(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
25 June 2015 (25.06.2015)

WIPO | PCT

(10) International Publication Number
WO 2015/094992 A1

(51) International Patent Classification:
C12Q 1/68 (2006.01)

(21) International Application Number:
PCT/US2014/070232

(22) International Filing Date:
15 December 2014 (15.12.2014)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/917,270 17 December 2013 (17.12.2013) US

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

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(54) Title: IFN-GAMMA GENE SIGNATURE BIOMARKERS OF TUMOR RESPONSE TO PD-1 ANTAGONISTS

(57) Abstract: The present disclosure describes IFN-γ gene signature biomarkers that are useful for identifying cancer patients who are most likely to benefit from treatment with a PD-1 antagonist. The disclosure also provides methods and kits for testing tumor samples for the biomarkers, as well as methods for treating subjects with a PD-1 antagonist based on the test results.

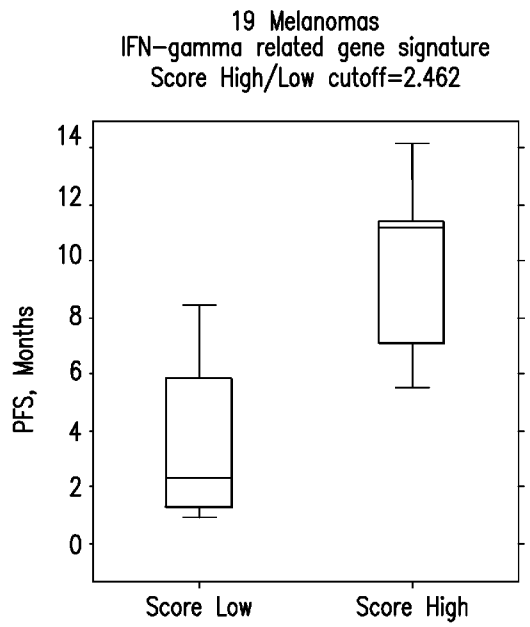

FIG.11

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(84) **Designated States** *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

Published:

— *with international search report (Art. 21(3))*

— *with sequence listing part of description (Rule 5.2(a))*

IFN-GAMMA GENE SIGNATURE BIOMARKERS OF TUMOR RESPONSE TO PD-1 ANTAGONISTS

FIELD OF THE INVENTION

The present invention relates generally to the treatment of cancer. In particular, the invention relates to methods for identifying patients who are likely to respond to treatment with an antagonist of Programmed Death 1 (PD-1).

BACKGROUND OF THE INVENTION

PD-1 is recognized as an important player in immune regulation and the maintenance of peripheral tolerance. PD-1 is moderately expressed on naive T, B and NKT cells and up-regulated by T/B cell receptor signaling on lymphocytes, monocytes and myeloid cells (1).

Two known ligands for PD-1, PD-L1 (B7-H1) and PD-L2 (B7-DC), are expressed in human cancers arising in various tissues. In large sample sets of *e.g.* ovarian, renal, colorectal, pancreatic, liver cancers and melanoma, it was shown that PD-L1 expression correlated with poor prognosis and reduced overall survival irrespective of subsequent treatment (2-13). Similarly, PD-1 expression on tumor infiltrating lymphocytes was found to mark dysfunctional T cells in breast cancer and melanoma (14-15) and to correlate with poor prognosis in renal cancer (16). Thus, it has been proposed that PD-L1 expressing tumor cells interact with PD-1 expressing T cells to attenuate T cell activation and evasion of immune surveillance, thereby contributing to an impaired immune response against the tumor.

One important aspect of PD-1 signaling may involve dependency on the interferon-gamma (IFN-γ or IFNG) / STAT1 (a key transcription factor) signaling pathway. IFN-γ, also called immune or type II interferon, is a pleiotropic cytokine involved in the regulation of nearly all phases of immune and inflammatory responses, including the activation, growth and differentiation of T-cells, B-cells, macrophages, NK cells and other cell types such as endothelial cells and fibroblasts. This cytokine enhances MHC expression on antigen-presenting cells, and also plays an important role in activating lymphocytes to enhance anti-tumor effects.

Several monoclonal antibodies that inhibit the interaction between PD-1 and one or both of its ligands PD-L1 and PD-L2 are in clinical development for treating cancer. These include nivolumab and MK-3475, which are antibodies that bind to PD-1, and MPDL3280A, which binds to PD-L1. While clinical studies with these antibodies have produced durable anti-tumor responses in some cancer types, a significant number of patients failed to exhibit

an anti-tumor response. Thus, a need exists for diagnostic tools to identify which cancer patients are most likely to achieve a clinical benefit to treatment with a PD-1 antagonist.

An active area in cancer research is the identification of gene expression patterns, commonly referred to as gene signatures or molecular signatures, which are characteristic of particular types or subtypes of cancer, and which may be associated with clinical outcomes.

SUMMARY OF THE INVENTION

The present invention provides IFN-γ gene signature biomarkers that are predictive of tumor response to therapy with PD-1 antagonists. A biomarker of the invention is a composite intratumoral RNA expression score (a "gene signature score") for a gene signature which comprises a specific set of at least about 5 to about 10 of the genes listed in Table 1 below. Each of the genes in Table 1 has a biological relationship to IFN-γ signaling and thus is referred to herein as an IFNG-related gene.

Table 1: IFNG-related Genes for IFN-γ Gene Signatures

Gene	Target Transcript
CCL4	NM_002984.2
CCL5	NM_002985.2
CCR5	NM_000579.1
CD2	NM_001767.2
CD86	NM_175862.3
CIITA	NM_000246.3
CXCL10	NM_001565.1
CXCL11	NM_005409.3
CXCL9	NM_002416.1
GZMA	NM_006144
HLA-DRA	NM_019111.3
IDO1	NM_002164.3
IFNG	NM_000619.2
KLRK1	NM_007360.1
PRF1	NM_001083116
STAT1	NM_007315.2

One exemplary IFN-γ gene signature of the invention comprises STAT1, CCR5, CXCL9, PRF1, and HLA-DRA. However, other combinations of at least about five of the genes in Table 1 may be selected for use as predictive IFN-γ gene signature biomarkers. One preferred IFN-γ gene signature of the invention consists of IFNG, STAT1, CCR5, CXCL9, PRF1, HLA-DRA, CXCL10, CXCL11, ID01 and GZMA.

The IFN-γ gene signature score for a tumor sample of interest is calculated as the arithmetic mean of normalized RNA expression levels, in the tumor sample, for each of the genes in the gene signature. Typically, the tumor sample is from a subject who is treatment naïve for anti-PD-1 therapy. To assess whether such a subject's tumor is likely to respond to a PD-1 antagonist, the calculated score for the tumor sample is compared to a reference score for the IFN-γ gene signature that has been pre-selected to divide at least the majority of responders to anti-PD-1 therapy from at least the majority of non-responders to anti-PD-1 therapy. If the subject has an IFN-γ gene signature score that is equal to or a greater than the reference IFN-γ gene signature score, the subject is more likely to respond, or to achieve a better response, to the PD-1 antagonist than if the subject's IFN-γ gene signature score is less than the reference score. The inventors contemplate that determining a subject's IFN-γ gene signature score will be useful in a variety of research and clinical applications.

Thus, in one aspect, the invention provides a method for testing a tumor for the presence or absence of a biomarker that predicts response to treatment with a PD-1 antagonist. The method comprises obtaining a sample from the tumor, measuring the RNA expression level in the tumor sample for each gene in an IFN-γ gene signature, and calculating a score for the IFN-γ gene signature from the measured RNA expression levels. In some embodiments, the method further comprises comparing the calculated score to a reference score for the IFN-γ gene signature, and classifying the tumor as biomarker positive or biomarker negative. If the calculated score is equal to or greater than the reference score, then the tumor is classified as biomarker positive, and if the calculated IFN-γ gene signature score is less than the reference IFN-γ gene signature score, then the tumor is classified as biomarker negative.

In another aspect, the invention provides a method for treating a subject having a tumor which comprises determining if the tumor is positive or negative for a IFN-γ gene signature biomarker and administering to the subject a PD-1 antagonist if the tumor is positive for the biomarker and administering to the subject a cancer treatment that does not include a PD-1 antagonist if the tumor is negative for the biomarker.

In yet another aspect, the invention provides a method for treating a subject having a tumor which comprises obtaining a sample from the tumor, measuring the expression level in the tumor sample for each gene in a IFN-γ gene signature, calculating a score for the IFN-γ gene signature from the measured expression levels, and administering to the subject a PD-1 antagonist if the calculated score is equal to or greater than a reference score for the IFN-γ

gene signature or administering to the subject a cancer therapy that does not contain a PD-1 antagonist if the calculated score is less than the reference score. In some preferred embodiments, the reference score is pre-selected to divide the majority of responders to the PD-1 antagonist from the majority of non-responders to the PD-1 antagonist. In other preferred embodiments, the reference score is pre-selected to divide the majority of good responders to the PD-1 antagonist from the majority of poor responders to the PD-1 antagonist.

In a still further aspect, the invention provides a pharmaceutical composition comprising a PD-1 antagonist for use in a subject who has a tumor that tests positive for an IFN-γ gene signature biomarker.

Yet another aspect of the invention is a drug product which comprises a pharmaceutical composition and prescribing information. The pharmaceutical composition comprises a PD-1 antagonist and at least one pharmaceutically acceptable excipient. The prescribing information states that the pharmaceutical composition is indicated for use in a subject who has a tumor that tests positive for an IFN-γ gene signature biomarker.

In another aspect, the invention provides a kit useful for assaying a tumor sample to determine an IFN-γ gene signature score for the tumor sample. The kit comprises a first set of probes for detecting expression of each gene in the IFN-γ gene signature. The kit comprises, for each target transcript in the gene signature, at least one probe for the target transcript. In some preferred embodiments, the target transcripts are the transcripts listed in Table 1 for IFNG, STAT1, CCR5, CXCL9, PRF1, HLA-DRA, CXCL10, CXCL11, ID01 and GZMA. In other preferred embodiments, the kit may also comprise a second set of probes for detecting expression of a set of normalization genes. The normalization gene set consists of 10 to 1000 genes, e.g., this gene set may consist of at least any of 25, 50, 75, 100, 150, 200, 300, 400, 500, 600, 700, 800 or 900 genes. The kit may also comprise a plurality of control tumor samples which may be assayed for expression of the IFN-γ gene signature and normalization genes in the same manner as the test tumor sample.

In some preferred embodiments of any of the above aspects of the invention, the test and reference IFN-γ gene signature scores are determined by performing quantile normalization of raw RNA expression values for the genes in the gene signature relative to the distribution of raw RNA expression values for a set of at least 200, 250, 300, 350 or 400 normalization genes, followed by a subsequent log10-transformation. In such embodiments, a reference score for an IFN-γ gene signature of IFNG, STAT1, CCR5, CXCL9, PRF1, HLA-

DRA, CXCL10, CXCL11, ID01 and GZMA is preferably between 2.255 and 2.483, between 2.305 and 2.473, between 2.450 and 2.473, or is about 2.462. In other embodiments, the reference score for an IFN-γ gene signature of STAT1, CCR5, CXCL9, PRF1, and HLA-DRA is preferably about 3.021.

In all of the above aspects and embodiments of the invention, the PD-1 antagonist inhibits the binding of PD-L1 to PD-1, and preferably also inhibits the binding of PD-L2 to PD-1. In some preferred embodiments, the PD-1 antagonist is a monoclonal antibody, or an antigen binding fragment thereof, which specifically binds to PD-1 or to PD-L1 and blocks the binding of PD-L1 to PD-1. In particularly preferred embodiments, the PD-1 antagonist is an anti-PD-1 antibody which comprises a heavy chain and a light chain, wherein the heavy and light chains comprise the amino acid sequences shown in Figure 6 (SEQ ID NO:21 and SEQ ID NO:22).

In some embodiments of any of the above aspects of the invention, the subject is a human and the cancer is a solid tumor and in some preferred embodiments, the solid tumor is bladder cancer, breast cancer, clear cell kidney cancer, head/neck squamous cell carcinoma, lung squamous cell carcinoma, malignant melanoma, non-small-cell lung cancer (NSCLC), ovarian cancer, pancreatic cancer, prostate cancer, renal cell cancer, small-cell lung cancer (SCLC) or triple negative breast cancer. In some particularly preferred embodiments, the human subject has ipilimumab-naïve advanced melanoma, while in other particularly preferred embodiments the human subject has ipilimumab-refractory advanced melanoma.

In other particularly preferred embodiments of any of the above aspects of the invention, the tumor is metastatic melanoma, the PD-1 antagonist is MK-3475, the IFN-γ gene signature consists essentially of IFNG, STAT1, CCR5, CXCL9, PRF1, HLA-DRA, CXCL10, CXCL11, ID01 and GZMA, and the reference score is 2.462.

In other particularly preferred embodiments of any of the above aspects of the invention, a responder achieves a partial response (PR) or complete response (CR) as measured by RECIST 1.1 criteria, and a non-responder does not achieve either a PR or CR.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGURE 1 shows amino acid sequences of the light chain and heavy chain CDRs for an exemplary anti-PD-1 monoclonal antibody useful in the present invention (SEQ ID NOs:1-6).

FIGURE 2 shows amino acid sequences of the light chain and heavy chain CDRs for another exemplary anti-PD-1 monoclonal antibody useful in the present invention (SEQ ID NOs:7-12).

FIGURE 3 shows amino acid sequences of the heavy chain variable region and full length heavy chain for an exemplary anti-PD-1 monoclonal antibody useful in the present invention (SEQ ID NO:13 and SEQ ID NO:14).

FIGURE 4 shows amino acid sequences of alternative light chain variable regions for an exemplary anti-PD-1 monoclonal antibody useful in the present invention (SEQ ID NOs:15-17).

FIGURE 5 shows amino acid sequences of alternative light chains for an exemplary anti-PD-1 monoclonal antibody useful in the present invention (SEQ ID NOs:18-20).

FIGURE 6 shows amino acid sequences of the heavy and light chains for MK-3475 (SEQ ID NOs. 21 and 22, respectively).

FIGURE 7 shows amino acid sequences of the heavy and light chains for nivolumab (SEQ ID NOs. 23 and 24, respectively).

FIGURE 8 shows a bar graph of response rates in a cohort of 19 melanoma patients treated with MK-3475 and who were classified as having either a low score or a high score for a preferred five-gene IFN-γ gene signature of the invention (STAT1, CCR5, CXCL9, PRF1, and HLA-DRA) based on a reference score (cut-off) of 3.021.

FIGURE 9 shows a box plot graph of PFS (in months) in a cohort of 19 melanoma patients treated with MK-3475 and who were classified as having either a low score or a high score for a preferred five-gene IFN-γ gene signature of the invention (STAT1, CCR5, CXCL9, PRF1, and HLA-DRA) based on a reference score (cut-off) of 3.021.

FIGURE 10 shows a bar graph of response rates in a cohort of 19 melanoma patients treated with MK-3475 and who were classified as having either a low score or a high score for a ten-gene IFN-γ gene signature (IFNG, STAT1, CCR5, CXCL9, PRF1, HLA-DRA, CXCL10, CXCL11, ID01 and GZMA) based on a reference score (cut-off) of 2.462.

FIGURE 11 shows a box plot graph of PFS (in months) in a cohort of 19 melanoma patients treated with MK-3475 and who were classified as having either a low score or a high score for a ten-gene IFN-γ gene signature (IFNG, STAT1, CCR5, CXCL9, PRF1, HLA-DRA, CXCL10, CXCL11, ID01 and GZMA) based on a reference score (cut-off) of 2.462.

DETAILED DESCRIPTION

Abbreviations. Throughout the detailed description and examples of the invention the following abbreviations will be used:

CCR5	Chemokine (C-C motif) receptor 5
CDR	Complementarity determining region
CHO	Chinese hamster ovary
CR	Complete Response
CXCL9	Chemokine (C-X-C motif) ligand 9
CXCL10	Chemokine (C-X-C motif) ligand 10
CXCL11	Chemokine (C-X-C motif) ligand 11
DFS	Disease free survival
FFPE	Formalin-fixed, paraffin-embedded
FR	Framework region
GZMA	Granzyme A
HLA-DRA	Major histocompatibility complex, class II, DR alpha
IDO1	Indoleamine 2,3-dioxygenase 1
IgG	Immunoglobulin G
IFNG or IFN-γ	Interferon gamma
IHC	Immunohistochemistry or immunohistochemical
LAG3	Lymphocyte activation gene 3
OR	Overall response
NCBI	National Center for Biotechnology Information
OS	Overall survival
PD	Progressive Disease
PD-1	Programmed Death 1
PD-L1	Programmed Cell Death 1 Ligand 1
PD-L2	Programmed Cell Death 1 Ligand 2
PFS	Progression free survival (PFS)
PR	Partial Response
PRF1	Perforin 1 (pore forming protein)
Q2W	One dose every two weeks
Q3W	One dose every three weeks
RECIST	Response Evaluation Criteria in Solid Tumors

SD	Stable Disease
STAT1	Signal transducer and activator of transcription 1
VH	Immunoglobulin heavy chain variable region
VK	Immunoglobulin kappa light chain variable region

I. DEFINITIONS

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

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

"About" when used to modify a numerically defined parameter (e.g., the gene signature score for a gene signature discussed herein, or the dosage of a PD-1 antagonist, or the length of treatment time with a PD-1 antagonist) means that the parameter may vary by as much as 10% above or below the stated numerical value for that parameter. For example, a gene signature consisting of about 10 genes may have between 9 and 11 genes. Similarly, a reference gene signature score of about 2.462 includes scores of and any score between 2.2158 and 2.708.

