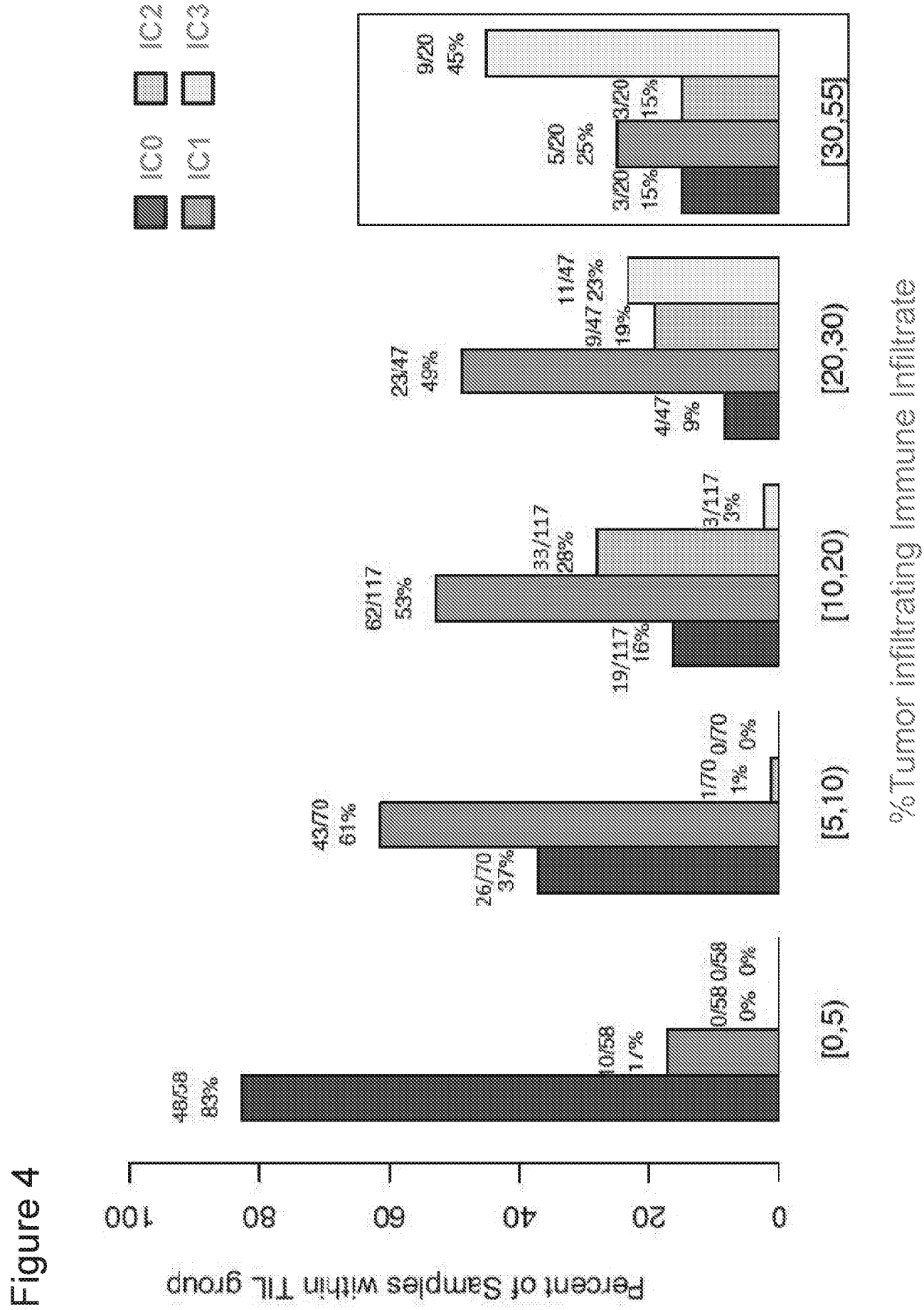

IC0
IC1
IC2
IC3
Percent of Samples within TIL group
0
20
40
60
80
100
48/58
83%
0/58
1%
0/58
0%
0/58
0%
[0,5)
43/70
61%
26/70
37%
1/70
1%
0/70
0%
[5,10)
62/117
53%
19/117
16%
33/117
28%
3/117
3%
[10,20)
23/47
49%
4/47
9%
9/47
19%
11/47
23%
[20,30)
9/20
45%
5/20
25%
3/20
15%
3/20
15%
[30,55]
%Tumor infiltrating immune infiltrate

Figure 4

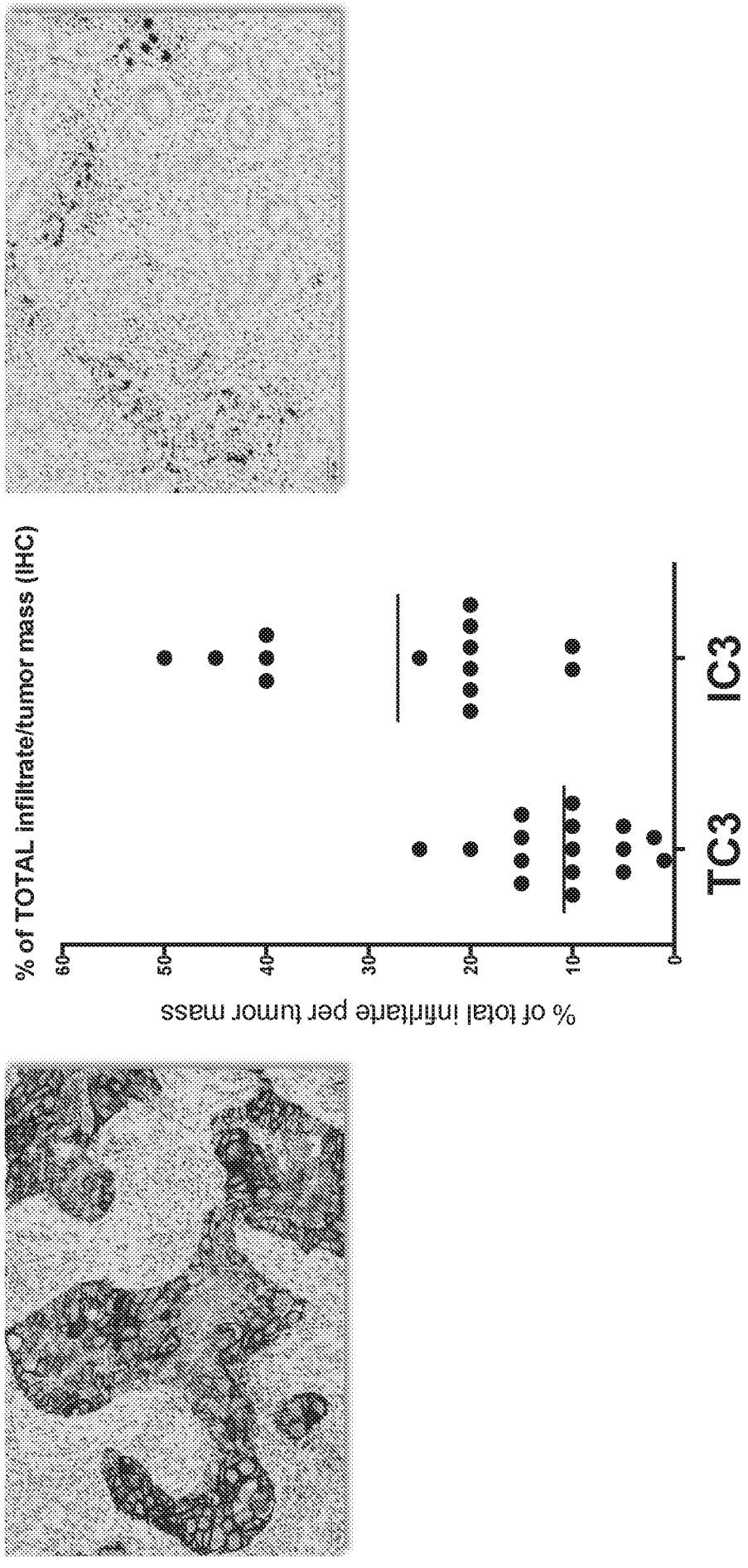

% of TOTAL infiltrate/tumor mass (IHC)
60
50
40
30
20
10
0
% of total infiltrate per tumor mass
TC3
IC3

Figure 5A

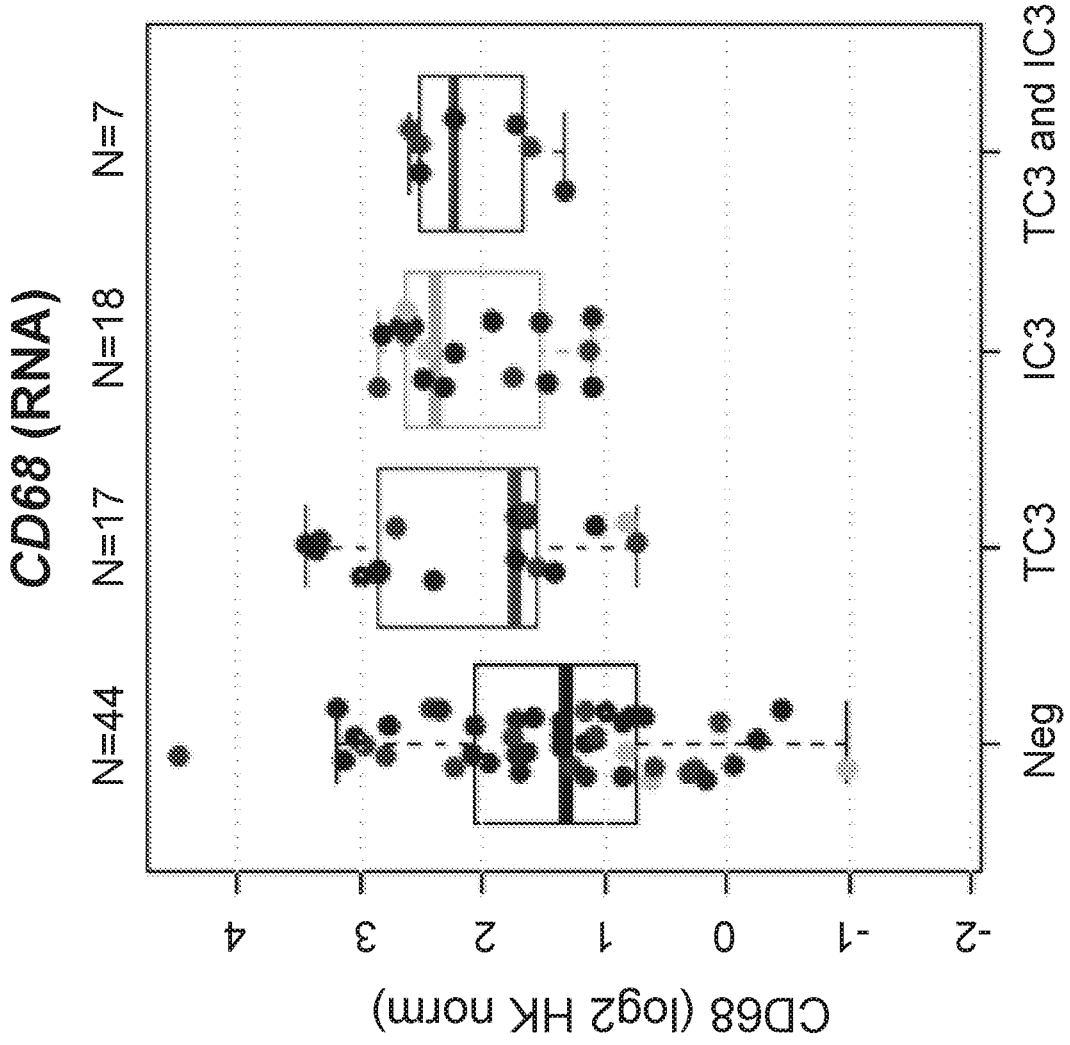

CD68 (RNA)
N=44
N=17
N=18
N=7
CD68 (log2 HK norm)
-2
-1
0
1
2
3
4
Neg
TC3
IC3
TC3 and IC3