"Administration" and "treatment," as it applies to an animal, human, experimental subject, cell, tissue, organ, or biological fluid, refers to contact of an exogenous pharmaceutical, therapeutic, diagnostic agent, or composition to the animal, human, subject, cell, tissue, organ, or biological fluid. Treatment of a cell encompasses contact of a reagent to the cell, as well as contact of a reagent to a fluid, where the fluid is in contact with the cell. "Administration" and "treatment" also means *in vitro* and *ex vivo* treatments, e.g., of a cell, by a reagent, diagnostic, binding compound, or by another cell. The term "subject" includes any organism, preferably an animal, more preferably a mammal (*e.g.*, rat, mouse, dog, cat, rabbit) and most preferably a human.

As used herein, the term "antibody" refers to any form of antibody that exhibits the desired biological or binding activity. Thus, it is used in the broadest sense and specifically covers, but is not limited to, monoclonal antibodies (including full length monoclonal antibodies), polyclonal antibodies, multispecific antibodies (*e.g.*, bispecific antibodies),

humanized, fully human antibodies, chimeric antibodies and camelized single domain antibodies. "Parental antibodies" are antibodies obtained by exposure of an immune system to an antigen prior to modification of the antibodies for an intended use, such as humanization of an antibody for use as a human therapeutic.

In general, the basic antibody structural unit comprises a tetramer. Each tetramer includes two identical pairs of polypeptide chains, each pair having one "light" (about 25 kDa) and one "heavy" chain (about 50-70 kDa). The amino-terminal portion of each chain includes a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The carboxy-terminal portion of the heavy chain may define a constant region primarily responsible for effector function. Typically, human light chains are classified as kappa and lambda light chains. Furthermore, human heavy chains are typically classified as mu, delta, gamma, alpha, or epsilon, and define the antibody's isotype as IgM, IgD, IgG, IgA, and IgE, respectively. Within light and heavy chains, the variable and constant regions are joined by a "J" region of about 12 or more amino acids, with the heavy chain also including a "D" region of about 10 more amino acids. See generally, Fundamental Immunology Ch. 7 (Paul, W., ed., 2nd ed. Raven Press, N.Y. (1989).

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

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

As used herein, the term "hypervariable region" refers to the amino acid residues of an antibody that are responsible for antigen-binding. The hypervariable region comprises amino acid residues from a "complementarity determining region" or "CDR" (*i.e.* CDRL1, CDRL2

and CDRL3 in the light chain variable domain and CDRH1, CDRH2 and CDRH3 in the heavy chain variable domain). See Kabat *et al.* (1991) Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (defining the CDR regions of an antibody by sequence); see also Chothia and Lesk (1987) *J. Mol. Biol.* 196: 901-917 (defining the CDR regions of an antibody by structure). As used herein, the term "framework" or "FR" residues refers to those variable domain residues other than the hypervariable region residues defined herein as CDR residues.

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

An antibody that "specifically binds to" a specified target protein is an antibody that exhibits preferential binding to that target as compared to other proteins, but this specificity does not require absolute binding specificity. An antibody is considered "specific" for its intended target if its binding is determinative of the presence of the target protein in a sample, e.g. without producing undesired results such as false positives. Antibodies, or binding fragments thereof, useful in the present invention will bind to the target protein with an affinity that is at least two fold greater, preferably at least ten times greater, more preferably at least 20-times greater, and most preferably at least 100-times greater than the affinity with non-target proteins. As used herein, an antibody is said to bind specifically to a polypeptide *comprising* a given amino acid sequence, e.g. the amino acid sequence of a mature human PD-1 or human PD-L1 molecule, if it binds to polypeptides comprising that sequence but does not bind to proteins lacking that sequence.

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

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

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

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

The terms "cancer", "cancerous", or "malignant" refer to or describe the physiological condition in mammals that is typically characterized by unregulated cell growth. Examples of cancer include but are not limited to, carcinoma, lymphoma, leukemia, blastoma, and sarcoma. More particular examples of such cancers include squamous cell carcinoma, myeloma, small-cell lung cancer, non-small cell lung cancer, glioma, hodgkin's lymphoma, non-hodgkin's lymphoma, acute myeloid leukemia (AML), multiple myeloma, gastrointestinal (tract) cancer, renal cancer, ovarian cancer, liver cancer, lymphoblastic leukemia, lymphocytic leukemia, colorectal cancer, endometrial cancer, kidney cancer, prostate cancer, thyroid cancer, melanoma, chondrosarcoma, neuroblastoma, pancreatic cancer, glioblastoma multiforme, cervical cancer, brain cancer, stomach cancer, bladder cancer, hepatoma, breast cancer, colon carcinoma, and head and neck cancer. Particularly preferred cancers that may be treated in accordance with the present invention include those

characterized by elevated expression of one or both of PD-L1 and PD-L2 in tested tissue samples.

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

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

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

"Conservatively modified variants" or "conservative substitution" refers to substitutions of amino acids in a protein with other amino acids having similar characteristics (e.g. charge, side-chain size, hydrophobicity/hydrophilicity, backbone conformation and rigidity, etc.), such that the changes can frequently be made without altering the biological activity or other desired property of the protein, such as antigen affinity and/or specificity. Those of skill in this art recognize that, in general, single amino acid substitutions in non-essential regions of a polypeptide do not substantially alter biological activity (*see, e.g.*, Watson *et al.* (1987) *Molecular Biology of the Gene*, The Benjamin/Cummings Pub. Co., p. 224 (4th Ed.)). In addition, substitutions of structurally or functionally similar amino acids are less likely to disrupt biological activity. Exemplary conservative substitutions are set forth in Table 2 below.

TABLE 2. Exemplary Conservative Amino Acid Substitutions

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

"Comprising" or variations such as "comprise", "comprises" or "comprised of" are used throughout the specification and claims in an inclusive sense, i.e., to specify the presence of the stated features but not to preclude the presence or addition of further features that may materially enhance the operation or utility of any of the embodiments of the invention, unless the context requires otherwise due to express language or necessary implication.

"Consists essentially of," and variations such as "consist essentially of" or "consisting essentially of," as used throughout the specification and claims, indicate the inclusion of any recited elements or group of elements, and the optional inclusion of other elements, of similar or different nature than the recited elements, that do not materially change the basic or novel properties of the specified dosage regimen, method, or composition. As a non-limiting example, if a gene signature score is defined as the composite RNA expression score for a set of genes that consists of a specified list of genes, the skilled artisan will understand that this gene signature score could include the RNA expression level determined for one or more additional genes, preferably no more than three additional genes, if such inclusion does not materially affect the predictive power.

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

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

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

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

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

"Monoclonal antibody" or "mAb" or "Mab", as used herein, refers to a population of substantially homogeneous antibodies, *i.e.*, the antibody molecules comprising the population are identical in amino acid sequence except for possible naturally occurring mutations that may be present in minor amounts. In contrast, conventional (polyclonal) antibody preparations typically include a multitude of different antibodies having different amino acid sequences in their variable domains, particularly their CDRs, which are often specific for different epitopes. The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by the hybridoma method first described by Kohler *et al.* (1975) *Nature* 256: 495, or may be made by recombinant DNA methods (*see, e.g.*, U.S. Pat. No. 4,816,567). The "monoclonal antibodies" may also be isolated from phage antibody libraries using the techniques described in Clackson *et al.* (1991) *Nature* 352: 624-628 and Marks *et al.* (1991) *J. Mol. Biol.* 222: 581-597, for example. *See also* Presta (2005) *J. Allergy Clin. Immunol.* 116:731.

"Oligonucleotide" refers to a nucleic acid that is usually between 5 and 100 contiguous bases in length, and most frequently between 10-50, 10-40, 10-30, 10-25, 10-20, 15-50, 15-40, 15-30, 15-25, 15-20, 20-50, 20-40, 20-30 or 20-25 contiguous bases in length.

"Patient" or "subject" refers to any single subject for which therapy is desired or that is participating in a clinical trial, epidemiological study or used as a control, including humans and mammalian veterinary patients such as cattle, horses, dogs, and cats.

"PD-1 antagonist" means any chemical compound or biological molecule that blocks binding of PD-L1 expressed on a cancer cell to PD-1 expressed on an immune cell (T cell, B cell or NKT cell) and preferably also blocks binding of PD-L2 expressed on a cancer cell to the immune-cell expressed PD-1. Alternative names or synonyms for PD-1 and its ligands include: PDCD1, PD1, CD279 and SLEB2 for PD-1; PDCD1L1, PDL1, B7H1, B7-4, CD274 and B7-H for PD-L1; and PDCD1L2, PDL2, B7-DC, Btdc and CD273 for PD-L2. In any of the various aspects and embodiments of the present invention in which a human individual is being treated, the PD-1 antagonist blocks binding of human PD-L1 to human PD-1, and preferably blocks binding of both human PD-L1 and PD-L2 to human PD-1. Human PD-1 amino acid sequences can be found in NCBI Locus No.: NP_005009. Human PD-L1 and PD-L2 amino acid sequences can be found in NCBI Locus No.: NP_054862 and NP_079515, respectively.

PD-1 antagonists useful in the any of the various aspects and embodiments of the present invention include a monoclonal antibody (mAb), or antigen binding fragment thereof, which specifically binds to PD-1 or PD-L1, and preferably specifically binds to human PD-1 or human PD-L1. The mAb may be a human antibody, a humanized antibody or a chimeric antibody, and may include a human constant region. In some embodiments, the human constant region is selected from the group consisting of IgG1, IgG2, IgG3 and IgG4 constant regions, and in preferred embodiments, the human constant region is an IgG1 or IgG4 constant region. In some embodiments, the antigen binding fragment is selected from the group consisting of Fab, Fab'-SH, $F(ab')_2$, scFv and Fv fragments.

Examples of mAbs that bind to human PD-1, and useful in the various aspects and embodiments of the present invention, are described in US7521051, US8008449, and US8354509. Specific anti-human PD-1 mAbs useful as the PD-1 antagonist various aspects and embodiments of the present invention include: MK-3475, a humanized IgG4 mAb with the structure described in *WHO Drug Information*, Vol. 27, No. 2, pages 161-162 (2013) and which comprises the heavy and light chain amino acid sequences shown in Figure 6, nivolumab (BMS-936558), a human IgG4 mAb with the structure described in *WHO Drug Information*, Vol. 27, No. 1, pages 68-69 (2013) and which comprises the heavy and light chain amino acid sequences shown in Figure 7; pidilizumab (CT-011, also known as hBAT or hBAT-1); and the humanized antibodies h409A11, h409A16 and h409A17, which are described in WO2008/156712.

Examples of mAbs that bind to human PD-L1, and useful in any of the various aspects and embodiments of the present invention, are described in WO2013/019906, W02010/077634 A1 and US8383796. Specific anti-human PD-L1 mAbs useful as the PD-1 antagonist in the various aspects and embodiments of the present invention include MPDL3280A, BMS-936559, MEDI4736, MSB0010718C and an antibody which comprises the heavy chain and light chain variable regions of SEQ ID NO:24 and SEQ ID NO:21, respectively, of WO2013/019906.

Other PD-1 antagonists useful in any of the various aspects and embodiments of the present invention include an immunoadhesin that specifically binds to PD-1 or PD-L1, and preferably specifically binds to human PD-1 or human PD-L1, e.g., a fusion protein containing the extracellular or PD-1 binding portion of PD-L1 or PD-L2 fused to a constant region such as an Fc region of an immunoglobulin molecule. Examples of immunoadhesion molecules that specifically bind to PD-1 are described in WO2010/027827 and WO2011/066342. Specific fusion proteins useful as the PD-1 antagonist in the treatment method, medicaments and uses of the present invention include AMP-224 (also known as B7-DCIg), which is a PD-L2-FC fusion protein and binds to human PD-1.

In some preferred embodiments of the various aspects of the present invention, the PD-1 antagonist is a monoclonal antibody, or antigen binding fragment thereof, which comprises: (a) light chain CDRs SEQ ID NOs: 1, 2 and 3 and heavy chain CDRs SEQ ID NOs: 4, 5 and 6; or (b) light chain CDRs SEQ ID NOs: 7, 8 and 9 and heavy chain CDRs SEQ ID NOs: 10, 11 and 12.

In other preferred embodiments of the various aspects of the present invention, the PD-1 antagonist is a monoclonal antibody, or antigen binding fragment thereof, which specifically binds to human PD-1 and comprises (a) a heavy chain variable region comprising SEQ ID NO:13 or a variant thereof, and (b) a light chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NO:15 or a variant thereof; SEQ ID NO:16 or a variant thereof; and SEQ ID NO: 17 or a variant thereof. A variant of a heavy chain variable region sequence is identical to the reference sequence except having up to 17 conservative amino acid substitutions in the framework region (i.e., outside of the CDRs), and preferably has less than ten, nine, eight, seven, six or five conservative amino acid substitutions in the framework region. A variant of a light chain variable region sequence is identical to the reference sequence except having up to five conservative amino acid

substitutions in the framework region (i.e., outside of the CDRs), and preferably has less than four, three or two conservative amino acid substitution in the framework region.

In another preferred embodiment of the various aspects of the present invention, the PD-1 antagonist is a monoclonal antibody which specifically binds to human PD-1 and comprises (a) a heavy chain comprising SEQ ID NO: 14 and (b) a light chain comprising SEQ ID NO:18, SEQ ID NO:19 or SEQ ID NO:20.

In yet another preferred embodiment of the aspects of the present invention, the PD-1 antagonist is a monoclonal antibody which specifically binds to human PD-1 and comprises (a) a heavy chain comprising SEQ ID NO: 14 and (b) a light chain comprising SEQ ID NO:18.

Table 3 below provides a list of the amino acid sequences of exemplary anti-PD-1 mAbs for use in the various aspects of the present invention, and the sequences are shown in Figures 1-5.

Table 3. Exemplary anti-human PD-1 antibodies	
A. Comprises light and heavy chain CDRs of hPD-1.08A in WO2008/156712	
CDRL1	SEQ ID NO:1
CDRL2	SEQ ID NO:2
CDRL3	SEQ ID NO:3
CDRH1	SEQ ID NO:4
CDRH2	SEQ ID NO:5
CDRH3	SEQ ID NO:6
B. Comprises light and heavy chain CDRs of hPD-1.09A in WO2008/156712	
CDRL1	SEQ ID NO:7
CDRL2	SEQ ID NO:8
CDRL3	SEQ ID NO:9
CDRH1	SEQ ID NO:10
CDRH2	SEQ ID NO:11
CDRH3	SEQ ID NO:12
C. Comprises the mature h109A heavy chain variable region and one of the mature K09A light chain variable regions in WO2008/156712	
Heavy chain VR	SEQ ID NO:13
Light chain VR	SEQ ID NO:15 or SEQ ID NO:16 or SEQ ID NO:17
D. Comprises the mature 409 heavy chain and one of the mature K09A light chains in WO2008/156712	
Heavy chain	SEQ ID NO:14
Light chain	SEQ ID NO:18 or SEQ ID NO:19 or SEQ ID NO:20

"Probe" as used herein means an oligonucleotide that is capable of specifically hybridizing under stringent hybridization conditions to a transcript expressed by a gene of interest listed in Table 1 or Table 4, and in some preferred embodiments, specifically hybridizes under stringent hybridization conditions to the particular transcript listed in Table 1 or Table 4 for the gene of interest.

"RECIST 1.1 Response Criteria" as used herein means the definitions set forth in Eisenhauer et al., E.A. et al., Eur. J Cancer 45:228-247 (2009) for target lesions or nontarget lesions, as appropriate based on the context in which response is being measured.

"Reference IFN-γ gene signature score" as used herein means the score for an IFN-γ gene signature that has been determined to divide at least the majority of responders from at least the majority of non-responders in a reference population of subjects who have the same tumor type as a test subject and who have been treated with a PD-1 antagonist. Preferably, at least any of 60%, 70%, 80%, or 90% of responders in the reference population will have an IFN-γ gene signature score that is above the selected reference score, while the IFN-γ gene signature score for at least any of 60%, 70% 80%, 90% or 95% of the non-responders in the reference population will be lower than the selected reference score. In some embodiments, the negative predictive value of the reference score is greater than the positive predictive value. In some preferred embodiments, responders in the reference population are defined as subjects who achieved a partial response (PR) or complete response (CR) as measured by RECIST 1.1 criteria and non-responders are defined as not achieving any RECIST 1.1 clinical response. In particularly preferred embodiments, subjects in the reference population were treated with substantially the same anti-PD-1 therapy as that being considered for the test subject, i.e., administration of the same PD-1 antagonist using the same or a substantially similar dosage regimen.

"Sample" when referring to a tumor or any other biological material referenced herein, means a sample that has been removed from the subject; thus, none of the testing methods described herein are performed in or on the subject.

"Sustained response" means a sustained therapeutic effect after cessation of treatment with a therapeutic agent, or a combination therapy described herein. In some embodiments, the sustained response has a duration that is at least the same as the treatment duration, or at least 1.5, 2.0, 2.5 or 3 times longer than the treatment duration.

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

"Treat" or "treating" a cancer as used herein means to administer a PD-1 antagonist other therapeutic agent to a subject having a cancer, or diagnosed with a cancer, to achieve at least one positive therapeutic effect, such as for example, reduced number of cancer cells, reduced tumor size, reduced rate of cancer cell infiltration into peripheral organs, or reduced rate of tumor metastasis or tumor growth. Positive therapeutic effects in cancer can be measured in a number of ways (See, W. A. Weber, J. Null. Med. 50:1S-10S (2009); Eisenhauer et al., *supra*). In some preferred embodiments, response to a PD-1 antagonist is assessed using RECIST 1.1 criteria. In some embodiments, the treatment achieved by a therapeutically effective amount is any of PR, CR, PFS, DFS, OR or OS. In some preferred embodiments, a gene signature biomarker of the invention predicts whether a subject with a solid tumor is likely to achieve a PR or a CR. The dosage regimen of a therapy described herein that is effective to treat a cancer patient may vary according to factors such as the disease state, age, and weight of the patient, and the ability of the therapy to elicit an anti-cancer response in the subject. While an embodiment of the treatment method, medicaments and uses of the present invention may not be effective in achieving a positive therapeutic effect in every subject, it should do so in a statistically significant number of subjects as determined by any statistical test known in the art such as the Student's t-test, the chi^2-test, the U-test according to Mann and Whitney, the Kruskal-Wallis test (H-test), Jonckheere-Terpstra-test and the Wilcoxon-test.

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

"Tumor burden" also referred to as "tumor load", refers to the total amount of tumor material distributed throughout the body. Tumor burden refers to the total number of cancer cells or the total size of tumor(s), throughout the body, including lymph nodes and bone narrow. Tumor burden can be determined by a variety of methods known in the art, such as, e.g. by measuring the dimensions of tumor(s) upon removal from the subject, e.g., using

calipers, or while in the body using imaging techniques, e.g., ultrasound, bone scan, computed tomography (CT) or magnetic resonance imaging (MRI) scans.

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

"Variable regions" or "V region" as used herein means the segment of IgG chains which is variable in sequence between different antibodies. It extends to Kabat residue 109 in the light chain and 113 in the heavy chain.

II. UTILITY OF GENE SIGNATURE BIOMARKERS OF THE INVENTION

An IFN-γ gene signature biomarker described herein is useful to identify cancer patients who are most likely to achieve a clinical benefit from treatment with a PD-1 antagonist. This utility supports the use of these biomarkers in a variety of research and commercial applications, including but not limited to, clinical trials of PD-1 antagonists in which patients are selected on the basis of their IFN-γ gene signature score, diagnostic methods and products for determining a patient's IFN-γ gene signature score or for classifying a patient as positive or negative for a IFN-γ gene signature biomarker, personalized treatment methods which involve tailoring a patient's drug therapy based on the patient's IFN-γ gene signature score, as well as pharmaceutical compositions and drug products comprising a PD-1 antagonist for use in treating patients who test positive for a IFN-γ gene signature biomarker.

The utility of any of the applications claimed herein does not require that 100% of the patients who test positive for a biomarker of the invention achieve an anti-tumor response to a PD-1 antagonist; nor does it require a diagnostic method or kit to have a specific degree of specificity or sensitivity in determining the presence or absence of a biomarker in every subject, nor does it require that a diagnostic method claimed herein be 100% accurate in predicting for every subject whether the subject is likely to have a beneficial response to a PD-1 antagonist. Thus, the inventors herein intend that the terms "determine", "determining" and "predicting" should not be interpreted as requiring a definite or certain result; instead these terms should be construed as meaning either that a claimed method provides an

accurate result for at least the majority of subjects or that the result or prediction for any given subject is more likely to be correct than incorrect.

Preferably, the accuracy of the result provided by a diagnostic method of the invention is one that a skilled artisan or regulatory authority would consider suitable for the particular application in which the method is used.

Similarly, the utility of the claimed drug products and treatment methods does not require that the claimed or desired effect is produced in every cancer patient; all that is required is that a clinical practitioner, when applying his or her professional judgment consistent with all applicable norms, decides that the chance of achieving the claimed effect of treating a given patient according to the claimed method or with the claimed composition or drug product.

A. *Testing for Biomarkers of the Invention*

An IFN-γ gene signature score is determined in a sample of tumor tissue removed from a subject. The tumor may be primary or recurrent, and may be of any type (as described above), any stage (e.g., Stage I, II, III, or IV or an equivalent of other staging system), and/or histology. The subject may be of any age, gender, treatment history and/or extent and duration of remission.

The tumor sample can be obtained by a variety of procedures including, but not limited to, surgical excision, aspiration or biopsy. The tissue sample may be sectioned and assayed as a fresh specimen; alternatively, the tissue sample may be frozen for further sectioning. In some preferred embodiments, the tissue sample is preserved by fixing and embedding in paraffin or the like.

The tumor tissue sample may be fixed by conventional methodology, with the length of fixation depending on the size of the tissue sample and the fixative used. Neutral buffered formalin, glutaraldehyde, Bouin's and paraformaldehyde are nonlimiting examples of fixatives. In preferred embodiments, the tissue sample is fixed with formalin. In some embodiments, the fixed tissue sample is also embedded in paraffin to prepare an FFPE tissue sample.

Typically, the tissue sample is fixed and dehydrated through an ascending series of alcohols, infiltrated and embedded with paraffin or other sectioning media so that the tissue sample may be sectioned. Alternatively, the tumor tissue sample is first sectioned and then the individual sections are fixed.

In some preferred embodiments, the IFN-γ gene signature score for a tumor is determined using FFPE tissue sections of about 3-4 millimeters, and preferably 4 micrometers, which are mounted and dried on a microscope slide.

Once a suitable sample of tumor tissue has been obtained, it is analyzed to quantitate the expression level of each of the genes that comprise the particular IFN-γ gene signature to be scored, e.g. each of IFNG, STAT1, CCR5, CXCL9, PRF1, HLA-DRA, CXCL10, CXCL11, ID01 and GZMA. The phrase "determine the expression level of a gene" as used herein refers to detecting and quantifying RNA transcribed from that gene or a protein translated from such RNA. The term "RNA transcript" includes mRNA transcribed from the gene, and/or specific spliced variants thereof and/or fragments of such mRNA and spliced variants. In preferred embodiments, the RNA transcripts whose expression is measured are the transcripts in Table 1.

A person skilled in the art will appreciate that a number of methods can be used to isolate RNA from the tissue sample for analysis. For example, RNA may be isolated from frozen tissue samples by homogenization in guanidinium isothiocyanate and acid phenol-chloroform extraction. Commercial kits are available for isolating RNA from FFPE samples.

If the tumor sample is an FFPE tissue section on a glass slide, it is preferable to perform gene expression analysis on whole cell lysates rather than on isolated total RNA. These lysates may be prepared as described in Example 1 below.

Persons skilled in the art are also aware of several methods useful for detecting and quantifying the level of RNA transcripts within the isolated RNA or whole cell lysates. Quantitative detection methods include, but are not limited to, arrays (i.e., microarrays), quantitative real time PCR (RT-PCR), multiplex assays, nuclease protection assays, and Northern blot analyses. Generally, such methods employ labeled probes that are complimentary to a portion of each transcript to be detected. Probes for use in these methods can be readily designed based on the known sequences of the genes and the transcripts expressed thereby. In some preferred embodiments, the probes are designed to hybridize to each of the gene signature transcripts identified in Table 1. Suitable labels for the probes are well-known and include, e.g., fluorescent, chemilumnescent and radioactive labels.

In some embodiments, assaying a tumor sample for a gene signature of the invention employs detection and quantification of RNA levels in real-time using nucleic acid sequence based amplification (NASBA) combined with molecular beacon detection molecules.

NASBA is described, e.g., in Compton J., *Nature* 350 (6313):91-92 (1991). NASBA is a single-step isothermal RNA-specific amplification method. Generally, the method involves the following steps: RNA template is provided to a reaction mixture, where the first primer attaches to its complementary site at the 3' end of the template; reverse transcriptase synthesizes the opposite, complementary DNA strand; RNAse H destroys the RNA template (RNAse H only destroys RNA in RNA-DNA hybrids, but not single-stranded RNA); the second primer attaches to the 3' end of the DNA strand, and reverse transcriptase synthesizes the second strand of DNA; and T7 RNA polymerase binds double-stranded DNA and produces a complementary RNA strand which can be used again in step 1, such that the reaction is cyclic.

In other embodiments, the assay format is a flap endonuclease-based format, such as the Invader™ assay (Third Wave Technologies). In the case of using the invader method, an invader probe containing a sequence specific to the region 3' to a target site, and a primary probe containing a sequence specific to the region 5' to the target site of a template and an unrelated flap sequence, are prepared. Cleavase is then allowed to act in the presence of these probes, the target molecule, as well as a FRET probe containing a sequence complementary to the flap sequence and an auto-complementary sequence that is labeled with both a fluorescent dye and a quencher. When the primary probe hybridizes with the template, the 3' end of the invader probe penetrates the target site, and this structure is cleaved by the Cleavase resulting in dissociation of the flap. The flap binds to the FRET probe and the fluorescent dye portion is cleaved by the Cleavase resulting in emission of fluorescence.

In yet other embodiments, the assay format employs direct mRNA capture with branched DNA (QuantiGene™, Panomics) or Hybrid Capture™ (Digene).

One example of an array technology suitable for use in measuring expression of the genes in an IFN-γ gene signature is the ArrayPlate™ assay technology sold by HTG Molecular, Tucson Arizona, and described in Martel, R.R., et al., Assay and Drug Development Technologies 1(1):61-71, 2002. In brief, this technology combines a nuclease protection assay with array detection. Cells in microplate wells are subjected to a nuclease protection assay. Cells are lysed in the presence of probes that bind targeted mRNA species. Upon addition of SI nuclease, excess probes and unhybridized mRNA are degraded, so that only mRNA:probe duplexes remain. Alkaline hydrolysis destroys the mRNA component of the duplexes, leaving probes intact. After the addition of a neutralization solution, the contents of the processed cell culture plate are transferred to another ArrayPlate™ called a

programmed ArrayPlate™. ArrayPlates™ contain a 16-element array at the bottom of each well. Each array element comprises a position- specific anchor oligonucleotide that remains the same from one assay to the next. The binding specificity of each of the 16 anchors is modified with an oligonucleotide, called a programming linker oligonucleotide, which is complementary at one end to an anchor and at the other end to a nuclease protection probe. During a hybridization reaction, probes transferred from the culture plate are captured by immobilized programming linker. Captured probes are labeled by hybridization with a detection linker oligonucleotide, which is in turn labeled with a detection conjugate that incorporates peroxidase. The enzyme is supplied with a chemiluminescent substrate, and the enzyme-produced light is captured in a digital image. Light intensity at an array element is a measure of the amount of corresponding target mRNA present in the original cells.

By way of further example, DNA microarrays can be used to measure gene expression. In brief, a DNA microarray, also referred to as a DNA chip, is a microscopic array of DNA fragments, such as synthetic oligonucleotides, disposed in a defined pattern on a solid support, wherein they are amenable to analysis by standard hybridization methods (see Schena, *BioEssays* 18:427 (1996)). Exemplary microarrays and methods for their manufacture and use are set forth in T.R. Hughes et al., *Nature Biotechnology* 9:342-347 (2001). A number of different microarray configurations and methods for their production are known to those of skill in the art and are disclosed in U.S. Patent Nos: 5,242,974; 5,384,261; 5,405,783; 5,412,087; 5,424,186; 5,429,807; 5,436,327; 5,445,934; 5,556,752; 5,405,783; 5,412,087; 5,424,186; 5,429,807; 5,436,327; 5,472,672; 5,527,681 ; 5,529,756; 5,545,531; 5,554,501 ; 5,561,071 ; 5,571,639; 5,593,839; 5,624,711 ; 5,700,637; 5,744,305; 5,770,456; 5,770,722; 5,837,832; 5,856,101; 5,874,219; 5,885,837; 5,919,523; 6,022,963; 6,077,674; and 6,156,501; Shena, et al., *Tibtech* 6:301-306, 1998; Duggan, et al., *Nat. Genet.* 2:10-14, 1999; Bowtell, et al., *Nat. Genet.* 21:25-32, 1999; Lipshutz, et al., *Nat. Genet.* 21:20-24, 1999; Blanchard, et al., *Biosensors and Bioelectronics* 77:687- 90, 1996; Maskos, et al., *Nucleic Acids Res.* 2:4663-69, 1993; and Hughes, et al., *Nat. Biotechnol.* 79:342-347, 2001. Patents describing methods of using arrays in various applications include: U.S. Patent Nos. 5,143,854; 5,288,644; 5,324,633; 5,432,049; 5,470,710; 5,492,806; 5,503,980; 5,510,270; 5,525,464; 5,547,839; 5,580,732; 5,661,028; 5,848,659; and 5,874,219; the disclosures of which are herein incorporated by reference.

In one embodiment, an array of oligonucleotides may be synthesized on a solid support. Exemplary solid supports include glass, plastics, polymers, metals, metalloids,

ceramics, organics, etc. Using chip masking technologies and photoprotective chemistry, it is possible to generate ordered arrays of nucleic acid probes. These arrays, which are known, for example, as "DNA chips" or very large scale immobilized polymer arrays ("VLSIPS®" arrays), may include millions of defined probe regions on a substrate having an area of about 1 cm^2 to several cm^2, thereby incorporating from a few to millions of probes (see, e.g., U.S. Patent No. 5,631,734).

To compare expression levels, labeled nucleic acids may be contacted with the array under conditions sufficient for binding between the target nucleic acid and the probe on the array. In one embodiment, the hybridization conditions may be selected to provide for the desired level of hybridization specificity; that is, conditions sufficient for hybridization to occur between the labeled nucleic acids and probes on the microarray.

Hybridization may be carried out in conditions permitting essentially specific hybridization. The length and GC content of the nucleic acid will determine the thermal melting point and thus, the hybridization conditions necessary for obtaining specific hybridization of the probe to the target nucleic acid. These factors are well known to a person of skill in the art, and may also be tested in assays. An extensive guide to nucleic acid hybridization may be found in Tijssen, et al. (Laboratory Techniques in Biochemistry and Molecular Biology, Vol. 24: Hybridization With Nucleic Acid Probes, P. Tijssen, ed.; Elsevier, N.Y. (1993)). The methods described above will result in the production of hybridization patterns of labeled target nucleic acids on the array surface. The resultant hybridization patterns of labeled nucleic acids may be visualized or detected in a variety of ways, with the particular manner of detection selected based on the particular label of the target nucleic acid. Representative detection means include scintillation counting, autoradiography, fluorescence measurement, calorimetric measurement, light emission measurement, light scattering, and the like.

One such method of detection utilizes an array scanner that is commercially available (Affymetrix, Santa Clara, Calif.), for example, the 417® Arrayer, the 418® Array Scanner, or the Agilent Gene Array® Scanner. This scanner is controlled from a system computer with an interface and easy-to-use software tools. The output may be directly imported into or directly read by a variety of software applications. Exemplary scanning devices are described in, for example, U.S. Patent Nos. 5,143,854 and 5,424,186.

A preferred assay method to measure biomarker transcript abundance includes using the nCounter® Analysis System marketed by NanoString® Technologies (Seattle, Washington

USA). This system, which is described by Geiss et al., Nature Biotechnol. 2(3):317-325 (2008), utilizes a pair of probes, namely, a capture probe and a reporter probe, each comprising a 35- to 50-base sequence complementary to the transcript to be detected. The capture probe additionally includes a short common sequence coupled to an immobilization tag, e.g. an affinity tag that allows the complex to be immobilized for data collection. The reporter probe additionally includes a detectable signal or label, e.g. is coupled to a color-coded tag. Following hybridization, excess probes are removed from the sample, and hybridized probe/target complexes are aligned and immobilized via the affinity or other tag in a cartridge. The samples are then analyzed, for example using a digital analyzer or other processor adapted for this purpose. Generally, the color-coded tag on each transcript is counted and tabulated for each target transcript to yield the expression level of each transcript in the sample. This system allows measuring the expression of hundreds of unique gene transcripts in a single multiplex assay using capture and reporter probes designed by NanoString.

In measuring expression of the genes in an IFN-γ gene signature described herein, the absolute expression of each of the genes in a tumor sample is compared to a control; for example, the control can be the average level of expression of each of the genes, respectively, in a pool of subjects. To increase the sensitivity of the comparison, however, the expression level values are preferably transformed in a number of ways.

For example, the expression level of each gene in the gene signature can be normalized by the average expression level of all of the genes, the expression level of which is determined, or by the average expression level of a set of control genes. Thus, in one embodiment, the genes are represented by a set of probes, and the expression level of each of the genes is normalized by the mean or median expression level across all of the genes represented, including any genes that are not part of the gene signature of interest. In a specific embodiment, the normalization is carried out by dividing the median or mean level of expression of all of the genes on the microarray. In another embodiment, the expression levels of the signature genes are normalized by the mean or median level of expression of a set of control genes. In a specific embodiment, the control genes comprise housekeeping genes. In another specific embodiment, the normalization is accomplished by dividing by the median or mean expression level of the control genes.

The sensitivity of a gene signature score will also be increased if the expression levels of individual genes in the gene signature are compared to the expression of the same genes in

a pool of tumor samples. Preferably, the comparison is to the mean or median expression level of each signature gene in the pool of samples. Such a comparison may be accomplished, for example, by dividing by the mean or median expression level of the pool for each of the genes from the expression level each of the genes in the subject sample of interest. This has the effect of accentuating the relative differences in expression between genes in the sample and genes in the pool as a whole, making comparisons more sensitive and more likely to produce meaningful results than the use of absolute expression levels alone. The expression level data may be transformed in any convenient way; preferably, the expression level data for all is log transformed before means or medians are taken.