Figure 5B

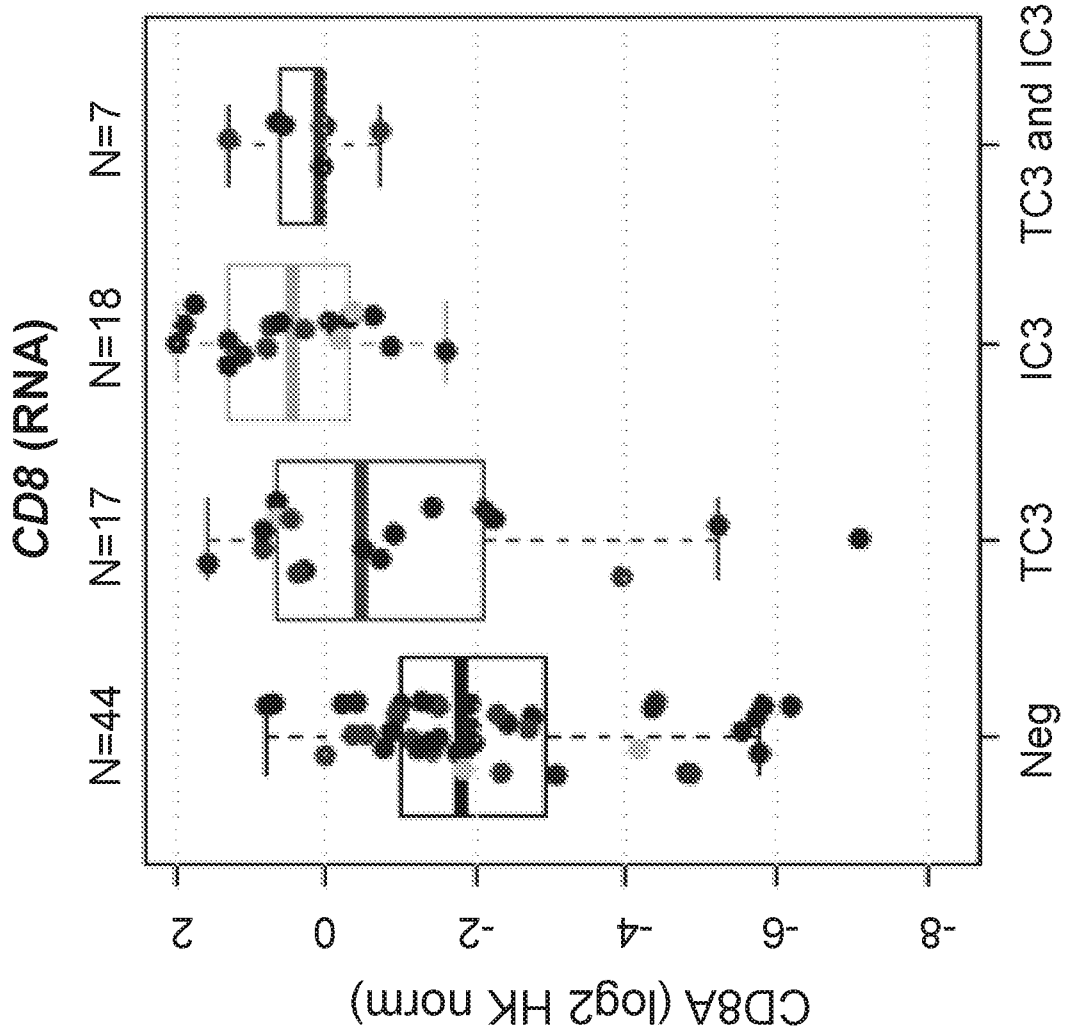

CD8A (log2 HK norm)
2
0
-2
-4
-6
-8
CD8 (RNA)
N=44
N=17
N=18
N=7
Neg
TC3
IC3
TC3 and IC3

Figure 5C

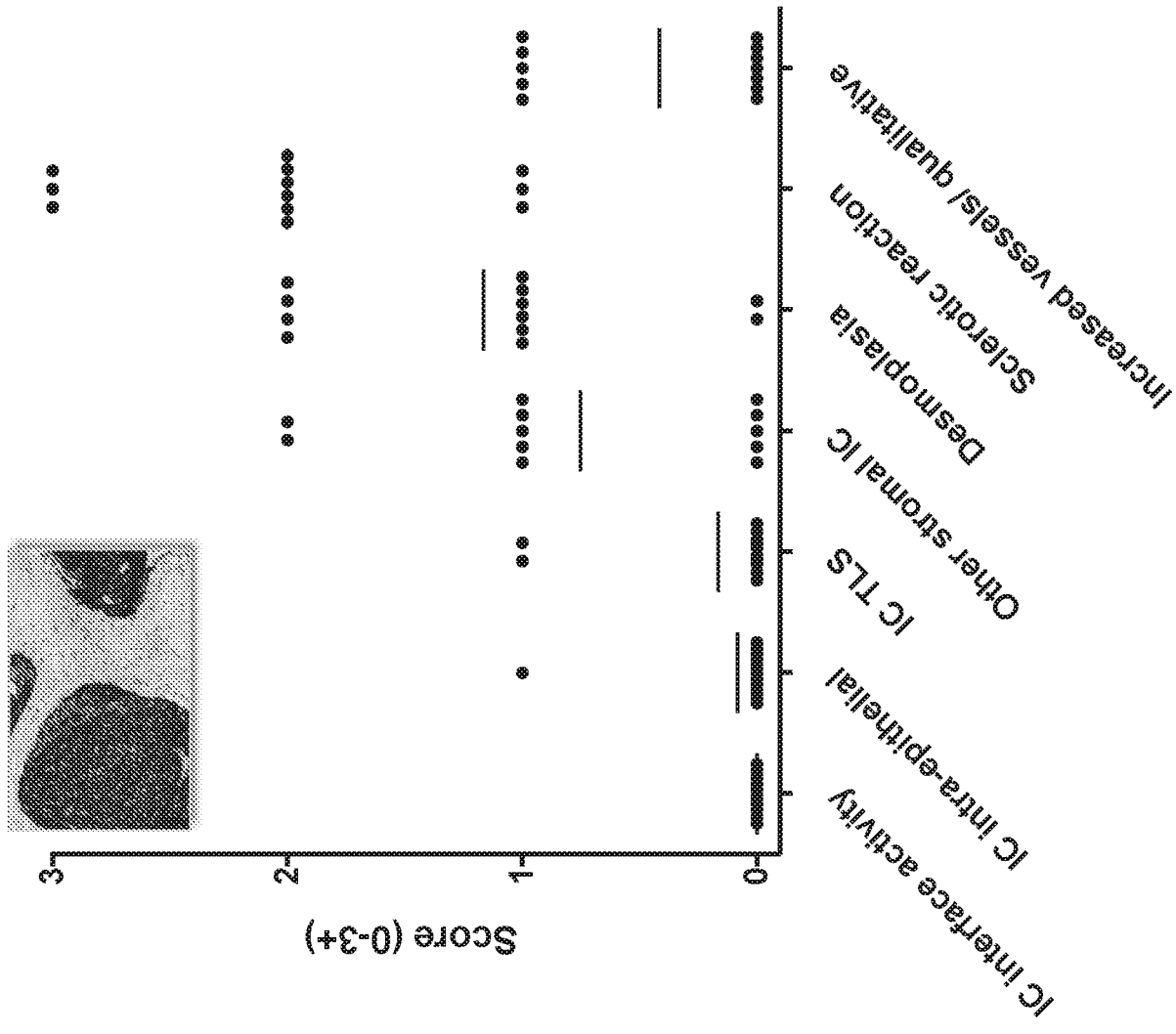

IC interface activity
IC intra-epithelial
IC TLS
Other stromal IC
Desmoplasia
Sclerotic reaction
Increased vessels/ qualitative
Score (0-3+)
0
1
2
3

Figure 6A

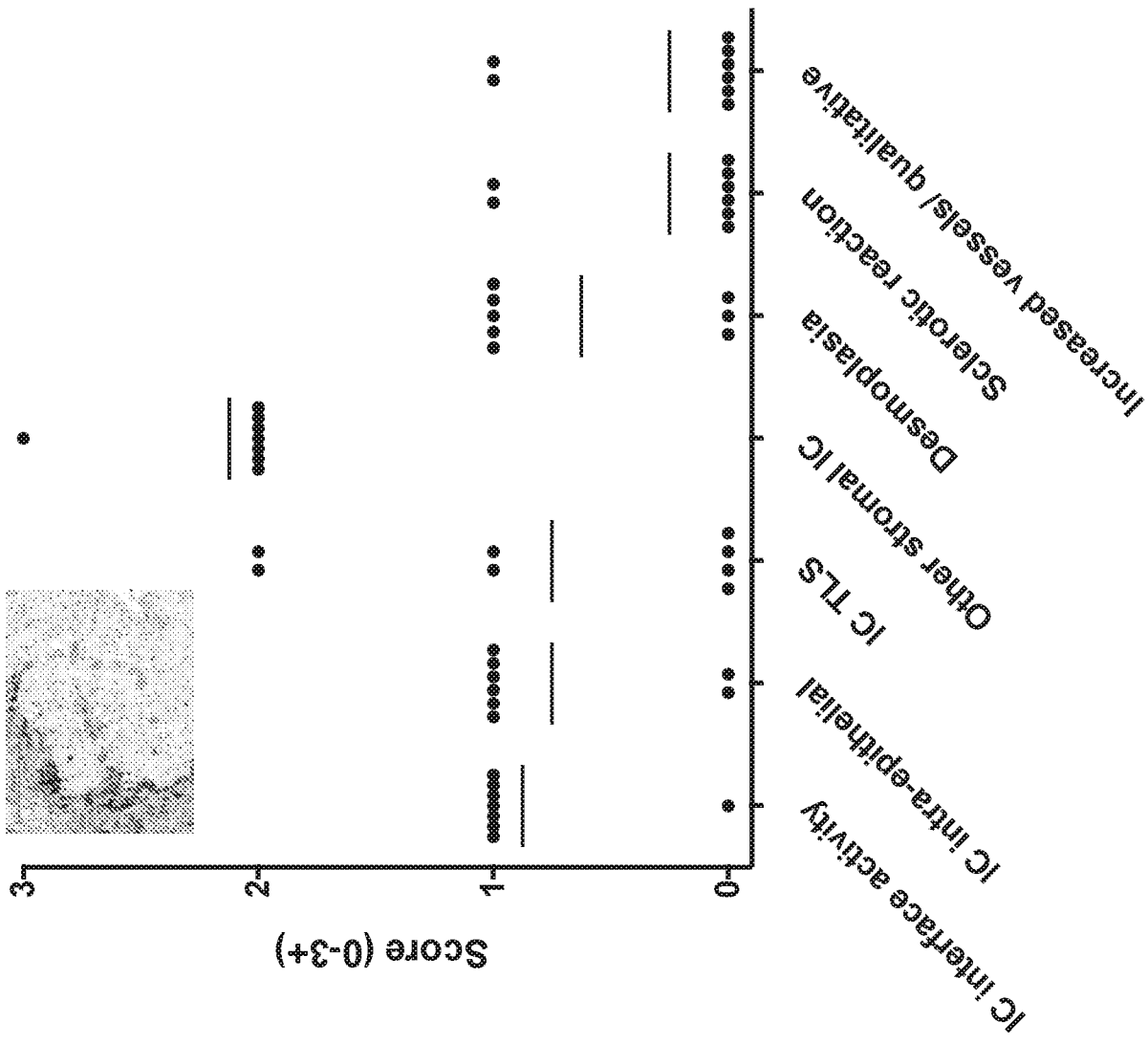
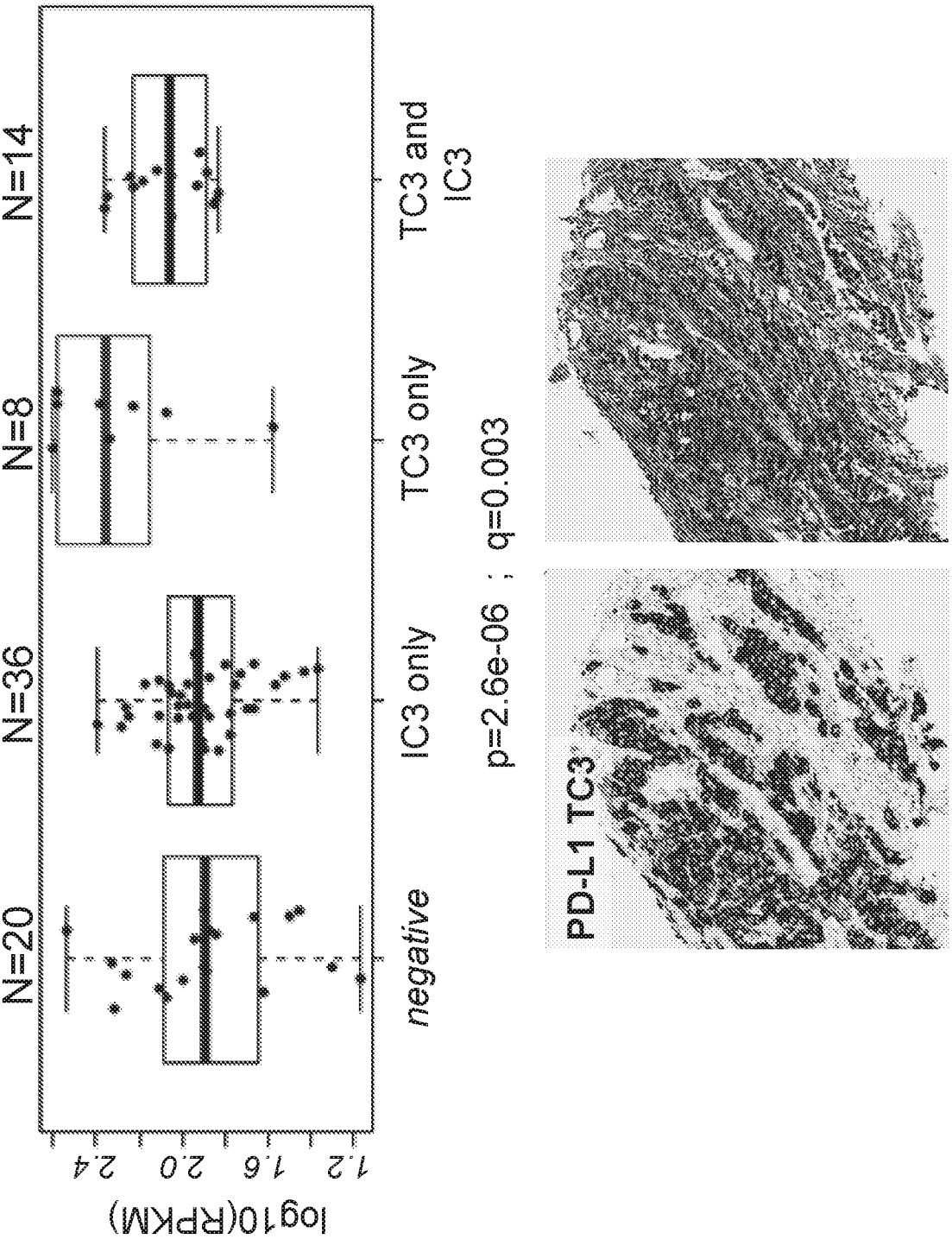