In performing comparisons to a pool, two approaches may be used. First, the expression levels of the signature genes in the sample may be compared to the expression level of those genes in the pool, where nucleic acid derived from the sample and nucleic acid derived from the pool are hybridized during the course of a single experiment. Such an approach requires that a new pool of nucleic acid be generated for each comparison or limited numbers of comparisons, and is therefore limited by the amount of nucleic acid available. Alternatively, and preferably, the expression levels in a pool, whether normalized and/or transformed or not, are stored on a computer, or on computer-readable media, to be used in comparisons to the individual expression level data from the sample (i.e., single-channel data).

When comparing a subject's tumor sample with a standard or control, the expression value of a particular gene in the sample is compared to the expression value of that gene in the standard or control. For each gene in a gene signature of the invention, the log(10) ratio is created for the expression value in the individual sample relative to the standard or control. A score for an IFN-γ gene signature is calculated by determining the mean log(10) ratio of the genes in the signature. If the gene signature score for the test sample is above a pre-determined threshold for that gene signature, then the sample is considered to be positive for an IFN-γ gene signature biomarker. In one embodiment of the invention, the pre-determined threshold is set at any number between 2.17 and 2.69 (i.e., 2.18, 2.19, 2.20 . . . . 2.66, 2.67, 2.68). The pre-determined threshold may also be the mean, median, or a percentile of scores for that gene signature in a collection of samples or a pooled sample used as a standard or control.

It will be recognized by those skilled in the art that other differential expression values, besides log(10) ratio, may be used for calculating a signature score, as long as the value represents an objective measurement of transcript abundance of the genes. Examples include, but are not limited to: xdev, error-weighted log (ratio), and mean subtracted log(intensity).

In one preferred embodiment, raw expression values are normalized by performing quantile normalization relative to the reference distribution and subsequent log10-transformation. When the gene expression is detected using the nCounter® Analysis System marketed by NanoString® Technologies, the reference distribution is generated by pooling reported (i.e., raw) counts for the test sample and one or more control samples (preferably at least 2 samples, more preferably at least any of 4, 8 or 16samples) after excluding values for technical (both positive and negative control) probes and without performing intermediate normalization relying on negative (background-adjusted) or positive (synthetic sequences spiked with known titrations). The IFN-γ signature score is then calculated as the arithmetic mean of normalized values for each of the genes in the gene signature, e.g., each of STAT1, CCR5, CXCL9, PRF1, and HLA-DRA or each of IFNG, STAT1, CCR5, CXCL9, PRF1, HLA-DRA, CXCL10, CXCL11, ID01 and GZMA.

In some preferred embodiments, the reference distribution is generated from raw expression counts for a normalization set of genes, which consists essentially of each of the genes in the set of 400 genes listed in Table 4, or a subset thereof. The subset may consist of at least any of 25, 50, 75, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375 or any whole number in between 25 and 400.

Table 4. Normalization Gene Set

Gene Id	Target Transcript NCBI Accession #	Gene Id	Target Transcript NCBI Accession #
ABCF1	NM_001090.2	IL2	NM_000586.2
ALAS1	NM_000688.4	IL4	NM_000589.2
AXL	NM_021913.2	ITGAL (CD11a)	NM_002209.2
Adipoq	NM_004797.2	ITGAM (CD11b)	NM_000632.3
Areg	NM_001657.2	Icam1	NM_000201.1
Arg1	NM_000045.2	Icos	NM_012092.2
Arg2	NM_001172.3	IcosL (B7-H2)	NM_015259.4
Atp6v0d2	NM_152565.1	Id2	NM_002166.4
Atp8b4	NM_024837.2	Ido1 (Indo)	NM_002164.3
B7-H3 (CD276)	NM_001024736.1	Ifi16	NM_005531.1
B7-H4 (VTCN1)	NM_024626.2	Ifitm1	NM_003641.3

Gene Id	Target Transcript NCBI Accession #	Gene Id	Target Transcript NCBI Accession #
BAGE	NM_001187.1	Ifngr2	NM_005534.3
BCL6	NM_138931.1	Igf1	NM_000618.3
BLNK	NM_013314.2	Igj	NM_144646.3
Batf	NM_006399.3	Ikzf3	NM_012481.3
Bcl11a	NM_022893.3	Ing1	NM_198219.1
Bcl11b	NM_022898.1	Ing2	NM_001564.2
Bst1	NM_004334.2	Insr	NM_000208.1
Btla	NM_181780.2	Irf1	NM_002198.1
CADM1	NM_014333.3	Irf2	NM_002199.2
CD112	NM_002856.2	Irf4	NM_002460.1
CD113	NM_015480.2	Irf6	NM_006147.2
CD127 (IL-7RA)	NM_002185.2	Irf7	NM_001572.3
CD14	NM_000591.2	Irf8	NM_002163.2
CD155	NM_006505.3	Itga1 (CD49)	NM_181501.1
CD160	NM_007053.2	Itga2 (CD49b)	NM_002203.2
CD163	NM_004244.4	Itgae (CD103)	NM_002208.4
CD167 DDR1	NM_001954.4	Itgax	NM_000887.3
CD2	NM_001767.2	Itk	NM_005546.3
CD200	NM_005944.5	Itm2a	NM_004867.4
CD200R1	NM_138939.2	Jak3	NM_000215.2
CD207 - CLEC4K Langerin	NM_015717.2	Jakmip1	NM_001099433.1
CD209	NM_021155.2	KIR2DL1	NM_014218.2
CD22 (Siglec-2)	NM_001771.2	KLK6	NM_002774.3
CD226	NM_006566.2	KLRG2 (CLEC15b)	NM_198508.2
CD244	NM_016382.2	Klrc1 (NKG2A)	NM_002259.3
CD24a	NM_013230.2	Klrc2 (NKG2c)	NM_002260.3
CD28	NM_001243078.1	Klrd1 (CD94)	NM_002262.3
CD3 delta	NM_000732.4	Klrk1-NKG2D	NM_007360.1
CD3 epsilon	NM_000733.2	LAIR1	NM_002287.3
CD3 zeta (CD247)	NM_198053.1	LIFR	NM_002310.3
CD300a	NM_007261.2	LILRA1 (CD85I)	NM_006863.1
CD300b (CD300LB IREM3)	NM_174892.2	LILRA2 v1-2 (CD85H)	NM_001130917.1
CD300e (IREM2)	NM_181449.1	LILRA4 (CD85G)	NM_012276.3
CD300f (IREM1)	NM_139018.3	LILRA5 v3-4 (CD85F)	NM_181879.1
CD317 (Bst2)	NM_004335.2	Lag3 (CD223)	NM_002286.5
CD33	NM_001177608.1	Lamp2	NM_002294.2
CD4	NM_000616.3	Lat	NM_001014987.1
CD40 (TNFRSF5)	NM_001250.4	Lat2-linker for activation of T cells family member 2	NM_014146.3
CD40L (TNFSF5)	NM_000074.2	Lax1	NM_001136190.1

Gene Id	Target Transcript NCBI Accession #	Gene Id	Target Transcript NCBI Accession #
CD44	NM_001001392.1	Lck	NM_005356.2
CD45 (PTPRC)	NM_080921.2	Lgals3	NM_001177388.1
CD47	NM_001777.3	Lgals3BP	NM_005567.3
CD48	NM_001778.2	Lgals9- lectin	NM_002308.3
CD5	NM_014207.2	LilRB4	NM_001081438.1
CD55	NM_000574.3	Lst1	NM_001166538.1
CD62L L-selectin Sell	NR_029467.1	Ltk	NM_002344.5
CD68 (SCARD1)	NM_001251.2	Ly6e	NM_002346.2
CD69	NM_001781.1	Ly6g6c	NM_025261.2
CD7	NM_006137.6	Ly6g6d	NM_021246.2
CD72	NM_001782.2	MAGEA1-melanoma antigen family A	NM_004988.4
CD79A	NM_001783.3	MBL2	NM_000242.2
CD80	NM_005191.3	MER (MERTK)	NM_006343.2
CD84	NM_001184879.1	MLANA (Mart1)	NM_005511.1
CD86	NM_175862.3	MON1B	NM_014940.2
CD8b	NM_172099.2	MSA41 (CD20)	NM_152866.2
CD90 (Thy1)	NM_006288.2	Maf	NM_001031804.2
CD96	NM_005816.4	Mafb	NM_005461.3
CDH1 (E Cadherin)	NM_004360.2	Marco (Scara2)	NM_006770.3
CLEC12A	NM_138337.5	Mica	NM_000247.1
CLEC15a (KLRG1 MAFA)	NM_005810.3	Micb	NM_005931.3
CLEC4A	NM_194448.2	Mn1	NM_002430.2
CLEC6A	NM_001007033.1	Mrc1	NM_002438.2
CSPG4	NM_001897.4	Myh4	NM_017533.2
CXCL11 - ITAC	NM_005409.3	NCR2 - NKp44	NM_004828.3
CXCL2 (GRO-beta MIP-2)	NM_002089.3	Nfatc1	NM_172389.1
CXCL9 - Mig	NM_002416.1	Nkg7	NM_005601.3
CXCR2	NM_001557.2	Nlrp10 (NOD)	NM_176821.3
Caspase 3	NM_032991.2	Nr4a2	NM_006186.3
Ccl19	NM_006274.2	Ny-eso-1 (CTAG1B)	NM_001327.2
Ccl21	NM_002989.2	OAZ1	NM_004152.2
Ccl24	NM_002991.2	OSCAR	NM_130771.3
Ccl27	NM_006664.2	PARK7	NM_001123377.1
Ccl3	NM_002983.2	PD-1 (Pdcd1)	NM_005018.1
Ccl4	NM_002984.2	PDCD4	NM_014456.3
Ccl5	NM_002985.2	POLR1B	NM_019014.3
Ccl8	NM_005623.2	POLR2A	NM_000937.2
Ccr2	NM_001123041.2	PPARG	NM_015869.3
Ccr3	NM_001837.2	PPIA	NM_021130.2
Ccr4	NM_005508.4	Pdcd1Lg1 (PD-L1)	NM_014143.2

Gene Id	Target Transcript NCBI Accession #	Gene Id	Target Transcript NCBI Accession #
Ccr5	NM_000579.1	Pdcd1Lg2 (PD-L2)	NM_025239.3
Ccr6	NM_031409.2	Pdgfra	NM_006206.3
Ccr7	NM_001838.2	Phactr2	NM_001100164.1
Cdo1	NM_001801.2	Pi3kCA	NM_006218.2
Chi3l1	NM_001276.2	Pi3kCB	NM_006219.1
Chi3l2	NM_004000.2	Pi3kCD	NM_005026.3
Ciita	NM_000246.3	Pi3kCG	NM_002649.2
Clca1	NM_001285.3	Pilra (FDF03 inhibited)	NM_178273.1
Clca2	NM_006536.5	Pilrb (FDF03 activated)	NM_178238.1
Clec10a (mouse also MGL1)	NM_182906.2	Postn	NM_001135935.1
Clec1b (Clec-2)	NM_016509.3	Ppp1r2	NM_006241.4
Clec2d (OCIL)	NM_001004419.3	Prf1	NM_005041.3
Clec3b	NM_003278.2	Psmb10	NM_002801.2
Clec4d (MCL)	NM_080387.4	Psmb8	NM_004159.4
Clec4e (Mincle)	NM_014358.2	Psmb9	NM_002800.4
Clec5a (MDL-1)	NM_013252.2	Psme1	NM_006263.2
Clec7a (dectin-1)	NM_197954.2	Psme2	NM_002818.2
Clec9a	NM_207345.2	Pstpip1	NM_003978.3
Cmklr1	NM_004072.1	Pstpip2	NM_024430.3
Cpd	NM_001304.4	Pten	NM_000314.3
Crtam	NM_019604.2	Ptger2	NM_000956.2
Csf1r	NM_005211.2	Ptger4	NM_000958.2
Csf2rb	NM_000395.2	Ptpn10 (Dusp1)	NM_004417.2
Cst6	NM_001323.3	Ptpn13	NM_080684.2
Cst7	NM_003650.3	Ptpn22	NM_015967.3
Ctla4	NM_005214.3	Ptpn3	NM_001145372.1
Ctsb	NM_000100.2	Ptpn6	NM_002831.5
Ctsg	NM_001911.2	Ptpn7	NM_002832.3
Ctsz	NM_001336.3	Ptprcap	NM_005608.2
Cx3cl1	NM_002996.3	Ptprf	NM_002840.3
Cx3cr1	NM_001337.3	Pvrig	NM_024070.3
Cxcl1 (GRO-alpha)	NM_001511.1	RGS16	NM_002928.2
Cxcl10 (IP-10)	NM_001565.1	RIKEN cDNA 4632428N05 (VISTA)	NM_022153.1
Cxcl13 (BCA-1)	NM_006419.2	RPL19	NM_000981.3
Cxcl14	NM_004887.4	Rarres2	NM_002889.3
Cxcl3	NM_002090.2	Retnlb (Relmb Fizz2)	NM_032579.2
Cxcl4 (Pf4)	NM_002619.2	Rgn	NM_152869.2
Cxcr3	NM_001504.1	Rora	NM_134261.2
Cxcr6	NM_006564.1	Rorc (RORg and T)	NM_001001523.1

Gene Id	Target Transcript NCBI Accession #	Gene Id	Target Transcript NCBI Accession #
Cxcr7	NM_020311.1	Runx1	NM_001754.4
DCK	NM_000788.2	Runx3	NM_004350.1
DCT	NM_001922.3	S100a8	NM_002964.3
Dab1	NM_021080.3	S100a9	NM_002965.2
Dap10 (HCST)	NM_001007469.1	SAMD3	NM_001017373.2
Dap12 (TYROBP)	NM_003332.2	SART3	NM_014706.3
Def6	NM_022047.3	SDHA	NM_004168.1
Defb1	NM_005218.3	SIGLEC14	NM_001098612.1
Defb2	NM_004942.2	SIGLEC15 (CD33L3)	NM_213602.2
Dgkz	NM_001105540.1	SIGLEC5 (CD170; CD33L2)	NM_003830.2
Dpp4 (CD26)	NM_001935.3	Samhd1	NM_015474.2
Dsc1	NM_024421.2	Sema4a	NM_001193300.1
Dsc2	NM_024422.3	Serpinf1	NM_002615.4
Dsg2	NM_001943.3	Sgpp2	NM_152386.2
EEF1G	NM_001404.4	Sh2d1b	NM_053282.4
EGF	NM_001963.3	Sh2d2a	NM_001161443.1
Efemp1	NM_004105.3	Sirpb1	NM_006065.3
Egfr	NM_201282.1	Sirpg	NM_001039508.1
Egr2	NM_000399.3	Sit1	NM_014450.2
Eomes	NM_005442.2	Sla1	NM_001045556.2
Epcam	NM_002354.1	Sla2	NM_032214.2
Ezr	NM_003379.4	Slamf1 (CD150 Slam)	NM_003037.2
F2R (PAR-1)	NM_001992.2	Slamf6 (ntba)	NM_001184714.1
F2RL1 (PAR-2)	NM_005242.3	Slamf7 (Cracc)	NM_021181.3
FCER1A	NM_002001.2	Socs3	NM_003955.3
FCGR2A (CD32)	NM_021642.2	Stat1	NM_007315.2
FN1	NM_212482.1	Stat6	NM_003153.3
Fap	NM_004460.2	TBP	NM_001172085.1
Fasl (TNFSF6)	NM_000639.1	TIMP3	NM_000362.4
Fcgr2b (CD32b)	NM_001002273.1	TIMP4	NM_003256.2
Fcrl3	NM_052939.3	TNFRSF10b - TRAIL R2 DR5	NM_003842.3
Folr4	NM_001199206.1	TNFRSF13B - TACI	NM_012452.2
Foxp3	NM_014009.3	TNFRSF8 - CD30	NM_152942.2
G6PD	NM_000402.2	TNFSF10 - TRAIL CD253	NM_003810.2
GAPDH	NM_002046.3	TNFSF13b - BLYS	NM_006573.4
GUSB	NM_000181.1	TNFSF8 - CD30L	NM_001244.2
Gas6	NM_000820.2	TREM1	NM_018643.3
Gata3	NM_001002295.1	TREM2	NM_018965.2
Gdf10	NM_004962.2	TREML1 (TLT-1)	NM_178174.2

Gene Id	Target Transcript NCBI Accession #	Gene Id	Target Transcript NCBI Accession #
Gfi1	NM_005263.2	TREML2 (TLT-2)	NM_024807.2
Gitr (Tnfrsf18)	NM_004195.2	TUBB	NM_178014.2
Gitrl (Tnfsf18)	NM_005092.2	TYR (Tyrosinase)	NM_000372.4
Gnly	NM_006433.2	TYRO3	NM_006293.2
Gpld1	NM_001503.2	Tagap	NM_054114.3
gpr18	NM_001098200.1	Tarp (TCR gamma alternate reading frame protein)	NM_001003799.1
Grap2	NM_004810.2	Tbx21 (Tbet)	NM_013351.1
Gzma	NM_006144.2	Tcn2	NM_000355.2
Gzmb	NM_004131.3	Tigit	NM_173799.2
Gzmk	NM_002104.2	Tmem2	NM_013390.2
HLA-A (HLA Class I)	NM_002116.5	Tnfa	NM_000594.2
HLA-B	NM_005514.6	Tnfaip3	NM_006290.2
HLA-C	NM_002117.4	Tnfaip6	NM_007115.2
HLA-DRA (HLA class II)	NM_019111.3	Tnfaip8L2	NM_024575.3
HLA-E	NM_005516.4	Tnfrsf14 (Hvem)	NM_003820.2
HPRT1	NM_000194.1	Tnfrsf4 (Ox40)	NM_003327.2
Havcr1-Tim1	NM_001099414.1	Tnfrsf7 (Cd27)	NM_001242.4
Havcr2-Tim3	NM_032782.3	Tnfrsf9 (CD137 4-1BB)	NM_001561.4
Hcls1	NM_005335.4	Tnfsf14 (LIGHT)	NM_003807.2
Hgfac	NM_001528.2	Tnfsf4	NM_003326.2
Hif1a	NM_001530.2	Tnfsf7 CD27L	NM_001252.2
Hopx	NM_001145460.1	Tnfsf9 (4-1BBL)	NM_003811.3
IFNg	NM_000619.2	Tox	NM_014729.2
IGSF6	NM_005849.2	Trat1	NM_016388.2
IL-10R1	NM_001558.2	UBB	NM_018955.2
IL-2RA	NM_000417.1	Ubash3a	NM_001001895.1
IL-2RB	NM_000878.2	Ubash3b	NM_032873.3
IL-2Rg	NM_000206.1	VCAM	NM_001078.3
IL-37	NM_014439.3	Xist	NR_001564.1
IL10	NM_000572.2	Zap70	NM_001079.3
IL18	NM_001562.2	Zbtb16	NM_006006.4
IL18R1	NM_003855.2	Zbtb32	NM_014383.1

Each of the steps of obtaining a tissue sample, preparing one or more tissue sections therefrom for a gene signature biomarker assay, performing the assay, and scoring the results may be performed by separate individuals/entities at separate locations. For example, a surgeon may obtain by biopsy a tissue sample from a cancer patient's tumor and then send the

tissue sample to a pathology lab, which may fix the tissue sample and then prepare one or more slides, each with a single tissue section, for the assay. The slide(s) may be assayed soon after preparation, or stored for future assay. The lab that prepared a tissue section may conduct the assay or send the slide(s) to a different lab to conduct the assay. A pathologist or trained professional who scores the slide(s) for an IFN-γ gene signature may work for the diagnostic lab, or may be an independent contractor. Alternatively, a single diagnostic lab obtains the tissue sample from the subject's physician or surgeon and then performs all of the steps involved in preparing tissue sections, assaying the slide(s) and calculating the gene signature score for the tissue section(s).

In some embodiments, the individuals involved with preparing and assaying the tissue section for a gene signature biomarker do not know the identity of the subject whose sample is being tested; i.e., the sample received by the laboratory is made anonymous in some manner before being sent to the laboratory. For example, the sample may be merely identified by a number or some other code (a "sample ID") and the results of the assay are reported to the party ordering the test using the sample ID. In preferred embodiments, the link between the identity of a subject and the subject's tissue sample is known only to the individual or to the individual's physician.

In some embodiments, after the test results have been obtained, the diagnostic laboratory generates a test report, which may comprise any one or both of the following results: the tissue sample was biomarker positive or negative, the gene signature score for the tumor sample and the reference score for that gene signature. The test report may also include a list of genes whose expression was analyzed in the assay.

In other embodiments, the test report may also include guidance on how to interpret the results for predicting if a subject is likely to respond to a PD-1 antagonist. For example, in one embodiment, the tested tumor sample is from a melanoma and has an IFN-γ gene signature score at or above a prespecified threshold, the test report may indicate that the subject has a score that is associated with response or better response to treatment with a PD-1 antagonist, while if the IFN-γ gene signature score is below the threshold, then the test report indicates that the patient has a score that is associated with no response or poor response to treatment with a PD-1 antagonist. In some embodiments, the prespecified threshold in melanoma tissue samples for the IFN-γ gene signature of Table 1 is equal to or greater than 2.462.

In some embodiments, the test report is a written document prepared by the diagnostic laboratory and sent to the patient or the patient's physician as a hard copy or via electronic mail. In other embodiments, the test report is generated by a computer program and displayed on a video monitor in the physician's office. The test report may also comprise an oral transmission of the test results directly to the patient or the patient's physician or an authorized employee in the physician's office. Similarly, the test report may comprise a record of the test results that the physician makes in the patient's file.

Detecting the presence or absence of an IFN-γ gene signature of the invention may be performed using a kit that has been specially designed for this purpose. In one embodiment, the kit comprises a set of oligonucleotide probes capable of hybridizing to the target transcripts in the gene signature. The kit may further comprise oligonucleotide probes capable of detecting transcripts of other genes, such as control genes, or genes used for normalization purposes. The set of oligonucleotide probes may comprise an ordered array of oligonucleotides on a solid surface, such as a microchip, silica beads (such as BeadArray technology from Illumina, San Diego, CA), or a glass slide (see, e.g., WO 98/20020 and WO 98/20019). In some embodiments, the oligonucleotide probes are provided in one or more compositions in liquid or dried form.

Oligonucleotides in kits of the invention must be capable of specifically hybridizing to a target region of a polynucleotide, such as for example, an RNA transcript or cDNA generated therefrom. As used herein, specific hybridization means the oligonucleotide forms an anti-parallel double-stranded structure with the target region under certain hybridizing conditions, while failing to form such a structure with non-target regions when incubated with the polynucleotide under the same hybridizing conditions. The composition and length of each oligonucleotide in the kit will depend on the nature of the transcript containing the target region as well as the type of assay to be performed with the oligonucleotide and is readily determined by the skilled artisan.

In some embodiments, each oligonucleotide in the kit is a perfect complement of its target region. An oligonucleotide is said to be a "perfect" or "complete" complement of another nucleic acid molecule if every nucleotide of one of the molecules is complementary to the nucleotide at the corresponding position of the other molecule. While perfectly complementary oligonucleotides are preferred for detecting transcripts in a gene signature, departures from complete complementarity are contemplated where such departures do not prevent the molecule from specifically hybridizing to the target region as defined above. For

example, an oligonucleotide probe may have one or more non-complementary nucleotides at its 5' end or 3' end, with the remainder of the probe being completely complementary to the target region. Alternatively, non-complementary nucleotides may be interspersed into the probe as long as the resulting probe is still capable of specifically hybridizing to the target region.

In some preferred embodiments, each oligonucleotide in the kit specifically hybridizes to its target region under stringent hybridization conditions. Stringent hybridization conditions are sequence-dependent and vary depending on the circumstances. Generally, stringent conditions are selected to be about 5° C lower than the thermal melting point (Tm) for the specific sequence at a defined ionic strength and pH. The Tm is the temperature (under defined ionic strength, pH, and nucleic acid concentration) at which 50% of the probes complementary to the target sequence hybridize to the target sequence at equilibrium. As the target sequences are generally present in excess, at Tm, 50% of the probes are occupied at equilibrium.

Typically, stringent conditions include a salt concentration of at least about 0.01 to 1.0 M sodium ion concentration (or other salts) at pH 7.0 to 8. 3 and the temperature is at least about 25° C for short oligonucleotide probes (e.g., 10 to 50 nucleotides). Stringent conditions can also be achieved with the addition of destabilizing agents such as formamide. For example, conditions of 5xSSPE (750 mM NaCl, 50 mM NaPhosphate, 5 mM EDTA, pH 7.4) and a temperature of 25-30° C are suitable for allele-specific probe hybridizations. Additional stringent conditions can be found in Molecular Cloning: A Laboratory Manual, Sambrook et al., Cold Spring Harbor Press, Cold Spring Harbor, NY (1989), chapters 7, 9, and 11, and in NUCLEIC ACID HYBRIDIZATION, A PRACTICAL APPROACH, Haymes et al., IRL Press, Washington, D.C., 1985.

One non-limiting example of stringent hybridization conditions includes hybridization in 4X sodium chloride/sodium citrate (SSC), at about 65-70°C (or alternatively hybridization in 4X SSC plus 50% formamide at about 42-50°C) followed by one or more washes in lX SSC, at about 65-70°C. A non-limiting example of highly stringent hybridization conditions includes hybridization in lX SSC, at about 65-70°C (or alternatively hybridization in 1X SSC plus 50% formamide at about 42-50°C) followed by one or more washes in 0.3X SSC, at about 65-70°C. A non-limiting example of reduced stringency hybridization conditions includes hybridization in 4X SSC, at about 50-60°C (or alternatively hybridization in 6X SSC plus 50% formamide at about 40-45°C) followed by one or more washes in 2X SSC, at about

50-60°C. Stringency conditions with ranges intermediate to the above-recited values, e.g., at 65-70°C or at 42-50°C are also intended to be encompassed by the present invention. SSPE (1xSSPE is 0.15M NaCl, 10mM NaH_2PO_4, and 1.25mM EDTA, pH 7.4) can be substituted for SSC (1X SSC is 0.15M NaCl and 15mM sodium citrate) in the hybridization and wash buffers; washes are performed for 15 minutes each after hybridization is complete.

The hybridization temperature for hybrids anticipated to be less than 50 base pairs in length should be 5-10°C less than the melting temperature (T_m) of the hybrid, where Tm is determined according to the following equations. For hybrids less than 18 base pairs in length, T_m (°C) = 2(# of A + T bases) + 4(# of G + C bases). For hybrids between 18 and 49 base pairs in length, T_m (°C) = 81.5 + 16.6($\log_{10}$[Na+]) + 0.41(%G+C)-(600/N), where N is the number of bases in the hybrid, and [Na+] is the concentration of sodium ions in the hybridization buffer ([Na+] for 1 X SSC = 0.165 M).

The oligonucleotides in kits of the invention may be comprised of any phosphorylation state of ribonucleotides, deoxyribonucleotides, and acyclic nucleotide derivatives, and other functionally equivalent derivatives. Alternatively, the oligonucleotides may have a phosphate-free backbone, which may be comprised of linkages such as carboxymethyl, acetamidate, carbamate, polyamide (peptide nucleic acid (PNA)) and the like (Varma, in MOLECULAR BIOLOGY AND BIOTEChNOLOGY, A COMPREHENSIVE DESK REFERENCE, Meyers, ed., pp. 6 17-20, VCH Publishers, Inc., 1995). The oligonucleotides may be prepared by chemical synthesis using any suitable methodology known in the art, or may be derived from a biological sample, for example, by restriction digestion. The oligonucleotides may contain a detectable label, according to any technique known in the art, including use of radiolabels, fluorescent labels, enzymatic labels, proteins, haptens, antibodies, sequence tags and the like. The oligonucleotides in the kit may be manufactured and marketed as analyte specific reagents (ASRs) or may be constitute components of an approved diagnostic device.

Kits of the invention may also contain other reagents such as hybridization buffer and reagents to detect when hybridization with a specific target molecule has occurred. Detection reagents may include biotin-or fluorescent-tagged oligonucleotides and/or an enzyme-labeled antibody and one or more substrates that generate a detectable signal when acted on by the enzyme. It will be understood by the skilled artisan that the set of oligonucleotides and reagents for performing the assay will be provided in separate receptacles placed in the kit

container if appropriate to preserve biological or chemical activity and enable proper use in the assay.

In other embodiments, each of the oligonucleotide probes and all other reagents in the kit have been quality tested for optimal performance in an assay designed to determine the IFN-γ gene signature score in a tumor sample, and preferably when the tumor sample is an FFPE tissue section. In some embodiments, the kit includes an instruction manual that describes how to use the determined gene signature score to assign, to the tested tumor sample, the presence or absence of a gene signature biomarker that predicts response to treatment with a PD-1 antagonist.

B. Pharmaceutical compositions, drug products and treatment regimens

An individual to be treated by any of the methods and products described herein is a human subject diagnosed with a tumor, and a sample of the subject's tumor is available or obtainable to use in testing for the presence or absence of any of the gene signature biomarkers described herein.

The tumor tissue sample can be collected from a subject before and/or after exposure of the subject to one or more therapeutic treatment regimens, such as for example, a PD-1 antagonist, a chemotherapeutic agent, radiation therapy. Accordingly, tumor samples may be collected from a subject over a period of time. The tumor sample can be obtained by a variety of procedures including, but not limited to, surgical excision, aspiration or biopsy.

A physician may use an IFN-γ gene signature score as a guide in deciding how to treat a patient who has been diagnosed with a type of cancer that is susceptible to treatment with a PD-1 antagonist or other chemotherapeutic agent(s). Prior to initiation of treatment with the PD-1 antagonist or the other chemotherapeutic agent(s), the physician would typically order a diagnostic test to determine if a tumor tissue sample removed from the patient is positive or negative for an IFN-γ gene signature biomarker. However, it is envisioned that the physician could order a first or subsequent diagnostic tests at any time after the individual is administered the first dose of the PD-1 antagonist or other chemotherapeutic agent(s). In some embodiments, a physician may be considering whether to treat the patient with a pharmaceutical product that is indicated for patients whose tumor tests positive for the gene signature biomarker. For example, if the reported score is at or above a pre-specified threshold score that is associated with response or better response to treatment with a PD-1 antagonist, the patient is treated with a therapeutic regimen that includes at least the PD-1

antagonist (optionally in combination with one or more chemotherapeutic agents), and if the reported gene signature score is below a pre-specified threshold score that is associated with no response or poor response to treatment with a PD-1 antagonist, the patient is treated with a therapeutic regimen that does not include any PD-1 antagonist.

In deciding how to use the gene signature test results in treating any individual patient, the physician may also take into account other relevant circumstances, such as the stage of the cancer, weight, gender, and general condition of the patient, including inputting a combination of these factors and the gene signature biomarker test results into a model that helps guide the physician in choosing a therapy and/or treatment regimen with that therapy.

The physician may choose to treat the patient who tests biomarker positive with a combination therapy regimen that includes a PD-1 antagonist and one or more additional therapeutic agents. The additional therapeutic agent may be, e.g., a chemotherapeutic, a biotherapeutic agent (including but not limited to antibodies to VEGF, EGFR, Her2/neu, VEGF receptors, other growth factor receptors, CD20, CD40, CD-40L, CTLA-4, OX-40, 4-1BB, and ICOS), an immunogenic agent (for example, attenuated cancerous cells, tumor antigens, antigen presenting cells such as dendritic cells pulsed with tumor derived antigen or nucleic acids, immune stimulating cytokines (for example, IL-2, IFNα2, GM-CSF), and cells transfected with genes encoding immune stimulating cytokines such as but not limited to GM-CSF).

Examples of chemotherapeutic agents include alkylating agents such as thiotepa and cyclosphosphamide; alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, trietylenephosphoramide, triethylenethiophosphoramide and trimethylolomelamine; acetogenins (especially bullatacin and bullatacinone); a camptothecin (including the synthetic analogue topotecan); bryostatin; callystatin; CC-1065 (including its adozelesin, carzelesin and bizelesin synthetic analogues); cryptophycins (particularly cryptophycin 1 and cryptophycin 8); dolastatin; duocarmycin (including the synthetic analogues, KW-2189 and CBI-TMI); 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, ranimustine; antibiotics such as the enediyne antibiotics (e.g. calicheamicin, especially

calicheamicin gamma1I and calicheamicin phiI1, see, e.g., Agnew, Chem. Intl. Ed. Engl., 33:183-186 (1994); dynemicin, including dynemicin A; bisphosphonates, such as clodronate; an esperamicin; as well as neocarzinostatin chromophore and related chromoprotein enediyne antibiotic chromomophores), aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, carabicin, caminomycin, carzinophilin, chromomycins, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, 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; elformithine; elliptinium acetate; an epothilone; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidamine; maytansinoids such as maytansine and ansamitocins; mitoguazone; mitoxantrone; mopidamol; nitracrine; pentostatin; phenamet; pirarubicin; losoxantrone; podophyllinic acid; 2-ethylhydrazide; procarbazine; razoxane; rhizoxin; sizofuran; spirogermanium; tenuazonic acid; triaziquone; 2, 2′,2″-trichlorotriethylamine; trichothecenes (especially T-2 toxin, verracurin A, roridin A and anguidine); urethan; vindesine; dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); cyclophosphamide; thiotepa; taxoids, e.g. paclitaxel and doxetaxel; chlorambucil; gemcitabine; 6-thioguanine; mercaptopurine; methotrexate; platinum analogs such as cisplatin and carboplatin; vinblastine; platinum; etoposide (VP-16); ifosfamide; mitoxantrone; vincristine; vinorelbine; novantrone; teniposide; edatrexate; daunomycin; aminopterin; xeloda; ibandronate; CPT-11; topoisomerase inhibitor RFS 2000; difluoromethylornithine (DMFO); retinoids such as retinoic acid; capecitabine; and pharmaceutically acceptable salts, acids or derivatives of any of the above. Also included are anti-hormonal agents that act to regulate or inhibit hormone

action on tumors such as anti-estrogens and selective estrogen receptor modulators (SERMs), including, for example, tamoxifen, raloxifene, droloxifene, 4-hydroxytamoxifen, trioxifene, keoxifene, LY117018, onapristone, and toremifene (Fareston); aromatase inhibitors that inhibit the enzyme aromatase, which regulates estrogen production in the adrenal glands, such as, for example, 4(5)-imidazoles, aminoglutethimide, megestrol acetate, exemestane, formestane, fadrozole, vorozole, letrozole, and anastrozole; and anti-androgens such as flutamide, nilutamide, bicalutamide, leuprolide, and goserelin; and pharmaceutically acceptable salts, acids or derivatives of any of the above.