Score (0-3+)
0
1
2
3
IC interface activity
IC intra-epithelial
IC TLS
Other stromal IC
Desmoplasia
Sclerotic reaction
Increased Vessels/ qualitative

Figure 6B

Figure 7


COL6A1
log10(RPKM)
1.2
1.6
2.0
2.4
N=20
N=36
N=8
N=14
negative
IC3 only
TC3 only
TC3 and IC3
p=2.6e-06 ; q=0.003
PD-L1 TC3

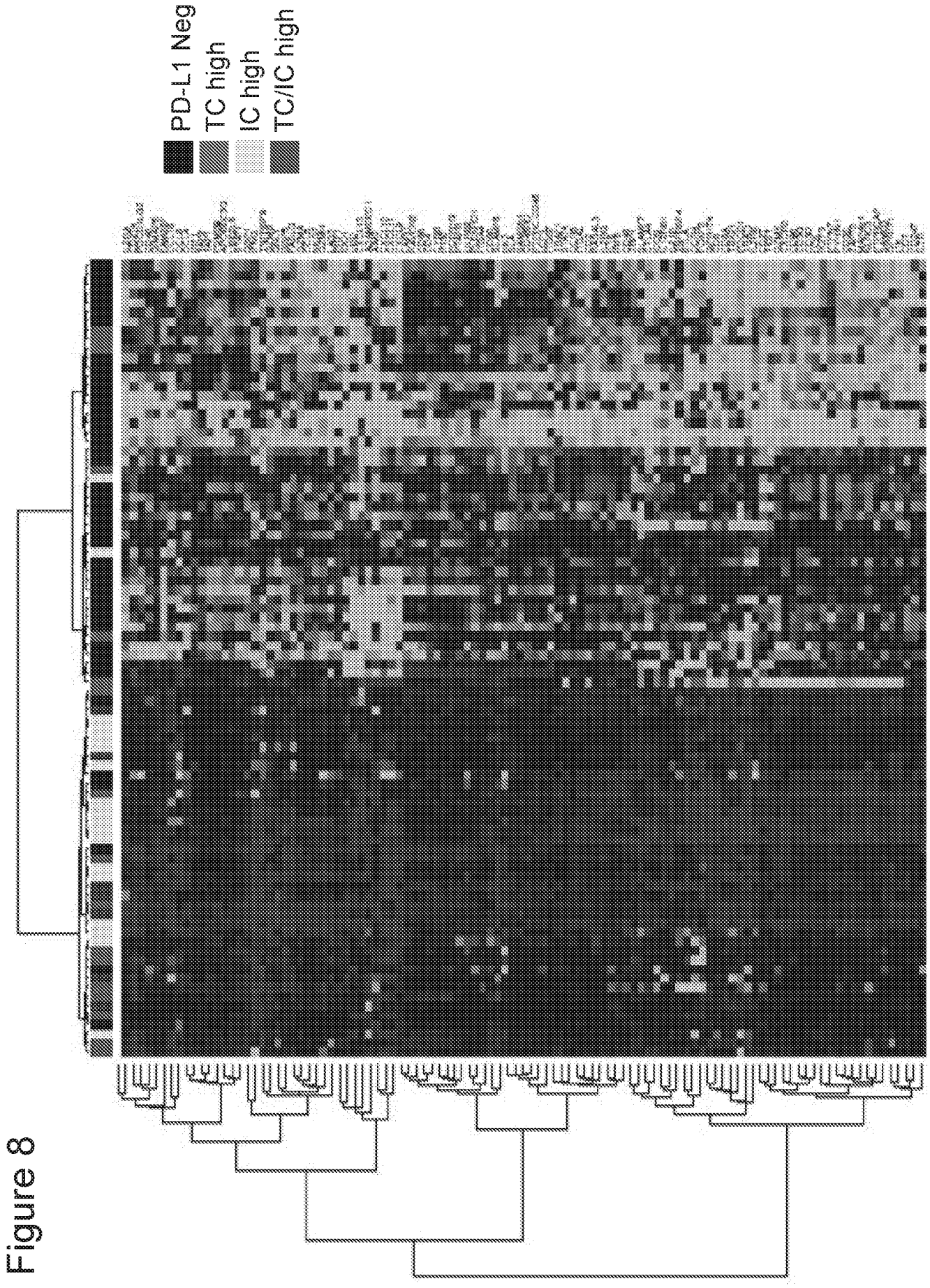
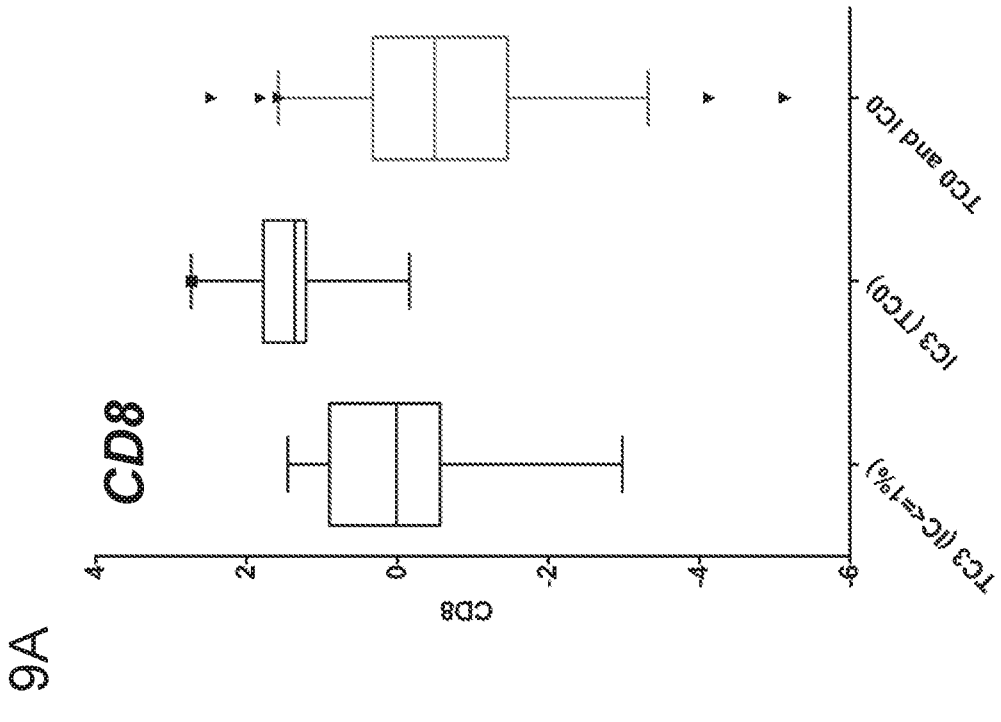
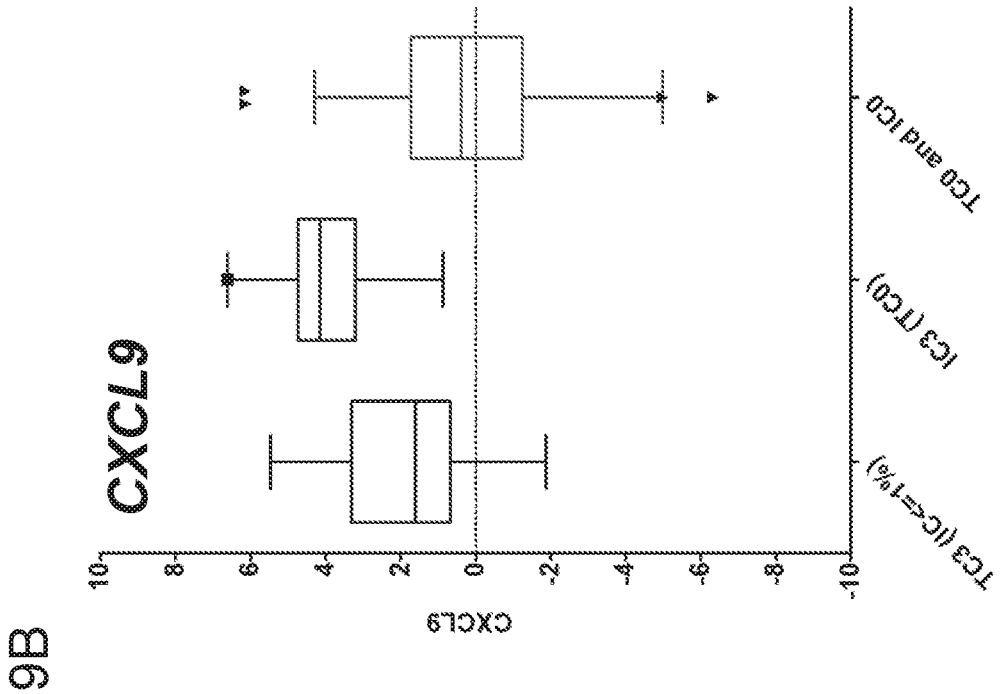