Each therapeutic agent in a combination therapy used to treat a biomarker positive patient may be administered either alone or in a medicament (also referred to herein as a pharmaceutical composition) which comprises the therapeutic agent and one or more pharmaceutically acceptable carriers, excipients and diluents, according to standard pharmaceutical practice.

Each therapeutic agent in a combination therapy used to treat a biomarker positive patient may be administered simultaneously (i.e., in the same medicament), concurrently (i.e., in separate medicaments administered one right after the other in any order) or sequentially in any order. Sequential administration is particularly useful when the therapeutic agents in the combination therapy are in different dosage forms (one agent is a tablet or capsule and another agent is a sterile liquid) and/or are administered on different dosing schedules, e.g., a chemotherapeutic that is administered at least daily and a biotherapeutic that is administered less frequently, such as once weekly, once every two weeks, or once every three weeks.

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

Each therapeutic agent in a combination therapy used to treat a biomarker positive patient can be administered orally or parenterally, including the intravenous, intramuscular, intraperitoneal, subcutaneous, rectal, topical, and transdermal routes of administration.

A patient may be administered a PD-1 antagonist prior to or following surgery to remove a tumor and may be used prior to, during or after radiation therapy.

In some embodiments, a PD-1 antagonist is administered to a patient who has not been previously treated with a biotherapeutic or chemotherapeutic agent, i.e., is treatment-naïve. In other embodiments, the PD-1 antagonist is administered to a patient who failed to achieve a sustained response after prior therapy with a biotherapeutic or chemotherapeutic agent, i.e., is treatment-experienced.

A therapy comprising a PD-1 antagonist is typically used to treat a tumor that is large enough to be found by palpation or by imaging techniques well known in the art, such as MRI, ultrasound, or CAT scan. In some preferred embodiments, the therapy is used to treat an advanced stage tumor having dimensions of at least about 200 mm^3, 300 mm^3, 400 mm^3, 500 mm^3, 750 mm^3, or up to 1000 mm^3.

Selecting a dosage regimen (also referred to herein as an administration regimen) for a therapy comprising a PD-1 antagonist depends on several factors, including the serum or tissue turnover rate of the entity, the level of symptoms, the immunogenicity of the entity, and the accessibility of the target cells, tissue or organ in the individual being treated. Preferably, a dosage regimen maximizes the amount of the PD-1 antagonist that is delivered to the patient consistent with an acceptable level of side effects. Accordingly, the dose amount and dosing frequency depends in part on the particular PD-1 antagonist, any other therapeutic agents to be used, and the severity of the cancer being treated, and patient characteristics. Guidance in selecting appropriate doses of antibodies, cytokines, and small molecules are available. *See, e.g.*, Wawrzynczak (1996) *Antibody Therapy*, Bios Scientific Pub. Ltd, Oxfordshire, UK; Kresina (ed.) (1991) *Monoclonal Antibodies, Cytokines and Arthritis*, Marcel Dekker, New York, NY; Bach (ed.) (1993) *Monoclonal Antibodies and Peptide Therapy in Autoimmune Diseases*, Marcel Dekker, New York, NY; Baert *et al.* (2003) *New Engl. J. Med.* 348:601-608; Milgrom *et al.* (1999) *New Engl. J. Med.* 341:1966-1973; Slamon *et al.* (2001) *New Engl. J. Med.* 344:783-792; Beniaminovitz *et al.* (2000) *New Engl. J. Med.* 342:613-619; Ghosh *et al.* (2003) *New Engl. J. Med.* 348:24-32; Lipsky *et al.* (2000) *New Engl. J. Med.* 343:1594-1602; Physicians' Desk Reference 2003 (Physicians' Desk Reference, 57th Ed); Medical Economics Company; ISBN: 1563634457; 57th edition (November 2002). Determination of the appropriate dosage regimen may be made by the clinician, *e.g.*, using parameters or factors known or suspected in the art to affect treatment or predicted to affect treatment, and will depend, for example, the patient's clinical history (e.g., previous therapy), the type and stage of the cancer to be treated and biomarkers of response to one or more of the therapeutic agents in the combination therapy.

Biotherapeutic agents used in combination with a PD-1 antagonist may be administered by continuous infusion, or by doses at intervals of, *e.g.*, daily, every other day, three times per week, or one time each week, two weeks, three weeks, monthly, bimonthly, etc. A total weekly dose is generally at least 0.05 μg/kg, 0.2 μg/kg, 0.5 μg/kg, 1 μg/kg, 10 μg/kg, 100 μg/kg, 0.2 mg/kg, 1.0 mg/kg, 2.0 mg/kg, 10 mg/kg, 25 mg/kg, 50 mg/kg body weight or more. *See, e.g.*, Yang *et al.* (2003) *New Engl. J. Med.* 349:427-434; Herold *et al.* (2002) *New Engl. J. Med.* 346:1692-1698; Liu *et al.* (1999) *J. Neurol. Neurosurg. Psych.* 67:451-456; Portielji *et al.* (20003) *Cancer Immunol. Immunother.* 52:133-144.

In some embodiments that employ an anti-human PD-1 mAb as the PD-1 antagonist, the dosing regimen will comprise administering the anti-human PD-1 mAb at a dose of 1, 2, 3, 5 or 10mg/kg at intervals of about 14 days (± 2 days) or about 21 days (± 2 days) or about 30 days (± 2 days) throughout the course of treatment.

In other embodiments that employ an anti-human PD-1 mAb as the PD-1 antagonist, the dosing regimen will comprise administering the anti-human PD-1 mAb at a dose of from about 0.005mg/kg to about 10mg/kg, with intra-patient dose escalation. In other escalating dose embodiments, the interval between doses will be progressively shortened, e.g., about 30 days (± 2 days) between the first and second dose, about 14 days (± 2 days) between the second and third doses. In certain embodiments, the dosing interval will be about 14 days (± 2 days), for doses subsequent to the second dose.

In certain embodiments, a subject will be administered an intravenous (IV) infusion of a medicament comprising any of the PD-1 antagonists described herein, and such administration may be part of a treatment regimen employing the PD-1 antagonist as a monotherapy regimen or as part of a combination therapy.

In one preferred embodiment of the invention, the PD-1 antagonist is nivolumab, which is administered intravenously at a dose selected from the group consisting of: 1 mg/kg Q2W, 2 mg/kg Q2W, 3 mg/kg Q2W, 5 mg/kg Q2W, 10 mg Q2W, 1 mg/kg Q3W, 2 mg/kg Q3W, 3 mg/kg Q3W, 5 mg/kg Q3W, and 10 mg Q3W.

In another preferred embodiment of the invention, the PD-1 antagonist is MK-3475, which is administered in a liquid medicament at a dose selected from the group consisting of 1 mg/kg Q2W, 2 mg/kg Q2W, 3 mg/kg Q2W, 5 mg/kg Q2W, 10 mg Q2W, 1 mg/kg Q3W, 2 mg/kg Q3W, 3 mg/kg Q3W, 5 mg/kg Q3W, and 10 mg Q3W. In some particularly preferred embodiments, MK-3475 is administered as a liquid medicament which comprises 25 mg/ml

MK-3475, 7% (w/v) sucrose, 0.02% (w/v) polysorbate 80 in 10 mM histidine buffer pH 5.5, and the selected dose of the medicament is administered by IV infusion over a time period of 30 minutes. The optimal dose for MK-3475 in combination with any other therapeutic agent may be identified by dose escalation starting with 2 mg/kg and going up to 10 mg/kg.

The present invention also provides a medicament which comprises a PD-1 antagonist as described above and a pharmaceutically acceptable excipient. When the PD-1 antagonist is a biotherapeutic agent, e.g., a mAb, the antagonist may be produced in CHO cells using conventional cell culture and recovery/purification technologies.

In some embodiments, a medicament comprising an anti-PD-1 antibody as the PD-1 antagonist may be provided as a liquid formulation or prepared by reconstituting a lyophilized powder with sterile water for injection prior to use. WO 2012/135408 describes the preparation of liquid and lyophilized medicaments comprising MK-3475 that are suitable for use in the present invention. In some preferred embodiments, a medicament comprising MK-3475 is provided in a glass vial which contains about 50 mg of MK-3475.

Exemplary Specific Embodiments of the Invention

1. A method for testing a tumor for the presence or absence of a biomarker that predicts response to treatment with a PD-1 antagonist, which comprises:

obtaining a sample from the tumor, measuring the raw RNA expression level in the tumor sample for each gene in an IFN-γ gene signature;

normalizing each of the measured raw RNA expression levels; and

calculating the arithmetic mean of the normalized RNA expression levels for each of the genes to generate a score for the IFN-γ gene signature;

wherein the IFN-γ gene signature comprises at least five of the genes in Table 1.

2. The method of embodiment 1, wherein the method further comprises:

comparing the calculated score to a reference score for the IFN-γ gene signature; and

classifying the tumor as biomarker positive or biomarker negative;

wherein if the calculated score is equal to or greater than the reference score, then the tumor is classified as biomarker positive, and if the calculated gene signature score is less than the reference IFN-γ gene signature score, then the tumor is classified as biomarker negative.

3. A method for treating a subject having a tumor which comprises:

determining if the tumor is positive or negative for an IFN-γ gene signature biomarker; and

administering to the subject a PD-1 antagonist if the tumor is positive for the biomarker; or

administering to the subject a cancer treatment that does not include a PD-1 antagonist if the tumor is negative for the biomarker;

wherein the IFN-γ gene signature comprises at least five of the genes in Table 1.

4. The method of embodiment 3, wherein the determining step comprises:

obtaining a sample from the subject's tumor;

sending the tumor sample to a laboratory with a request to test the sample for the presence or absence of an IFN-γ gene signature biomarker; and

receiving a report from the laboratory that states whether the tumor sample is biomarker positive or biomarker negative.

5. A method for treating a subject having a tumor which comprises:

obtaining a sample from the tumor;

measuring the raw RNA expression level in the tumor sample for each gene in a IFN-γ gene signature;

normalizing each of the measured raw RNA expression levels;

calculating the arithmetic mean of the normalized RNA expression levels for each of the genes to generate a score for the IFN-γ gene signature; and

administering to the subject a PD-1 antagonist if the calculated score is equal to or greater than a reference score for the IFN-γ gene signature; or

administering to the subject a cancer therapy that does not include a PD-1 antagonist if the calculated score is less than the reference score;

wherein the IFN-γ gene signature comprises at least five of the genes in Table 1.

6. A pharmaceutical composition comprising a PD-1 antagonist for use in a subject who has a tumor that tests positive for a IFN-γ gene signature biomarker, wherein the gene signature in the biomarker comprises at least five of the genes in Table 1.

7. A drug product which comprises a pharmaceutical composition and prescribing information, wherein the pharmaceutical composition comprises a PD-1 antagonist and at least one pharmaceutically acceptable excipient and the prescribing information states that

the pharmaceutical composition is indicated for use in a subject who has a tumor that tests positive for an IFN-γ gene signature biomarker.

8. The pharmaceutical composition of embodiment 6 or the drug product of embodiment 7, wherein the positive biomarker test result was generated by a method comprising:

obtaining a sample from the tumor,

measuring the raw RNA expression level in the tumor sample for each gene in an IFN-γ gene signature;

normalizing each of the measured raw RNA expression levels;

calculating the arithmetic mean of the normalized RNA expression levels for each of the genes to generate a score for the IFN-γ gene signature;

comparing the calculated score to a reference score for the IFN-γ gene signature; and

classifying the tumor as biomarker positive or biomarker negative;

wherein if the calculated score is equal to or greater than the reference score, then the tumor is classified as biomarker positive, and if the calculated IFN-γ gene signature score is less than the reference IFN-γ gene signature score, then the tumor is classified as biomarker negative.

9. A kit for assaying a tumor sample to determine an IFN-γ gene signature score for the tumor sample, wherein the kit comprises a first set of probes for detecting expression of each gene in the IFN-γ gene signature, wherein the IFN-γ gene signature comprises at least five of the genes in Table 1.

10. The kit of embodiment 9, wherein the first set of probes is designed to detect expression of the transcripts listed in Table 1 for each of IFNG, STAT1, CCR5, CXCL9, PRF1, HLA-DRA, CXCL10, CXCL11, ID01 and GZMA.

11. The kit of embodiments 9 or 10, which further comprises a second set of probes for detecting target transcripts expressed in the tumor sample by a set of normalization genes.

12. The method, composition, drug product or kit of any of the above embodiments, wherein the measuring step comprises contacting RNA molecules in the sample with at least one probe for the transcript listed in Table 1 for each gene whose expression is to be

measured, wherein the contacting is performed under stringent hybridization conditions, and quantitating the number of probe-RNA hybrids generated in the contacting step.

13. The method, composition, drug product or kit of any of the above embodiments, wherein the measuring step comprises amplifying and quantifying the transcript listed in Table 1 for each gene whose expression is to be measured.

14. The method, composition, drug product or kit of any of the above embodiments, wherein the normalizing step comprises performing quantile normalization of raw RNA expression values relative to the distribution of raw RNA expression values in the test sample and a plurality of control samples for a set of normalization genes, followed by a subsequent log10-transformation.

15. The method, composition, drug product or kit of any of the above embodiments, wherein the normalization gene set consists essentially of at least 100 or 200 genes in the 400 gene set listed in Table 4.

16. The method, composition, drug product or kit of any of the above embodiments, wherein the set of normalization genes consists essentially of at least 300 or 400 genes in the 400 gene set listed in Table 4.

17. The method, composition, drug product or kit of any of the above embodiments, wherein the IFN-γ gene signature consists essentially of IFNG, STAT1, CCR5, CXCL9, PRF1, HLA-DRA, CXCL10, CXCL11, ID01 and GZMA or a subset thereof, wherein the subset consists essentially of five, six, seven, eight or nine genes.

18. The method, composition, drug product or kit of any of the above embodiments, wherein the reference score is pre-selected to divide the majority of responders to the PD-1 antagonist from the majority of non-responders to the PD-1 antagonist.

19. The method, composition, drug product or kit of any of the above embodiments, wherein the majority of responders achieved at least a partial response to the PD-1 antagonist as measured by RECIST 1.1.

20. The method, composition, drug product or kit of any of the above embodiments, wherein the majority of responders achieved a complete response to the PD-1 antagonist as measured by RECIST 1.1.

21. The method, composition, drug product or kit of any of the above embodiments, wherein the IFN-γ gene signature consists essentially of IFNG, STAT1, CCR5, CXCL9, PRF1, HLA-DRA, CXCL10, CXCL11, ID01 and GZMA, the test and reference IFN-γ gene signature scores are determined by performing quantile normalization of raw RNA expression values relative to the distribution of raw RNA expression values for a set of at least 300 normalization genes in the test tumor sample and in a plurality of control tumor samples followed by a subsequent log10-transformation.

22. The method, composition, drug product or kit of embodiment 21, wherein the tumor is metastatic melanoma, the set of normalization genes consists essentially of the 400 genes in Table 4 and the reference score is between 2.255 and 2.483, between 2.305 and 2.473, between 2.450 and 2.469, or is 2.462.

23. The method, composition, drug product or kit of any of the above embodiments, wherein the PD-1 antagonist is a monoclonal antibody, or an antigen binding fragment thereof, which specifically binds to PD-1 or to PD-L1 and blocks the binding of PD-L1 to PD-1.

24. The method, composition, drug product or kit of embodiment 23, wherein the PD-1 antagonist is an anti-PD-1 monoclonal antibody which comprises a heavy chain and a light chain, wherein the heavy and light chains comprise SEQ ID NO:21 and SEQ ID NO:22.

25. The method, composition, drug product or kit of embodiment 22, wherein the PD-1 antagonist is MK-3475 and the reference score is about 2.1.

26. The method, composition, drug product or kit of embodiment 22, wherein the PD-1 antagonist is MPDL3280A, BMS-936559, MEDI4736, MSB0010718C or a monoclonal antibody which comprises the heavy chain and light chain variable regions of SEQ ID NO:24 and SEQ ID NO:21, respectively, of WO2013/019906.

27. The method, composition, drug product or kit of embodiment 22, wherein the monoclonal antibody, or antigen binding fragment thereof, comprises: (a) light chain CDRs of SEQ ID NOs: 1, 2 and 3 and heavy chain CDRs of SEQ ID NOs: 4, 5 and 6; or (b) light chain CDRs of SEQ ID NOs: 7, 8 and 9 and heavy chain CDRs of SEQ ID NOs: 10, 11 and 12.

28. The method, composition, drug product or kit of embodiment 22, wherein the PD-1 antagonist is an anti-PD-1 monoclonal antibody which comprises a heavy chain and a light chain, and wherein the heavy chain comprises SEQ ID NO:23 and the light chain comprises SEQ ID NO:24.

29. The method, composition, drug product or kit of any of the above embodiments, wherein the tumor sample is from a subject with ipilimumab-naïve advanced melanoma or ipilimumab-refractory advanced melanoma.

30. The method, composition, drug product or kit of any of the above embodiments, wherein the PD-1 antagonist is MK-3475 or nivolumab.

31. The method, composition, drug product or kit of any of the above embodiments, wherein the reference score is selected to provide a negative predictive value that is greater than the positive predictive value.

GENERAL METHODS

Standard methods in molecular biology are described Sambrook, Fritsch and Maniatis (1982 & 1989 2nd Edition, 2001 3rd Edition) *Molecular Cloning, A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY; Sambrook and Russell (2001) *Molecular Cloning, 3rd ed.,* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY; Wu (1993) *Recombinant DNA*, Vol. 217, Academic Press, San Diego, CA). Standard methods also appear in Ausbel, *et al.* (2001) *Current Protocols in Molecular Biology, Vols.1-4*, John Wiley and Sons, Inc. New York, NY, which describes cloning in bacterial cells and DNA mutagenesis (Vol. 1), cloning in mammalian cells and yeast (Vol. 2), glycoconjugates and protein expression (Vol. 3), and bioinformatics (Vol. 4).

Methods for protein purification including immunoprecipitation, chromatography, electrophoresis, centrifugation, and crystallization are described (Coligan, *et al.* (2000) *Current Protocols in Protein Science, Vol. 1*, John Wiley and Sons, Inc., New York). Chemical analysis, chemical modification, post-translational modification, production of fusion proteins, glycosylation of proteins are described (see, *e.g.*, Coligan, *et al.* (2000) *Current Protocols in Protein Science, Vol. 2*, John Wiley and Sons, Inc., New York; Ausubel, *et al.* (2001) *Current Protocols in Molecular Biology, Vol. 3*, John Wiley and Sons, Inc., NY, NY, pp. 16.0.5-16.22.17; Sigma-Aldrich, Co. (2001) *Products for Life Science Research*, St. Louis, MO; pp. 45-89; Amersham Pharmacia Biotech (2001) *BioDirectory*, Piscataway, N.J.,

pp. 384-391). Production, purification, and fragmentation of polyclonal and monoclonal antibodies are described (Coligan, *et al.* (2001) *Current Protcols in Immunology, Vol. 1*, John Wiley and Sons, Inc., New York; Harlow and Lane (1999) *Using Antibodies*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY; Harlow and Lane, *supra*). Standard techniques for characterizing ligand/receptor interactions are available (see, *e.g.*, Coligan, *et al.* (2001) *Current Protocols in Immunology, Vol. 4*, John Wiley, Inc., New York).

Monoclonal, polyclonal, and humanized antibodies can be prepared (see, *e.g.*, Sheperd and Dean (eds.) (2000) *Monoclonal Antibodies*, Oxford Univ. Press, New York, NY; Kontermann and Dubel (eds.) (2001) *Antibody Engineering*, Springer-Verlag, New York; Harlow and Lane (1988) *Antibodies A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, pp. 139-243; Carpenter, *et al.* (2000) *J. Immunol.* 165:6205; He, *et al.* (1998) *J. Immunol.* 160:1029; Tang *et al.* (1999) J. Biol. Chem. 274:27371-27378; Baca *et al.* (1997) *J. Biol. Chem.* 272:10678-10684; Chothia *et al.* (1989) *Nature* 342:877-883; Foote and Winter (1992) *J. Mol. Biol.* 224:487-499; U.S. Pat. No. 6,329,511).

An alternative to humanization is to use human antibody libraries displayed on phage or human antibody libraries in transgenic mice (Vaughan *et al.* (1996) *Nature* Biotechnol. 14:309-314; Barbas (1995) *Nature Medicine* 1:837-839; Mendez *et al.* (1997) *Nature Genetics* 15:146-156; Hoogenboom and Chames (2000) *Immunol. Today* 21:371-377; Barbas *et al.* (2001) *Phage Display: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York; Kay *et al.* (1996) *Phage Display of Peptides and Proteins: A Laboratory Manual*, Academic Press, San Diego, CA; de Bruin *et al.* (1999) *Nature Biotechnol.* 17:397-399).

Purification of antigen is not necessary for the generation of antibodies. Animals can be immunized with cells bearing the antigen of interest. Splenocytes can then be isolated from the immunized animals, and the splenocytes can fused with a myeloma cell line to produce a hybridoma (see, *e.g.*, Meyaard *et al.* (1997) *Immunity* 7:283-290; Wright *et al.* (2000) *Immunity* 13:233-242; Preston *et al.*, *supra*; Kaithamana *et al.* (1999) *J. Immunol.* 163:5157-5164).

Antibodies can be conjugated, *e.g.*, to small drug molecules, enzymes, liposomes, polyethylene glycol (PEG). Antibodies are useful for therapeutic, diagnostic, kit or other purposes, and include antibodies coupled, *e.g.*, to dyes, radioisotopes, enzymes, or metals, *e.g.*, colloidal gold (see, *e.g.*, Le Doussal *et al.* (1991) *J. Immunol.* 146:169-175; Gibellini *et*

al. (1998) *J. Immunol.* 160:3891-3898; Hsing and Bishop (1999) *J. Immunol.* 162:2804-2811; Everts *et al.* (2002) *J. Immunol.* 168:883-889).

Fluorescent reagents suitable for modifying nucleic acids, including nucleic acid primers and probes, polypeptides, and antibodies, for use, *e.g.*, as diagnostic reagents, are available (Molecular Probesy (2003) *Catalogue*, Molecular Probes, Inc., Eugene, OR; Sigma-Aldrich (2003) *Catalogue*, St. Louis, MO).

Standard methods of histology of the immune system are described (see, *e.g.*, Muller-Harmelink (ed.) (1986) *Human Thymus: Histopathology and Pathology*, Springer Verlag, New York, NY; Hiatt, *et al.* (2000) *Color Atlas of Histology*, Lippincott, Williams, and Wilkins, Phila, PA; Louis, *et al.* (2002) *Basic Histology: Text and Atlas*, McGraw-Hill, New York, NY).

Software packages and databases for determining, *e.g.*, antigenic fragments, leader sequences, protein folding, functional domains, glycosylation sites, and sequence alignments, are available (see, *e.g.*, GenBank, Vector NTI® Suite (Informax, Inc, Bethesda, MD); GCG Wisconsin Package (Accelrys, Inc., San Diego, CA); DeCypher® (TimeLogic Corp., Crystal Bay, Nevada); Menne, *et al.* (2000) *Bioinformatics* 16: 741-742; Menne, *et al.* (2000) *Bioinformatics Applications Note* 16:741-742; Wren, *et al.* (2002) *Comput. Methods Programs Biomed.* 68:177-181; von Heijne (1983) *Eur. J. Biochem.* 133:17-21; von Heijne (1986) *Nucleic Acids Res.* 14:4683-4690).

EXAMPLES

Example 1. Preparation of FFPE whole cell lysates and subsequent gene expression analysis using the NanoString nCounter™System.

This example describes the methods used to analyze gene expression in the FFPE tumor samples discussed in the Examples below. Whole cell lysates were prepared from slides of FFPE tissue for analysis on the NanoString nCounter™ gene expression platform (NanoString Technologies, Seattle, WA). Prior to making the cell lysate, each tissue section was deparaffinized in xylene for 3 x 5 min and then rehydrated by immersing consecutively in 100% ethanol for 2 x 2 min, 95% ethanol for 2 min, 70% ethanol for 2 min and then immersed in dH_2O until ready to be processed. Tissue was lysed on the slide by adding 10-50ul of PKD buffer (Qiagen catalog #73504). Tissue was scraped from the slide and transferred to a 1.5 ml eppendorf tube. Proteinase K (Qiagen catalog #73504) was added at no more than 10% final volume and the RNA lysate was incubated for 15 min at 55°C and

then 15 min at 80°C. The RNA lysate was stored at -80°C until gene expression profiling was performed using the NanoString nCounter™ system.

For each tumor sample, 5 ul of cellular lysate was mixed with a set of 400 capture and reporter probe pairs designed by NanoString for a set of 400 genes specified by the inventors herein. Each capture probe was biotinylated on its 3′ end and the 5′ end of each reporter probe was tagged with a fluorescent barcode. Probes and lysate were hybridized overnight at 65 °C for 12-16 hours as per NanoString's recommendations. Hybridized samples were run on the NanoString nCounter™ preparation station using NanoString's high sensitivity protocol, in which excess capture and reporter probes are removed and transcript-specific ternary complexes are immobilized on a streptavidin-coated cartridge. The samples were scanned at maximum scan resolution capabilities using the nCounter™ Digital Analyzer.

Example 2. Discovery of IFN-γ gene signatures.

The inventors herein selected the 400 gene set listed in Table 4 to investigate whether a gene expression signature could be derived that would be useful in predicting which patients are more likely to have an anti-tumor response to therapy with a PD-1 antagonist. This gene set employed tumor samples from a cohort of 19 melanoma patients who had been treated with MK-3475 for which clinical response data was available.

Tumor samples that had been obtained from the patients prior to treatment with MK-3475 were assayed for expression of the 400 gene set in Table 4 using the NanoString nCounter® Analysis System and a CodeSet designed by NanoString to measure expression of the gene set in a single multiplex reaction for each FFPE tumor sample. The CodeSet included the target transcript listed in Table 4 and a pair of capture and reporter probes for that transcript for each of the 400 genes. For each patient tumor sample, the raw transcript expression counts data were normalized by performing quantile normalization relative to the reference distribution and subsequent log10-transformation. The reference distribution was generated by pooling reported counts for all samples after excluding values for technical (both positive and negative control) probes, and without performing intermediate normalization relying on negative (background-adjusted) or positive (synthetic sequences spiked with known titrations).

A two sided t-test analysis was performed using the expression results for all 400 genes in the 19 patients and their best overall response to MK-3475, which was reported as a complete response (CR), partial response (PR) of length of PFS, each as determined by an

independent reviewer using RECIST 1.1 criteria). This analysis resulted in the discovery that increased expression levels of 51 of the 400 genes were associated with a better response (PR or CR) and/or longer PFS (P-value < 0.05, after correction for multiplicity testing). These 51 genes and the target transcripts are listed in Table 5 below.

Table 5: Up-regulated Genes

	Gene	Target Transcript*
1	CCR5	NM_000579
2	HLA-DRA	NM_019111
3	CXCL13	NM_006419
4	CCL5	NM_002985
5	STAT1	NM_007315
6	KLRK1-NKG2D	NM_007360
7	NKG7	NM_005601
8	CXCL9	NM_002416
9	LAIR1	NM_002287
10	LAG3	NM_002286
11	CXCR6	NM_006564
12	KLRD1	NM_002262
13	GZMA	NM_006144
14	PRF1	NM_005041
15	SIGLEC14	NM_001098612
16	PTPN22	NM_015967
17	CD86	NM_175862
18	SLA	NM_001045556
19	SIRPG	NM_001039508
20	CD72	NM_001782
21	HAVCR2	NM_032782
22	PSTPIP2	NM_024430
23	SLAMF6	NM_001184714
24	CD84	NM_001184879
25	CD300LF	NM_139018
26	CD3D	NM_000732
27	IFNG	NM_000619
28	CXCL11	NM_005409
29	CD2	NM_001767
30	CTSZ	NM_001336
31	GZMB	NM_004131
32	IL2RG	NM_000206
33	CXCL10	NM_001565
34	LILRB4	NM_001081438

Table 5: Up-regulated Genes		
35	PDCD1	NM_005018
36	CCL8	NM_005623
37	CIITA	NM_000246
38	CCL4	NM_002984
39	IGSF6	NM_005849
40	PTPRC	NM_080921
41	CLEC9A	NM_207345
42	CST7	NM_003650
43	IDO1	NM_002164
44	ITGAL	NM_002209
45	CDH1	NM_004360
46	PSTPIP1	NM_003978
47	GZMK	NM_002104
48	HLA-E	NM_005516
49	CD3E	NM_000733
50	TAGAP	NM_054114
51	TNFRSF9	NM_001561

The inventors hypothesized that gene signature biomarkers, which would provide a clinically relevant cutoff point for predicting response to MK-3475, could be generated from the genes in Table 5 that are related to IFN-γ signaling. To test their hypothesis, the inventors divided the 19 patient cohort into a group of 11 responders (patients whose best overall response (OR) was a complete response (CR) or partial response (PR) to MK-3475, each as determined by an independent reviewer using RECIST 1.1 criteria) and a group of 8 non-responders (whose best OR was not a CR or PR), and then tested various combinations of IFNG related genes in Table 5 for the ability to separate the majority of responders from the majority of non-responders.

Tumor samples that had been obtained from the patients prior to treatment with MK-3475 were assayed for expression of the 400 gene set in Table 4 using the NanoString nCounter® Analysis System. An IFN-γ gene signature score for each patient tumor sample was calculated as the arithmetic mean of the quantile normalized gene expression amount for each of the transcripts in the candidate gene signature. Association between IFN-γ gene signature score and best overall response to MK-3475 treatment was assessed using a one-sided *t-test* analysis for Response vs. Non-response and a cox-regression analysis for length

of progression free survival (PFS). A cut-off analysis was then performed on two of the IFN-γ gene signatures that demonstrated statistically significant associations between higher gene signature scores and better response to MK-3475: (1) a five gene signature of STAT1, CCR5, CXCL9, PRF1, and HLA-DRA and (2) a ten gene signature of IFNG, STAT1, CCR5, CXCL9, PRF1, HLA-DRA, CXCL10, CXCL11, ID01 and GZMA.

The results of these analyses are shown in Figures 8 to 11 below. For the figures containing box plots, the horizontal line in a box is the median, the top and bottom edges of the box represent the 25th and 75th percentiles, the whiskers extend to the most extreme data points not considered outliers, and outliers are plotted individually.

As shown in Figure 8, when 3.0214 was chosen as a reference (cut-off) score for the five gene IFN-γ gene signature, the response rate was greater than 80% in patients from the 19 patient cohort who were classified as biomarker positive (i.e., score at or above the cut-off) but less than 20% in patients classified as biomarker negative (i.e., score below the cut-off). Also, the mean length of PFS in this cohort was significantly longer in biomarker positive patients than in biomarker negative patients (i.e., less than 3.0214) (see Figure 9).

The ten gene signature performed better than the five gene signature in identifying patients most likely to achieve a clinical benefit to MK-3475, as shown in Figures 10 and 11. Specifically, when a cut-off of 2.462 was selected, there was a 0% response rate and a mean PFS of less than 6 months in biomarker negative patients (e.g., score below the cut-off), compared to a greater than 80% response rate and mean PFS of greater than 10 months in biomarker positive patients (e.g., score at or above the cut-off).

The inventors herein further evaluated the utility of the ten gene IFN-γ gene signature in selecting melanoma patients for therapy with a PD-1 antagonist by comparing the signature scores for samples from the 19 patient cohort with scores for the same IFN-γ gene signature determined for an independent set of melanoma tumors. The range of IFN-γ gene signature scores determined for these two tumor groups are shown in Table 6, with the shaded rows indicating a set of scores that may be useful as a cut-off point, or reference gene signature score, to classify between about 30% and 60% of melanoma tumor samples as biomarker positive, and thus more likely to respond to treatment with MK-3475.

Table 6. Range of IFN-γ Gene Signature Scores in 2 Different Melanoma Patient Sets

IFN-gamma related gene signature	Melanoma-19	Melanoma-71 Independent
1.6794	0%	0%
1.7801	0%	0%
1.8009	0%	0%
1.8263	0%	0%
1.8442	0%	0%
1.8491	0%	0%
1.8647	0%	0%
1.88	0%	0%
1.8863	0%	1%
1.8961	0%	1%
1.9064	0%	1%
1.9144	0%	1%
1.9234	0%	1%
1.9373	0%	1%
1.9513	0%	1%
1.9577	0%	1%
1.9625	0%	1%
1.9687	0%	1%
1.9835	0%	1%
1.9942	5%	3%
1.9962	5%	3%
2.0063	5%	3%
2.0108	5%	4%
2.0192	5%	4%
2.0329	5%	4%
2.0451	5%	4%
2.0609	5%	6%
2.0682	5%	6%
2.0796	5%	7%
2.0919	5%	8%
2.099	5%	8%
2.108	5%	8%
2.1117	5%	8%
2.1174	5%	11%
2.131	5%	11%
2.1405	5%	11%
2.1483	5%	13%
2.1602	5%	14%
2.171	5%	14%
2.1832	10%	15%
2.188	10%	15%
2.1987	10%	17%
2.2038	10%	18%
2.2084	10%	20%
2.2143	10%	23%
2.2248	10%	24%
2.2312	10%	25%
2.2399	10%	28%
2.2475	15%	28%
2.2552	20%	28%
2.2613	20%	31%

Table 6 (Continued).

IFN-gamma related gene signature	Melanoma-19	Melanoma-71 Independent
2.2683	20%	32%
2.2899	20%	32%
2.305	25%	34%
2.3103	25%	37%
2.3196	25%	37%
2.323	25%	37%
2.3384	25%	39%
2.3447	25%	41%
2.3491	25%	44%
2.3562	25%	44%
2.3613	25%	45%
2.3683	25%	46%
2.3798	25%	48%
2.3882	25%	49%
2.3938	25%	51%
2.4031	25%	52%
2.4126	25%	54%
2.4221	25%	54%
2.429	25%	54%
2.4398	25%	55%
2.45	30%	55%
2.4575	30%	55%
2.4691	30%	56%
2.473	35%	56%
2.4826	35%	58%
2.4862	40%	59%
2.4998	40%	62%
2.5167	40%	65%
2.5281	40%	65%
2.5406	40%	69%
2.5698	45%	69%
2.5852	45%	69%
2.5991	45%	73%
2.6189	45%	75%
2.6344	45%	77%
2.6373	45%	79%
2.6477	45%	82%
2.6543	50%	83%
2.6706	50%	86%
2.6862	60%	89%
2.6934	70%	89%
2.7112	70%	90%
2.7324	85%	90%
2.7509	85%	90%
2.7876	85%	93%
2.8222	85%	96%
2.8648	90%	97%
2.8853	90%	97%
2.9575	95%	99%
3.1421	100%	100%

Table 7 provides a brief description of the sequences in the sequence listing.