Figure 8

Figures 9A-9B





Figures 10A-10C

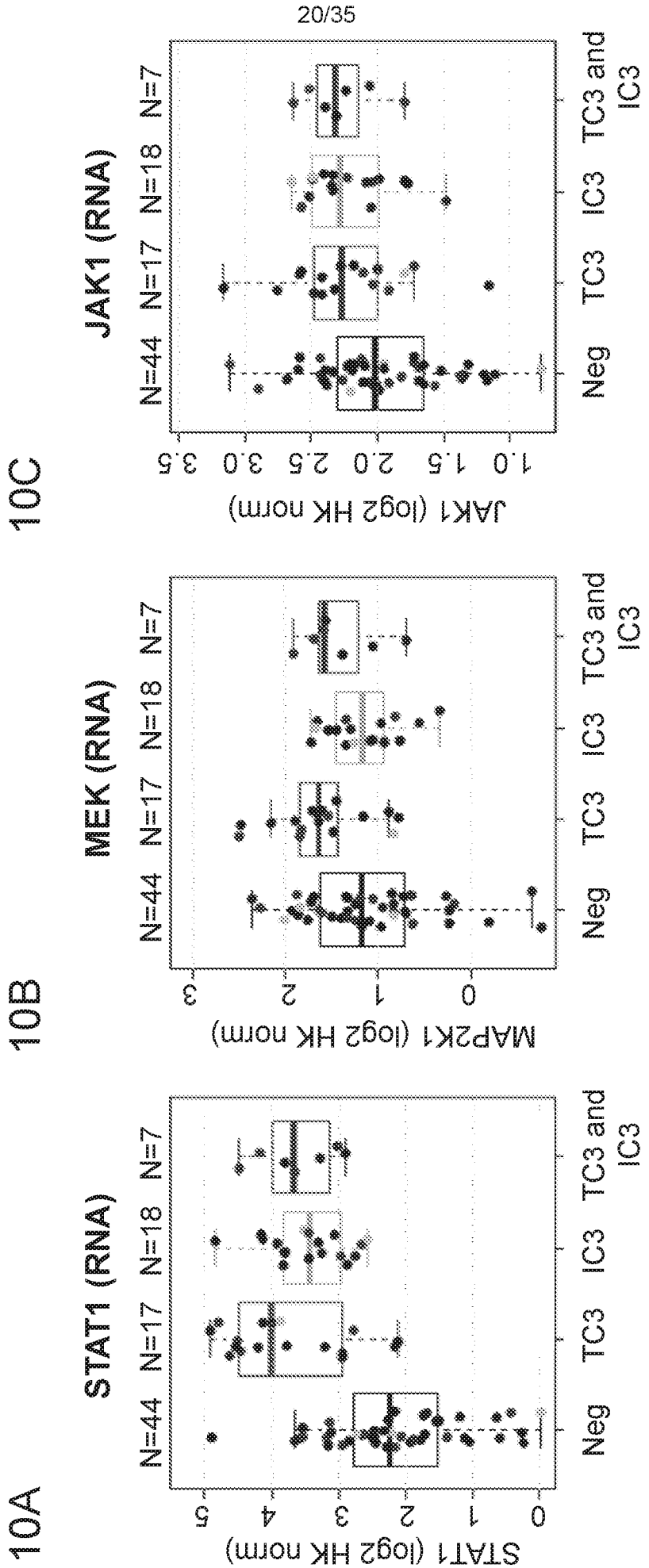


Figure 10D

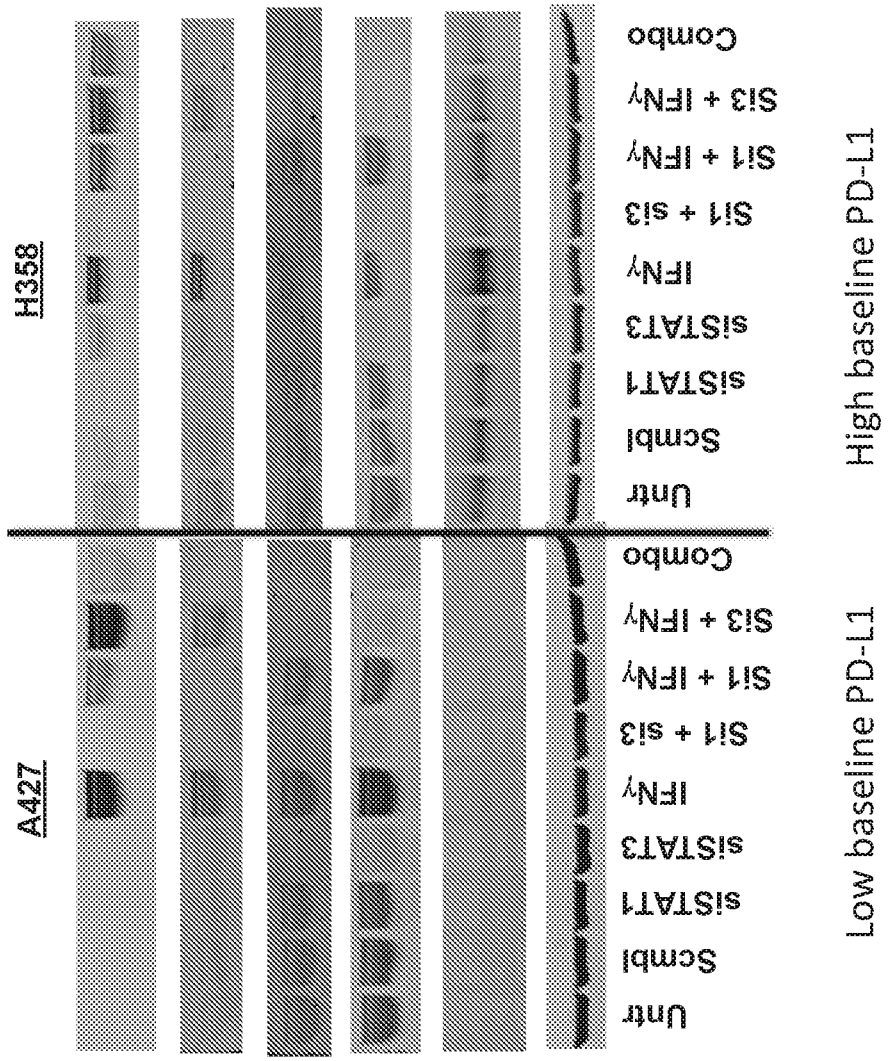
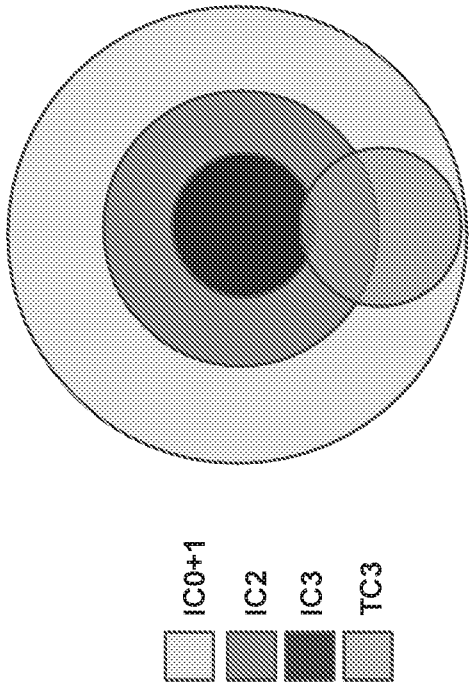

A427
H358
Untr
Scrbl
siSTAT1
siSTAT3
IFNγ
Si1 + si3
Si1 + IFNγ
Si3 + IFNγ
Combo
p-STAT1 (84, 91 kD)
t-STAT1 (84, 91 kD)
p-STAT3-S727 (86 kD)
t-STAT3 (86 kD)
PD-L1 (55 kD)
β-actin (44 kD)
Low baseline PD-L1
High baseline PD-L1

Figure 11

	Adjuvant	11	21+
TC1/2/3 or IC1/2/3	~61-68%	~66%	~65-69%
TC2/3 or IC2/3	~39-50%	~33%	~34-37%
TC3 or IC3	~21-28%	~16%	~16-20%



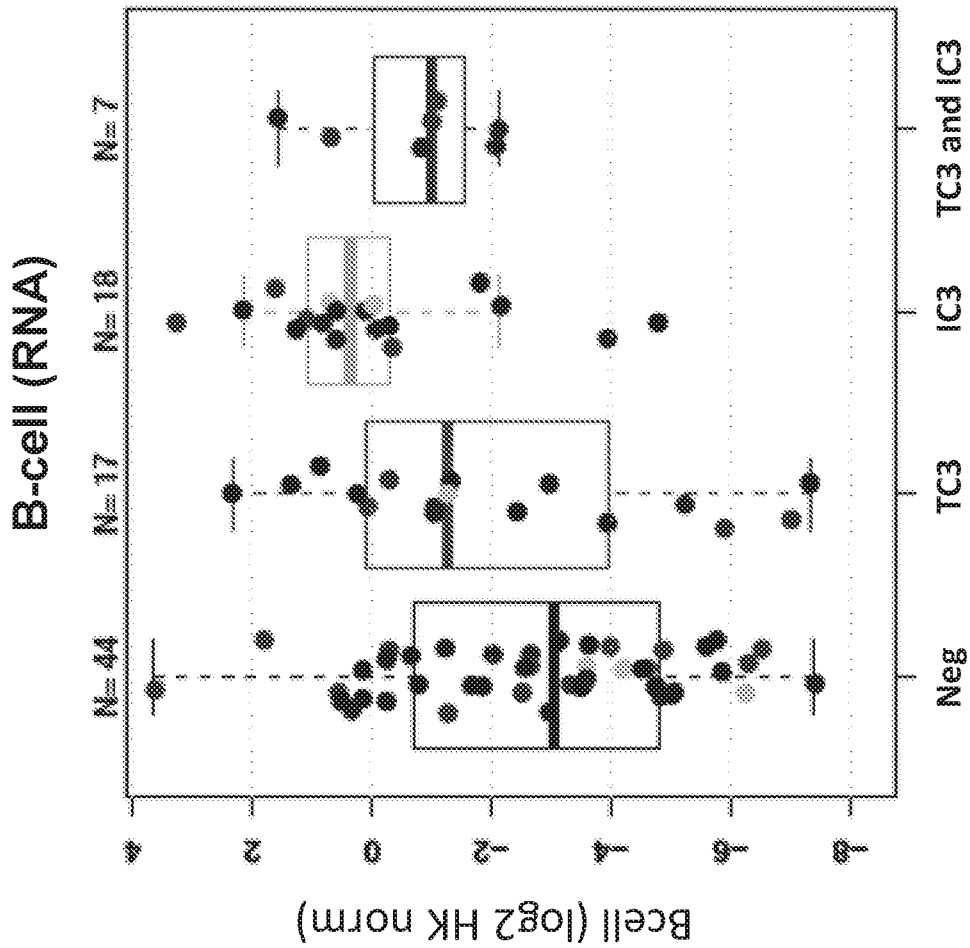

B-cell (RNA)
Bcell (log2 HK norm)
4
2
0
-2
-4
-6
-8
N= 44
N= 17
N= 18
N= 7
Neg
TC3
IC3
TC3 and IC3

Figure 12A

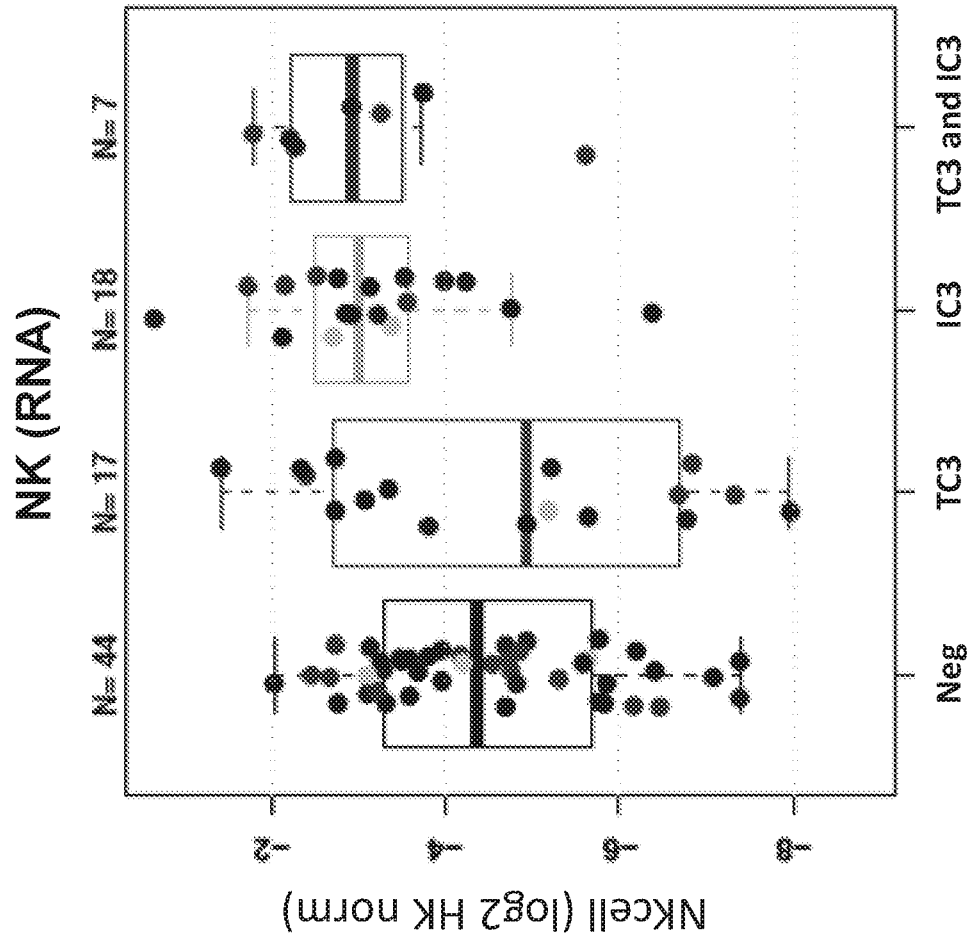

NK (RNA)
N= 44
N= 17
N= 18
N= 7
Neg
TC3
IC3
TC3 and IC3
NKcell (log2 HK norm)
-2
-4
-6
-8

Figure 12B

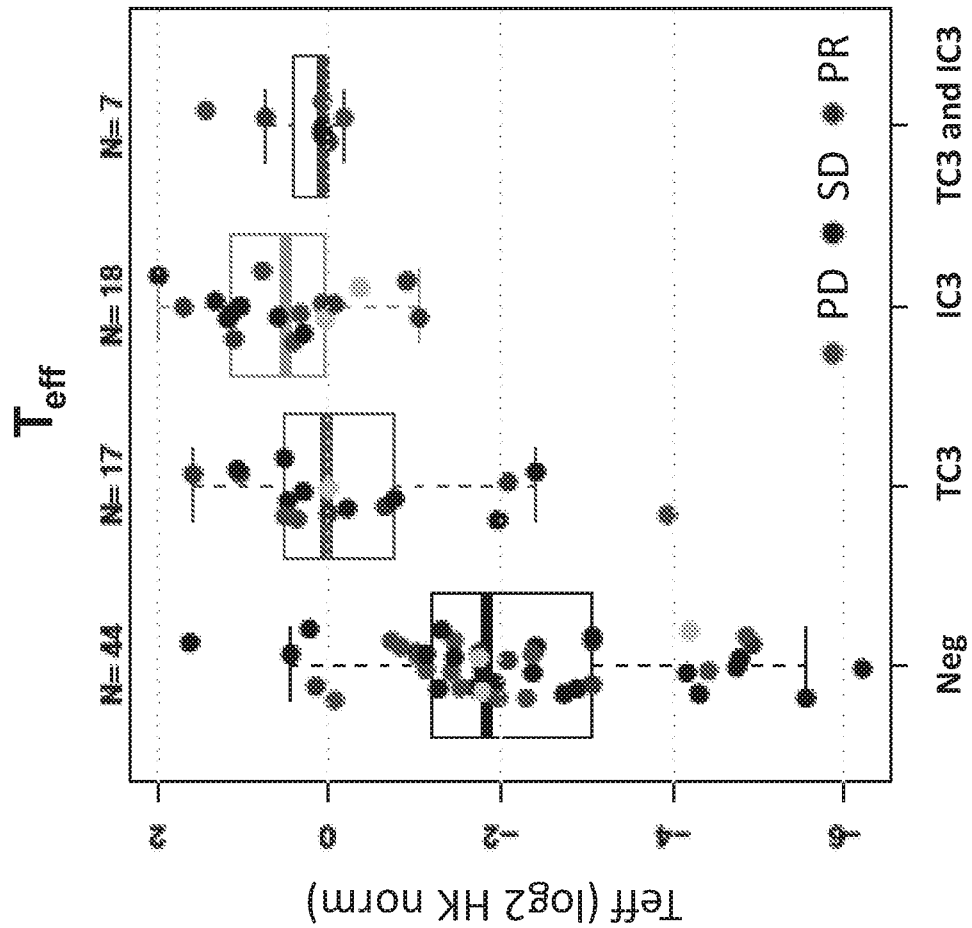

Teff (log2 HK norm)
2
0
-2
-4
-6
N= 44
N= 17
N= 18
N= 7
Neg
TC3
IC3
TC3 and IC3
PD
SD
PR
Teff

Figure 13A

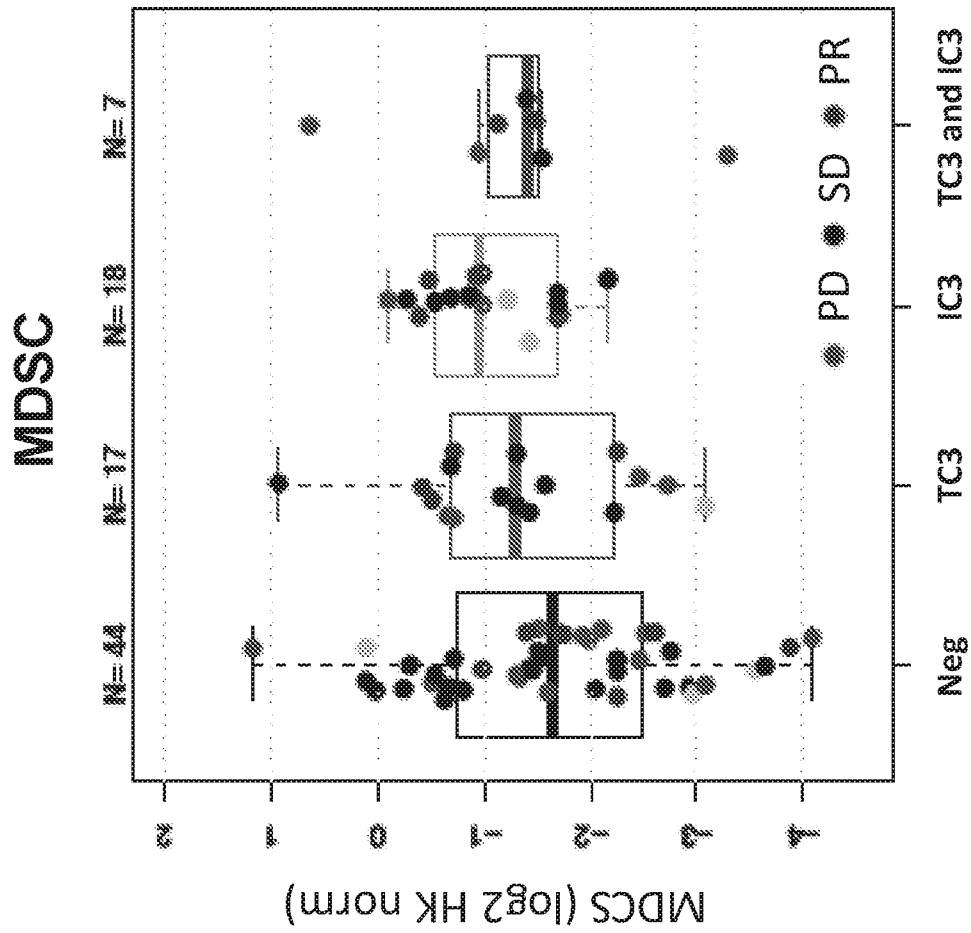

MDSC
MDCS (log2 HK norm)
2
1
0
-1
-2
-3
-4
N= 44
N= 17
N= 18
N= 7
Neg
TC3
IC3
TC3 and IC3
PD
SD
PR

Figure 13B

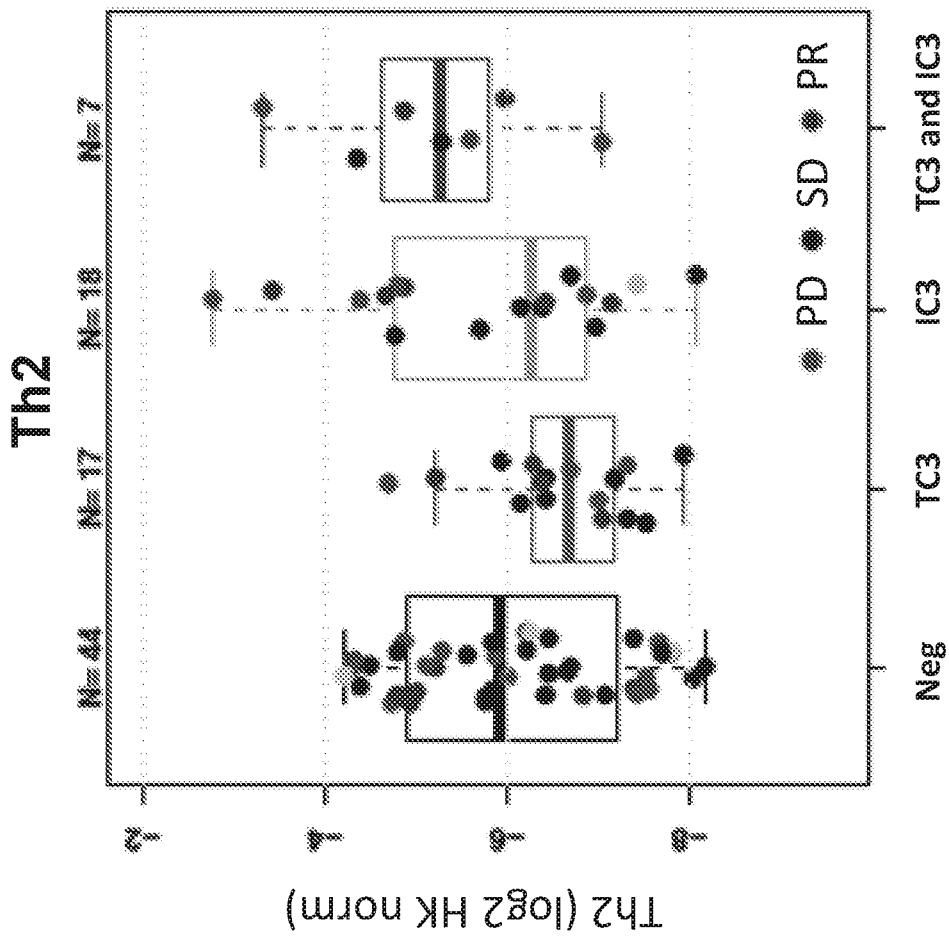

Th2
N=44
N=17
N=18
N=7
PD
SD
PR
Neg
TC3
IC3
TC3 and IC3
Th2 (log2 HK norm)

Figure 13C

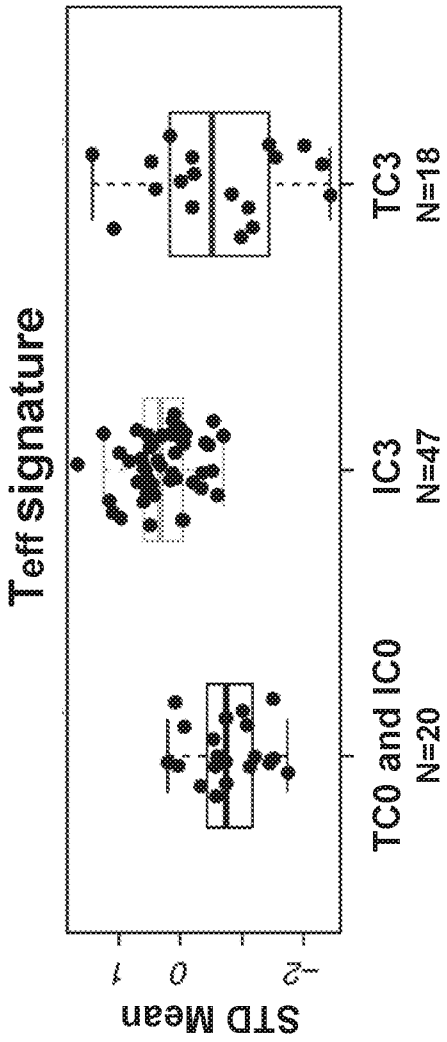
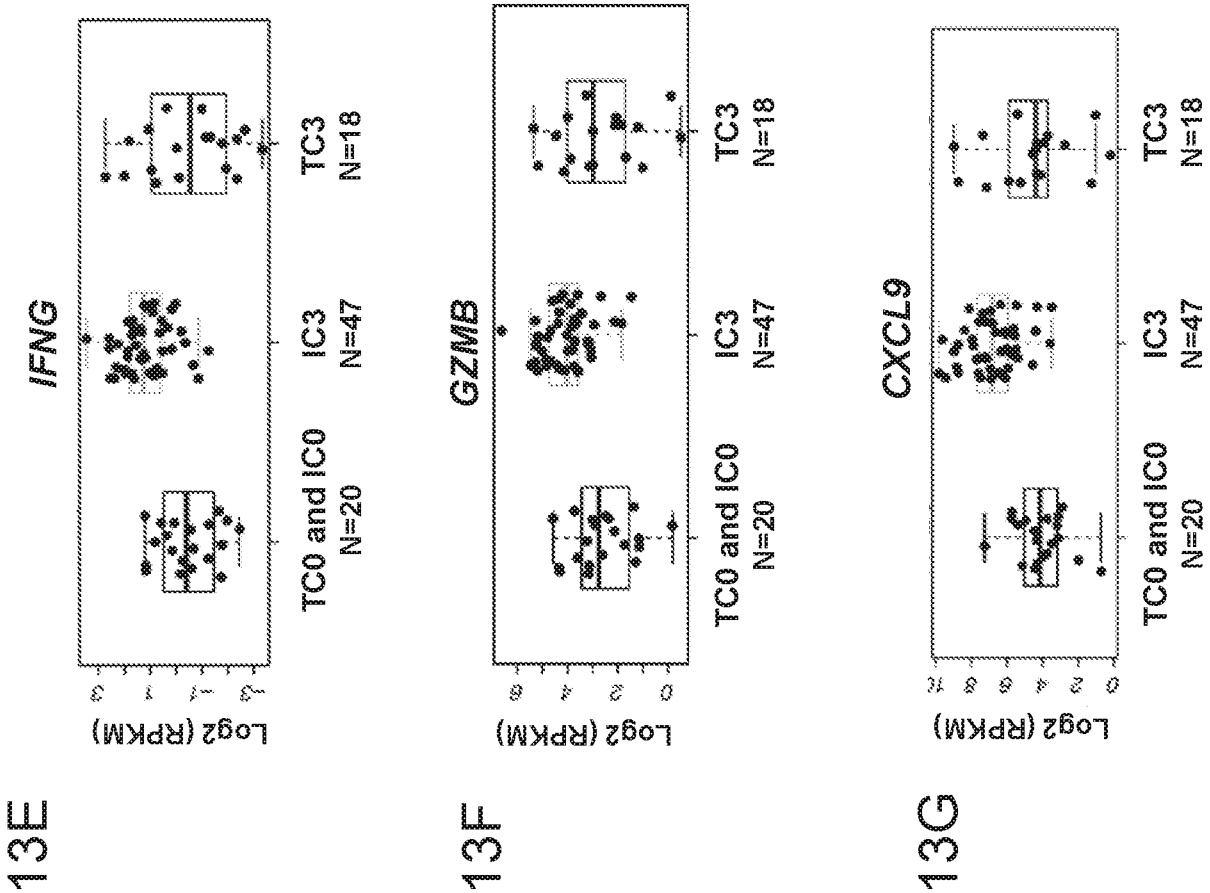


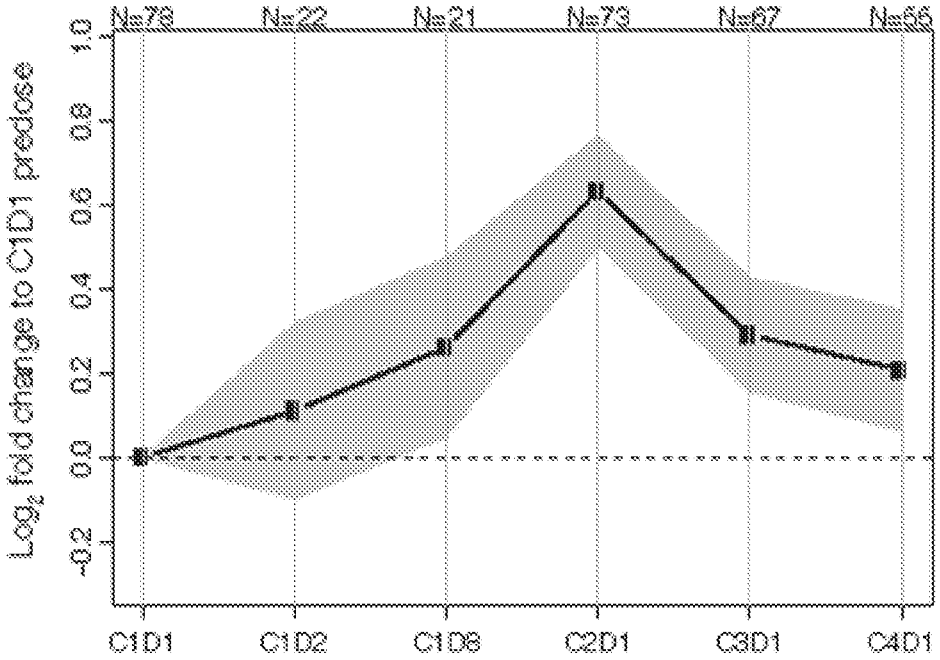
Figure 13D

Figures 13E-13G

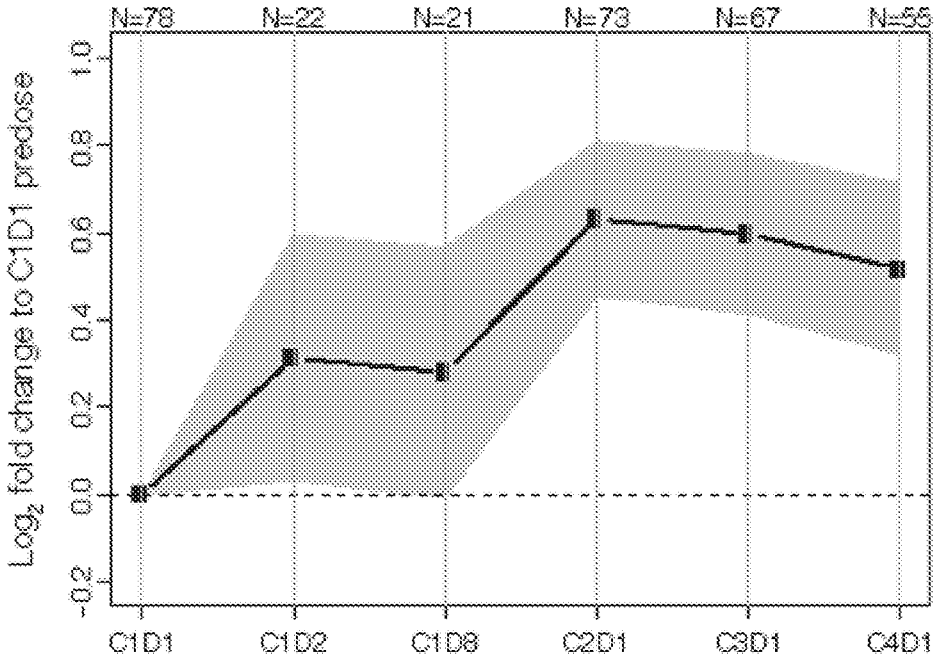


Figures 14A-14B

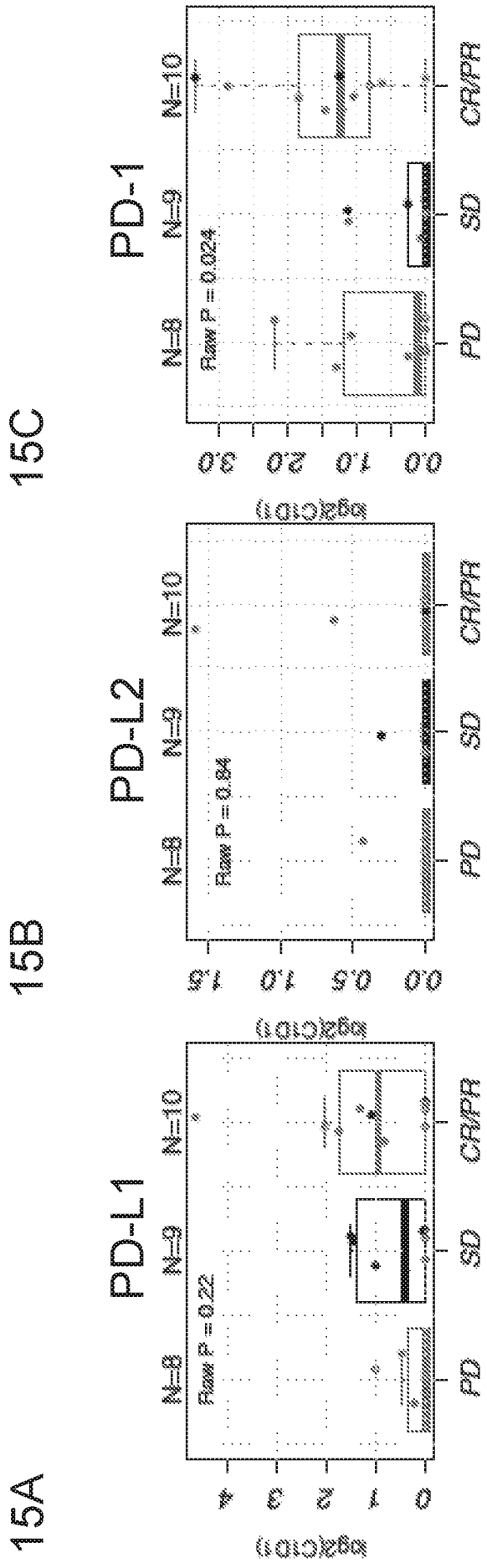
14A



14B



Figures 15A-15C



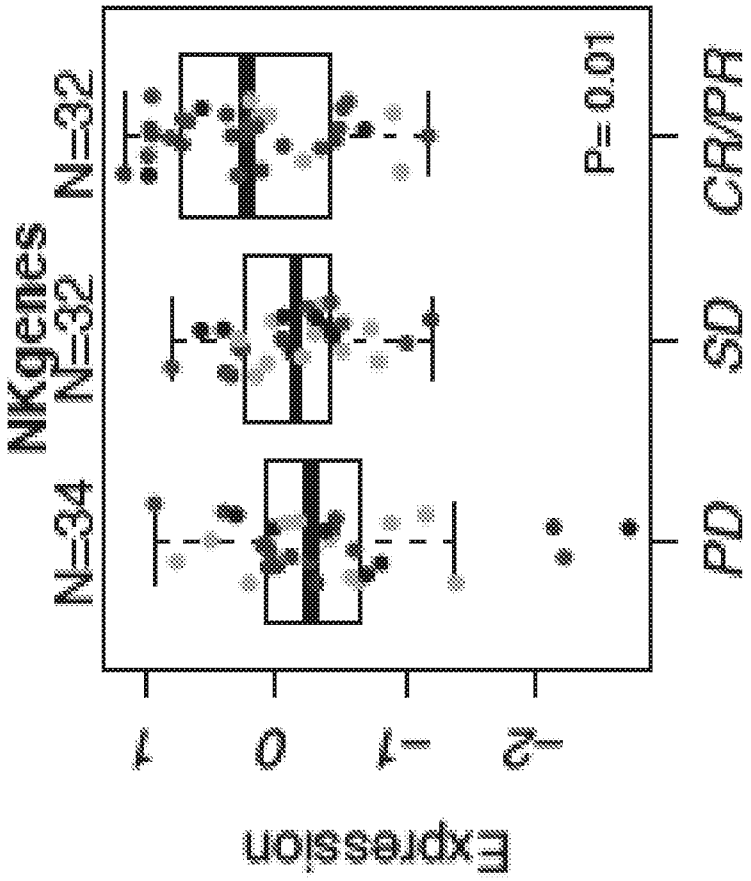


Figure 16A

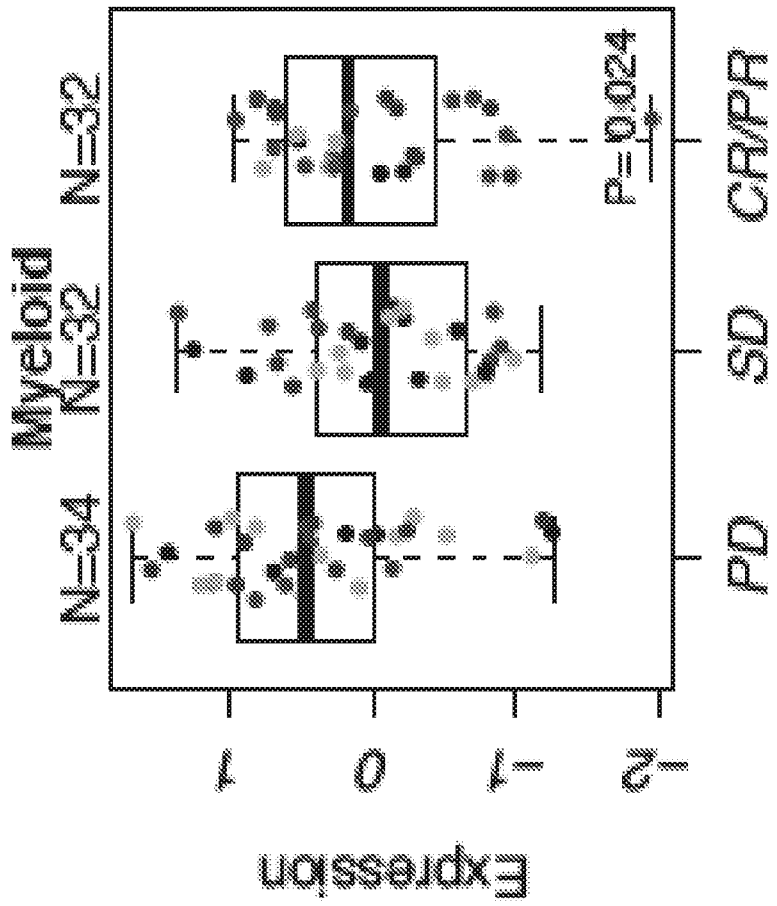
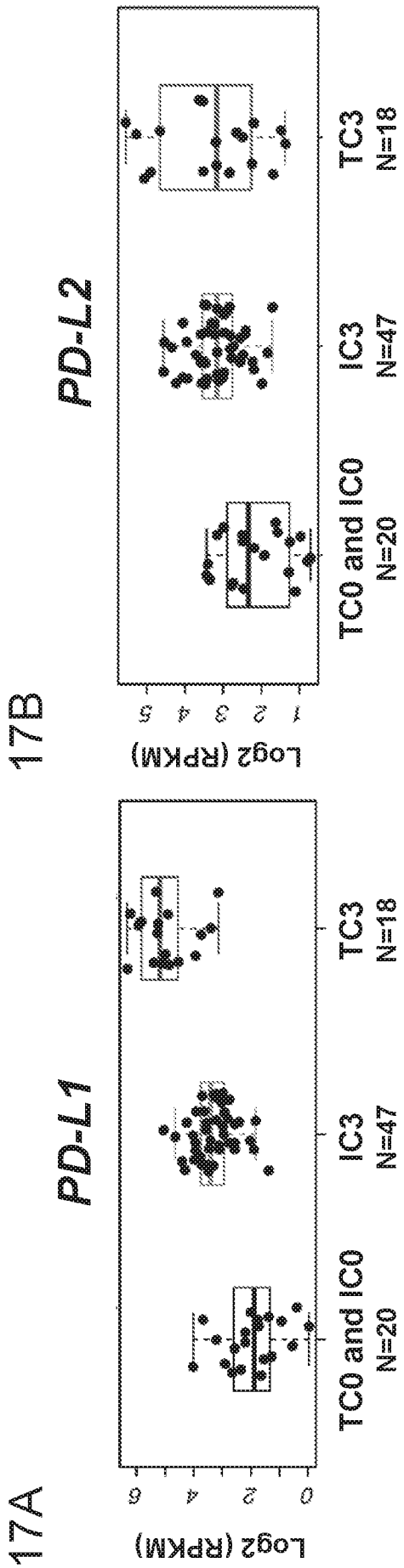
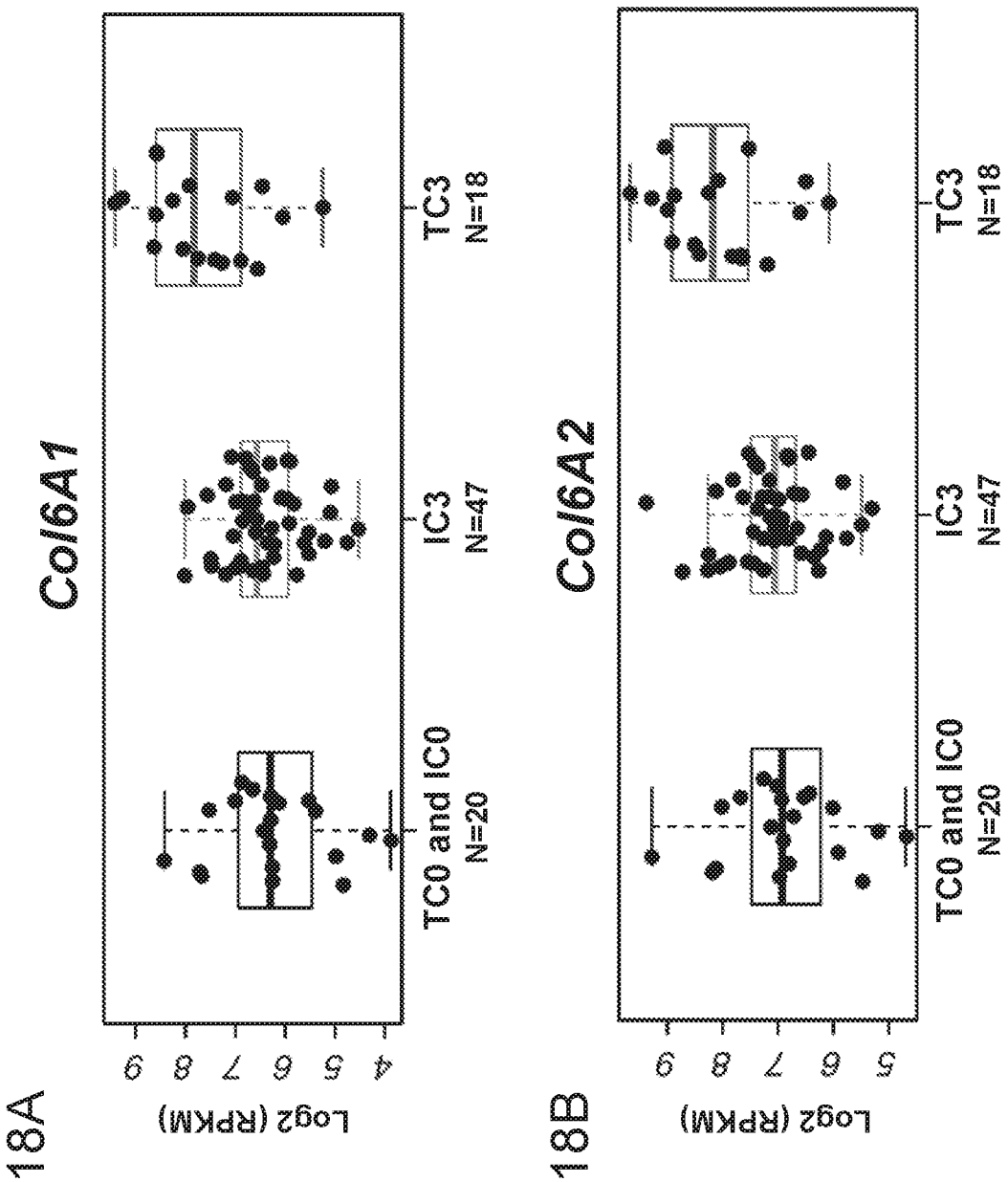


Figure 16B

Figures 17A-17B



Figures 18A-18B



INTERNATIONAL SEARCH REPORT

International application No
PCT/US2016/032112

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K16/28 G01N33/574
ADD. A61K39/00

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)
C07K A61K G01N

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2014/151006 A2 (GENENTECH INC [US]; HOFFMANN LA ROCHE [CH]) 25 September 2014 (2014-09-25) e.g. Figure 30(a); claims 1,30,41; paragraph 95, 432; the whole document -----	1-5,8, 10, 12-23, 31-44, 54-66
A	WO 2014/197369 A1 (DANA FARBER CANCER INST INC [US]) 11 December 2014 (2014-12-11) e.g. claim 2; table 1 starting on page 42; the whole document -----	1-5,8, 10, 12-23, 31-44, 54-66
	-/--	

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
3 August 2016	04/10/2016

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

1

Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	A I Spira ET AL: "Efficacy, safety and predictive biomarker results from a randomized phase II study comparing MPDL3280A vs docetaxel in 2L/3L NSCLC (POPLAR).", J Clin Oncol 33, 2015 (suppl; abstr 8010), 31 May 2015 (2015-05-31), XP055292176, Retrieved from the Internet: URL:http://meetinglibrary.asco.org/print/1999666 [retrieved on 2016-07-29] the whole document -----	1,8,10, 18,64-66
X,P	Anonymous: "Roche's investigational immunotherapy MPDL3280A doubled the likelihood of survival compared with chemotherapy in people with a specific type of lung cancer", , 14 May 2015 (2015-05-14), XP055292182, Retrieved from the Internet: URL:http://www.roche.com/med-cor-2015-05-14b-e.pdf [retrieved on 2016-07-29] the whole document -----	1,8,10, 18,64-66

1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2016/032112

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

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

   a. [X] forming part of the international application as filed:

      [X] in the form of an Annex C/ST.25 text file.

      [ ] on paper or in the form of an image file.

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

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

      [ ] in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

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

2. [ ] In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that 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 No.
PCT/US2016/032112

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-5, 8, 10, 12-23, 31-44, 54-66(all partially)

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-5, 8, 10, 12-23, 31-44, 54-66(all partially)

treating a patient suffering from a non-small cell lung cancer, the method comprising administering to the patient a therapeutically effective amount of a PD-L1 axis binding antagonist, wherein a tumor sample obtained from the patient has been determined to have a detectable expression level of PD-L1 in 5% or more of the tumor cells in the tumor sample, method of claims 1,8,10,18, antagonist of claim 64, use of claim 65, composition of claim 66, wherein the PD-L1 axis binding antagonist is a PD-L1 binding antagonist

---

2. claims: 1-5, 8, 10, 12-23, 31-35, 45-66(all partially)

treating a patient suffering from a non-small cell lung cancer, the method comprising administering to the patient a therapeutically effective amount of a PD-L1 axis binding antagonist, wherein a tumor sample obtained from the patient has been determined to have a detectable expression level of PD-L1 in 5% or more of the tumor cells in the tumor sample, method of claims 1,8,10,18, antagonist of claim 64, use of claim 65, composition of claim 66, wherein the PD-L1 axis binding antagonist is a PD-1 binding antagonist

---

3. claims: 1-5, 8, 10, 12-23, 31-35, 54-66(all partially)

treating a patient suffering from a non-small cell lung cancer, the method comprising administering to the patient a therapeutically effective amount of a PD-L1 axis binding antagonist, wherein a tumor sample obtained from the patient has been determined to have a detectable expression level of PD-L1 in 5% or more of the tumor cells in the tumor sample, method of claims 1,8,10,18, antagonist of claim 64, use of claim 65, composition of claim 66, wherein the PD-L1 axis binding antagonist is a PD-L2 binding antagonist

---

4. claims: 6, 7, 9, 11-17, 24-30, 34-44, 54-63, 67-69(all partially)

treating a patient suffering from a non-small cell lung cancer, the method comprising administering to the patient a therapeutically effective amount of a PD-L1 axis binding antagonist, wherein a tumor sample obtained from the patient has been determined to have a detectable expression level of PD-L1 in tumor-infiltrating immune cells that comprise 5% or more of the tumor sample, and a detectable expression level of PD-L1 in less than 50% of the tumor cells in the tumor sample, method of claims 6,9,11,24, antagonist of claim 67, use of claim 68, composition of claim 69, wherein the PD-L1

axis binding antagonist is a PD-L1 binding antagonist
---

5. claims: 6, 7, 9, 11-17, 24-30, 34, 35, 45-63, 67-69(all partially)

treating a patient suffering from a non-small cell lung cancer, the method comprising administering to the patient a therapeutically effective amount of a PD-L1 axis binding antagonist, wherein a tumor sample obtained from the patient has been determined to have a detectable expression level of PD-L1 in tumor-infiltrating immune cells that comprise 5% or more of the tumor sample, and a detectable expression level of PD-L1 in less than 50% of the tumor cells in the tumor sample, method of claims 6,9,11,24, antagonist of claim 67, use of claim 68, composition of claim 69, wherein the PD-L1 axis binding antagonist is a PD-1 binding antagonist
---

6. claims: 6, 7, 9, 11-17, 24-30, 34, 35, 54-63, 67-69(all partially)

treating a patient suffering from a non-small cell lung cancer, the method comprising administering to the patient a therapeutically effective amount of a PD-L1 axis binding antagonist, wherein a tumor sample obtained from the patient has been determined to have a detectable expression level of PD-L1 in tumor-infiltrating immune cells that comprise 5% or more of the tumor sample, and a detectable expression level of PD-L1 in less than 50% of the tumor cells in the tumor sample, method of claims 6,9,11,24, antagonist of claim 67, use of claim 68, composition of claim 69, wherein the PD-L1 axis binding antagonist is a PD-L2 binding antagonist
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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2016/032112

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2014151006 A2	25-09-2014	AU 2014235453 A1	08-10-2015
		CA 2905798 A1	25-09-2014
		CN 105209919 A	30-12-2015
		EP 2972373 A2	20-01-2016
		HK 1212769 A1	17-06-2016
		JP 2016520800 A	14-07-2016
		KR 20150131269 A	24-11-2015
		SG 11201507333X A	29-10-2015
		US 2016222118 A1	04-08-2016
		WO 2014151006 A2	25-09-2014
WO 2014197369 A1	11-12-2014	AU 2014275166 A1	28-01-2016
		CA 2913490 A1	11-12-2014
		EP 3004877 A1	13-04-2016
		US 2016122829 A1	05-05-2016
		WO 2014197369 A1	11-12-2014

SEQUENCE LISTING

<110> Genentech, Inc.
F. Hoffmann-La Roche AG

<120> THERAPEUTIC AND DIAGNOSTIC METHODS FOR CANCER

<130> 50474-111WO3

<150> US 62/168,700
<151> 2015-05-29

<150> US 62/160,561
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<160> 33

<170> PatentIn version 3.5

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Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ala Val Ile Trp Tyr Asp Gly Ser Lys Arg Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Phe
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Thr Asn Asp Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser
100 105 110

Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser
115 120 125

Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly Cys Leu Val Lys Asp
130 135 140

Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr
145 150 155 160

Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr
165 170 175

Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Lys
180 185 190

Thr Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn Thr Lys Val Asp
195 200 205

Lys Arg Val Glu Ser Lys Tyr Gly Pro Pro Cys Pro Pro Cys Pro Ala
210 215 220

Pro Glu Phe Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro
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Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
245 250 255

Val Asp Val Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val
260 265 270

Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
275 280 285

Phe Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
290 295 300

Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly
305 310 315 320

Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro
325 330 335

Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met Thr

340 345 350

Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser
355 360 365

Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr
370 375 380

Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr
385 390 395 400

Ser Arg Leu Thr Val Asp Lys Ser Arg Trp Gln Glu Gly Asn Val Phe
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420 425 430

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20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile
35 40 45

Tyr Asp Ala Ser Asn Arg Ala Thr Gly Ile Pro Ala Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu Pro
65 70 75 80

Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Ser Ser Asn Trp Pro Arg
85 90 95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala
100 105 110

Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
115 120 125

Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
130 135 140

Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
145 150 155 160

Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
165 170 175

Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
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20 25 30

Trp Ile His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ala Trp Ile Ser Pro Tyr Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val
50 55 60

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

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Arg His Trp Pro Gly Gly Phe Asp Tyr Trp Gly Gln Gly Thr
100 105 110

Leu Val Thr Val Ser Ala
115

<210> 4
<211> 108
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<400> 4

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Val Ser Thr Ala
20 25 30

Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Ser Ala Ser Phe Leu Tyr Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Leu Tyr His Pro Ala
85 90 95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg
100 105

<210> 5
<211> 10
<212> PRT
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<220>
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<220>
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<222> (6)..(6)
<223> Xaa is Asp or Gly

<400> 5

Gly Phe Thr Phe Ser Xaa Ser Trp Ile His
1 5 10

<210> 6
<211> 18
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<220>
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<220>
<221> MOD_RES
<222> (4)..(4)
<223> Xaa is Ser or Leu

<220>
<221> MOD_RES
<222> (10)..(10)
<223> Xaa is Thr or Ser

<400> 6

Ala Trp Ile Xaa Pro Tyr Gly Gly Ser Xaa Tyr Tyr Ala Asp Ser Val
1 5 10 15

Lys Gly

<210> 7
<211> 9
<212> PRT
<213> Artificial Sequence

<220>

<223> Synthetic Polypeptide

<400> 7

Arg His Trp Pro Gly Gly Phe Asp Tyr
1 5

<210> 8
<211> 25
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<400> 8

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser
20 25

<210> 9
<211> 13
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<400> 9

Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
1 5 10

<210> 10
<211> 32
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<400> 10

Arg Phe Thr Ile Ser Ala Asp Thr Ser Lys Asn Thr Ala Tyr Leu Gln
1 5 10 15

Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg
20 25 30

<210> 11
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<400> 11

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ala
1 5 10

<210> 12
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
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<220>
<221> MOD_RES
<222> (5)..(5)
<223> Xaa is Asp or Val

<220>
<221> MOD_RES
<222> (6)..(6)
<223> Xaa is Val or Ile

<220>
<221> MOD_RES
<222> (7)..(7)
<223> Xaa is Ser or Asn

<220>
<221> MOD_RES
<222> (9)..(9)
<223> Xaa is Ala or Phe

<220>
<221> MOD_RES
<222> (10)..(10)
<223> Xaa is Val or Leu

<400> 12

Arg Ala Ser Gln Xaa Xaa Xaa Thr Xaa Xaa Ala
1 5 10

<210> 13
<211> 7

<212> PRT
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<220>
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<220>
<221> MOD_RES
<222> (4)..(4)
<223> Xaa is Phe or Thr

<220>
<221> MOD_RES
<222> (6)..(6)
<223> Xaa is Tyr or Ala

<400> 13

Ser Ala Ser Xaa Leu Xaa Ser
1 5

<210> 14
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
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<220>
<221> MOD_RES
<222> (3)..(3)
<223> Xaa is Tyr, Gly, Phe, or Ser

<220>
<221> MOD_RES
<222> (4)..(4)
<223> Xaa is Leu, Tyr, Phe, or Trp

<220>
<221> MOD_RES
<222> (5)..(5)
<223> Xaa is Tyr, Asn, Ala, Thr, Gly, Phe, or Ile

<220>
<221> MOD_RES
<222> (6)..(6)
<223> Xaa is His, Val, Pro, Thr, or Ile

<220>
<221> MOD_RES
<222> (8)..(8)
<223> Xaa is Ala, Trp, Arg, Pro, or Thr

<400> 14

Gln Gln Xaa Xaa Xaa Xaa Pro Xaa Thr
1 5

<210> 15
<211> 23
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<400> 15

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys
20

<210> 16
<211> 15
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<400> 16

Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr
1 5 10 15

<210> 17
<211> 32
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<400> 17

Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr
1 5 10 15

Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys
20 25 30

<210> 18

<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<400> 18

Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg
1 5 10

<210> 19
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<400> 19

Gly Phe Thr Phe Ser Asp Ser Trp Ile His
1 5 10

<210> 20
<211> 18
<212> PRT
<213> Artificial Sequence

<220>
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<400> 20

Ala Trp Ile Ser Pro Tyr Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val
1 5 10 15

Lys Gly

<210> 21
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<400> 21

Arg His Trp Pro Gly Gly Phe Asp Tyr
1 5

<210> 22
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<400> 22

Arg Ala Ser Gln Asp Val Ser Thr Ala Val Ala
1 5 10

<210> 23
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<400> 23

Ser Ala Ser Phe Leu Tyr Ser
1 5

<210> 24
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<400> 24

Gln Gln Tyr Leu Tyr His Pro Ala Thr
1 5

<210> 25
<211> 118
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<400> 25

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Ser
20 25 30

Trp Ile His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ala Trp Ile Ser Pro Tyr Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val
50 55 60

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

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Arg His Trp Pro Gly Gly Phe Asp Tyr Trp Gly Gln Gly Thr
100 105 110

Leu Val Thr Val Ser Ser
115

<210> 26
<211> 122
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<400> 26

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Ser
20 25 30

Trp Ile His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ala Trp Ile Ser Pro Tyr Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val
50 55 60

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

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Arg His Trp Pro Gly Gly Phe Asp Tyr Trp Gly Gln Gly Thr
100 105 110

Leu Val Thr Val Ser Ser Ala Ser Thr Lys
115 120

<210> 27
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<400> 27

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
1 5 10

<210> 28
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<400> 28

Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
1 5 10

<210> 29
<211> 30
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<400> 29

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser

20 25 30

<210> 30
<211> 14
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<400> 30

Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ala
1 5 10

<210> 31
<211> 15
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<400> 31

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys
1 5 10 15

<210> 32
<211> 447
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<400> 32

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Ser
20 25 30

Trp Ile His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ala Trp Ile Ser Pro Tyr Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val
50 55 60

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

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Arg His Trp Pro Gly Gly Phe Asp Tyr Trp Gly Gln Gly Thr
100 105 110

Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro
115 120 125

Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly
130 135 140

Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn
145 150 155 160

Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln
165 170 175

Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser
180 185 190

Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser
195 200 205

Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr
210 215 220

His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser
225 230 235 240

Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg
245 250 255

Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro
260 265 270

Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala
275 280 285

Lys Thr Lys Pro Arg Glu Glu Gln Tyr Ala Ser Thr Tyr Arg Val Val

290 295 300

Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr
305 310 315 320

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

Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu
340 345 350

Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys
355 360 365

Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser
370 375 380

Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp
385 390 395 400

Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser
405 410 415

Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala
420 425 430

Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
435 440 445

<210> 33
<211> 214
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<400> 33

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Val Ser Thr Ala
20 25 30

Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Ser Ala Ser Phe Leu Tyr Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Leu Tyr His Pro Ala
85 90 95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala
100 105 110

Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
115 120 125

Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
130 135 140

Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
145 150 155 160

Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
165 170 175

Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
180 185 190

Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
195 200 205

Phe Asn Arg Gly Glu Cys
210

摘要

本发明提供用于癌症，例如非小细胞肺癌(NSCLC)的治疗和诊断方法和组合物。本发明提供基于本发明的生物标志物的表达水平(例如肿瘤细胞和/或肿瘤浸润性免疫细胞中的PD-L1表达水平)，治疗NSCLC的方法，确定罹患NSCLC的患者是否有可能响应包含PD-L1轴结合拮抗剂的治疗的方法，预测罹患NSCLC的患者对包含PD-L1轴结合拮抗剂的治疗的响应性的方法，和为罹患NSCLC的患者选择疗法的方法。