SEQ ID NO:	Description
1	hPD-1.08A light chain CDR1
2	hPD-1.08A light chain CDR2
3	hPD-1-08A light chain CDR3
4	hPD-1.08A heavy chain CDR1
5	hPD-1.08A heavy chain CDR2
6	hPD-1.08A heavy chain CDR3
7	hPD-1.09A light chain CDR1
8	hPD-1.09A light chain CDR2
9	hPD-1.09A light chain CDR3
10	hPD-1.09A heavy chain CDR1
11	hPD-1.09A heavy chain CDR2
12	hPD-1.09A heavy chain CDR3
13	109A-H heavy chain variable region
14	409A-H heavy chain full length
15	K09A-L-11 light chain variable region
16	K09A-L-16 light chain variable region
17	K09A-L-17 light chain variable region
18	K09A-L-11 light chain full length
19	K09A-L-16 light chain full length
20	K09A-L-17 light chain full length
21	MK-3475 Heavy chain
22	MK-3475 Light chain
23	Nivolumab Heavy chain
24	Nivolumab light chain

5

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All references cited herein are incorporated by reference to the same extent as if each individual publication, database entry (e.g. Genbank sequences or GeneID entries), patent application, or patent, was specifically and individually indicated to be incorporated by reference. This statement of incorporation by reference is intended by Applicants, pursuant to 37 C.F.R. §1.57(b)(1), to relate to each and every individual publication, database entry (e.g. Genbank sequences or GeneID entries), patent application, or patent, each of which is clearly identified in compliance with 37 C.F.R. §1.57(b)(2), even if such citation is not immediately adjacent to a dedicated statement of incorporation by reference. The inclusion of dedicated statements of incorporation by reference, if any, within the specification does not in any way weaken this general statement of incorporation by reference. Citation of the references herein is not intended as an admission that the reference is pertinent prior art, nor does it constitute any admission as to the contents or date of these publications or documents.

CLAIMS

1. A method for testing a tumor for the presence or absence of a biomarker that predicts response to treatment with a PD-1 antagonist, which comprises:

obtaining a sample from the tumor, measuring the raw RNA expression level in the tumor sample for each gene in a IFN-γ gene signature;

normalizing each of the measured raw RNA expression levels; and

calculating the arithmetic mean of the normalized RNA expression levels for each of the genes to generate a score for the IFN-γ gene signature;

wherein the IFN-γ gene signature comprises at least five of the genes in Table 1 below:

Table 1

Gene	Target Transcript
CCL4	NM_002984.2
CCL5	NM_002985.2
CCR5	NM_000579.1
CD2	NM_001767.2
CD86	NM_175862.3
CIITA	NM_000246.3
CXCL10	NM_001565.1
CXCL11	NM_005409.3
CXCL9	NM_002416.1
GZMA	NM_006144
HLA-DRA	NM_019111.3
IDO1	NM_002164.3
IFNG	NM_000619.2
KLRK1	NM_007360.1
PRF1	NM_001083116
STAT1	NM_007315.2

.

2. The method of claim 1, wherein the method further comprises:

comparing the calculated score to a reference score for the IFN-γ gene signature; and

classifying the tumor as biomarker positive or biomarker negative;

wherein if the calculated score is equal to or greater than the reference score, then the tumor is classified as biomarker positive, and if the calculated IFN-γ gene signature score is less than the reference IFN-γ gene signature score, then the tumor is classified as biomarker negative.

3. A method for treating a subject having a tumor which comprises:

determining if the tumor is positive or negative for an IFN-γ gene signature biomarker; and

administering to the subject a PD-1 antagonist if the tumor is positive for the biomarker; or

administering to the subject a cancer treatment that does not include a PD-1 antagonist if the tumor is negative for the biomarker;

wherein the IFN-γ gene signature comprises at least five of the genes in Table 1.

Table 1

Gene	Target Transcript
CCL4	NM_002984.2
CCL5	NM_002985.2
CCR5	NM_000579.1
CD2	NM_001767.2
CD86	NM_175862.3
CIITA	NM_000246.3
CXCL10	NM_001565.1
CXCL11	NM_005409.3
CXCL9	NM_002416.1
GZMA	NM_006144
HLA-DRA	NM_019111.3
IDO1	NM_002164.3
IFNG	NM_000619.2
KLRK1	NM_007360.1
PRF1	NM_001083116
STAT1	NM_007315.2

4. The method of claim 3, wherein the determining step comprises:

obtaining a sample from the subject's tumor;

sending the tumor sample to a laboratory with a request to test the sample for the presence or absence of an IFN-γ gene signature biomarker; and

receiving a report from the laboratory that states whether the tumor sample is biomarker positive or biomarker negative.

5. A method for treating a subject having a tumor which comprises:

obtaining a sample from the tumor;

measuring the raw RNA expression level in the tumor sample for each gene in an IFN-γ gene signature;

normalizing each of the measured raw RNA expression levels;

calculating the arithmetic mean of the normalized RNA expression levels for each of the genes to generate a score for the IFN-γ gene signature; and

administering to the subject a PD-1 antagonist if the calculated score is equal to or greater than a reference score for the IFN-γ gene signature; or

administering to the subject a cancer therapy that does not include a PD-1 antagonist if the calculated score is less than the reference score;

wherein the IFN-γ gene signature comprises at least five of the genes in Table 1.

Table 1

Gene	Target Transcript
CCL4	NM_002984.2
CCL5	NM_002985.2
CCR5	NM_000579.1
CD2	NM_001767.2
CD86	NM_175862.3
CIITA	NM_000246.3
CXCL10	NM_001565.1
CXCL11	NM_005409.3
CXCL9	NM_002416.1
GZMA	NM_006144
HLA-DRA	NM_019111.3
IDO1	NM_002164.3
IFNG	NM_000619.2
KLRK1	NM_007360.1
PRF1	NM_001083116
STAT1	NM_007315.2

6. The method of any one of the above claims, wherein the gene signature consists essentially of IFNG, STAT1, CCR5, CXCL9, PRF1, HLA-DRA, CXCL10, CXCL11, ID01 and GZMA.

7. The method of any one of the above claims, wherein the PD-1 antagonist is nivolumab or MK-3475.

8. A pharmaceutical composition comprising a PD-1 antagonist for use in a subject who has a tumor that tests positive for an IFN-γ gene signature biomarker, wherein the IFN-γ gene signature comprises at least five of the genes in Table 1.

Table 1

Gene	Target Transcript
CCL4	NM_002984.2
CCL5	NM_002985.2
CCR5	NM_000579.1
CD2	NM_001767.2
CD86	NM_175862.3
CIITA	NM_000246.3
CXCL10	NM_001565.1
CXCL11	NM_005409.3
CXCL9	NM_002416.1
GZMA	NM_006144
HLA-DRA	NM_019111.3
IDO1	NM_002164.3
IFNG	NM_000619.2
KLRK1	NM_007360.1
PRF1	NM_001083116
STAT1	NM_007315.2

9. The composition of claim 8, wherein the gene signature consists essentially of IFNG, STAT1, CCR5, CXCL9, PRF1, HLA-DRA, CXCL10, CXCL11, ID01 and GZMA.

10. The composition of claim 8 or 9, wherein the tumor sample is a melanoma tumor sample and the PD-1 antagonist is MK-3475.

11. The pharmaceutical composition of any one of claims 8 to 10, wherein the positive biomarker test result was generated by a method comprising:

obtaining a sample from the tumor,

measuring the raw RNA expression level in the tumor sample for each gene in an IFN-γ gene signature;

normalizing each of the measured raw RNA expression levels;

calculating the arithmetic mean of the normalized RNA expression levels for each of the genes to generate a score for the IFN-γ gene signature;

comparing the calculated score to a reference score for the IFN-γ gene signature; and

classifying the tumor as biomarker positive or biomarker negative;

wherein if the calculated score is equal to or greater than the reference score, then the tumor is classified as biomarker positive, and if the calculated IFN-γ gene signature score is less than the reference IFN-γ gene signature score, then the tumor is classified as biomarker negative.

12. A drug product which comprises a pharmaceutical composition and prescribing information, wherein the pharmaceutical composition comprises a PD-1 antagonist and at least one pharmaceutically acceptable excipient and the prescribing information states that the pharmaceutical composition is indicated for use in a subject who has a tumor that tests positive for an IFN-γ gene signature biomarker.

13. A kit for assaying a tumor sample to determine an IFN-γ gene signature score for the tumor sample, wherein the kit comprises a first set of probes for detecting expression of each gene in the IFN-γ gene signature, wherein the IFN-γ gene signature comprises at least five of the genes in Table 1.

Table 1

Gene	Target Transcript
CCL4	NM_002984.2
CCL5	NM_002985.2
CCR5	NM_000579.1
CD2	NM_001767.2
CD86	NM_175862.3
CIITA	NM_000246.3
CXCL10	NM_001565.1
CXCL11	NM_005409.3
CXCL9	NM_002416.1
GZMA	NM_006144
HLA-DRA	NM_019111.3
IDO1	NM_002164.3
IFNG	NM_000619.2
KLRK1	NM_007360.1
PRF1	NM_001083116
STAT1	NM_007315.2

14. The kit of claim 15, wherein the first set of probes is designed to detect expression of the transcripts listed in Table 1 for each of IFNG, STAT1, CCR5, CXCL9, PRF1, HLA-DRA, CXCL10, CXCL11, ID01 and GZMA.

15. The kit of claims 15 or 16, which further comprises a second set of probes for detecting target transcripts expressed in the tumor sample by a set of normalization genes.

hPD-1.08A light chain CDR1 (SEQ ID NO:1)

Arg Ala Ser Lys Ser Val Ser Thr Ser Gly Phe Ser Tyr Leu His

hPD-1.08A light chain CDR2 (SEQ ID NO:2)

Leu Ala Ser Asn Leu Glu Ser

hPD-1-08A light chain CDR3 (SEQ ID NO:3)

Gln His Ser Trp Glu Leu Pro Leu Thr

hPD-1.08A heavy chain CDR1 (SEQ ID NO:4)

Ser Tyr Tyr Leu Tyr

hPD-1.08A heavy chain CDR2 (SEQ ID NO:5)

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

hPD-1.08A heavy chain CDR3 (SEQ ID NO:6)

Arg Asp Ser Asn Tyr Asp Gly Gly Phe Asp Tyr

FIG.1

hPD-1.09A light chain CDR1 (SEQ ID NO:7)

Arg Ala Ser Lys Gly Val Ser Thr Ser Gly Tyr Ser Tyr Leu His

hPD-1.09A light chain CDR2 (SEQ ID NO:8)

Leu Ala Ser Tyr Leu Glu Ser

hPD-1.09A light chain CDR3 (SEQ ID NO:9)

Gln His Ser Arg Asp Leu Pro Leu Thr

hPD-1.09A heavy chain CDR1 (SEQ ID NO:10)

Asn Tyr Tyr Met Tyr

hPD-1.09A heavy chain CDR2 (SEQ ID NO:11)

Gly Ile Asn Pro Ser Asn Gly Gly Thr Asn Phe Asn Glu Lys Phe Lys Asn

hPD-1.09A heavy chain CDR3 (SEQ ID NO:12)

Arg Asp Tyr Arg Phe Asp Met Gly Phe Asp Tyr

FIG.2

109A-H heavy chain variable region (SEQ ID NO:13)

Gln Val Gln Leu Val Gln Ser Gly Val Glu Val Lys Lys Pro Gly Ala Ser Val Lys
Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr Tyr Met Tyr Trp Val Arg
Gln Ala Pro Gly Gln Gly Leu Glu Trp Met Gly Gly Ile Asn Pro Ser Asn Gly Gly
Thr Asn Phe Asn Glu Lys Phe Lys Asn Arg Val Thr Leu Thr Thr Asp Ser Ser Thr
Thr Thr Ala Tyr Met Glu Leu Lys Ser Leu Gln Phe Asp Asp Thr Ala Val Tyr Tyr
Cys Ala Arg Arg Asp Tyr Arg Phe Asp Met Gly Phe Asp Tyr Trp Gly Gln Gly Thr
Thr Val Thr Val Ser Ser

409A-H heavy chain full length (SEQ ID NO:14)

Gln Val Gln Leu Val Gln Ser Gly Val Glu Val Lys Lys Pro Gly Ala Ser Val Lys
Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr Tyr Met Tyr Trp Val Arg
Gln Ala Pro Gly Gln Gly Leu Glu Trp Met Gly Gly Ile Asn Pro Ser Asn Gly Gly
Thr Asn Phe Asn Glu Lys Phe Lys Asn Arg Val Thr Leu Thr Thr Asp Ser Ser Thr
Thr Thr Ala Tyr Met Glu Leu Lys Ser Leu Gln Phe Asp Asp Thr Ala Val Tyr Tyr
Cys Ala Arg Arg Asp Tyr Arg Phe Asp Met Gly Phe Asp Tyr Trp Gly Gln Gly Thr
Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro
Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr
Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His
Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr
Val Pro Ser Ser Ser Leu Gly Thr Lys Thr Tyr Thr Cys Asn Val Asp His Lys Pro
Ser Asn Thr Lys Val Asp Lys Arg Val Glu Ser Lys Tyr Gly Pro Pro Cys Pro Pro
Cys Pro Ala Pro Glu Phe Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro
Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val
Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val Ser
Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val
Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln
Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met Thr Lys Asn
Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp
Ser Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser Arg Trp Gln
Glu Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr
Gln Lys Ser Leu Ser Leu Ser Leu Gly Lys

FIG.3

K09A-L-11 light chain variable region (SEQ ID NO:15)

Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly Glu Arg Ala
Thr Leu Ser Cys Arg Ala Ser Lys Gly Val Ser Thr Ser Gly Tyr Ser Tyr Leu His
Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile Tyr Leu Ala Ser Tyr
Leu Glu Ser Gly Val Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr
Leu Thr Ile Ser Ser Leu Glu Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln His Ser
Arg Asp Leu Pro Leu Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys

K09A-L-16 light chain variable region (SEQ ID NO:16)

Glu Ile Val Leu Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Pro Gly Glu Pro Ala
Ser Ile Ser Cys Arg Ala Ser Lys Gly Val Ser Thr Ser Gly Tyr Ser Tyr Leu His
Trp Tyr Leu Gln Lys Pro Gly Gln Ser Pro Gln Leu Leu Ile Tyr Leu Ala Ser Tyr
Leu Glu Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr
Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Gln His Ser
Arg Asp Leu Pro Leu Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys

K09A-L-17 light chain variable region (SEQ ID NO:17)

Asp Ile Val Met Thr Gln Thr Pro Leu Ser Leu Pro Val Thr Pro Gly Glu Pro Ala
Ser Ile Ser Cys Arg Ala Ser Lys Gly Val Ser Thr Ser Gly Tyr Ser Tyr Leu His
Trp Tyr Leu Gln Lys Pro Gly Gln Ser Pro Gln Leu Leu Ile Tyr Leu Ala Ser Tyr
Leu Glu Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Ala Phe Thr
Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Val Gly Leu Tyr Tyr Cys Gln His Ser
Arg Asp Leu Pro Leu Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys

FIG.4

K09A-L-11 light chain full length (SEQ ID NO:18)

Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly Glu Arg Ala
Thr Leu Ser Cys Arg Ala Ser Lys Gly Val Ser Thr Ser Gly Tyr Ser Tyr Leu His
Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile Tyr Leu Ala Ser Tyr
Leu Glu Ser Gly Val Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr
Leu Thr Ile Ser Ser Leu Glu Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln His Ser
Arg Asp Leu Pro Leu Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg Thr Val
Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr
Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp
Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp
Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr
Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val
Thr Lys Ser Phe Asn Arg Gly Glu Cys

K09A-L-16 light chain full length (SEQ ID NO:19)

Glu Ile Val Leu Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Pro Gly Glu Pro Ala
Ser Ile Ser Cys Arg Ala Ser Lys Gly Val Ser Thr Ser Gly Tyr Ser Tyr Leu His
Trp Tyr Leu Gln Lys Pro Gly Gln Ser Pro Gln Leu Leu Ile Tyr Leu Ala Ser Tyr
Leu Glu Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr
Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Gln His Ser
Arg Asp Leu Pro Leu Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg Thr Val
Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr
Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp
Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp
Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr
Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val
Thr Lys Ser Phe Asn Arg Gly Glu Cys

FIG.5A

K09A-L-17 light chain full length (SEQ ID NO:20)

Asp Ile Val Met Thr Gln Thr Pro Leu Ser Leu Pro Val Thr Pro Gly Glu Pro Ala
Ser Ile Ser Cys Arg Ala Ser Lys Gly Val Ser Thr Ser Gly Tyr Ser Tyr Leu His
Trp Tyr Leu Gln Lys Pro Gly Gln Ser Pro Gln Leu Leu Ile Tyr Leu Ala Ser Tyr
Leu Glu Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Ala Phe Thr
Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Val Gly Leu Tyr Tyr Cys Gln His Ser
Arg Asp Leu Pro Leu Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg Thr Val
Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr
Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp
Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp
Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr
Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val
Thr Lys Ser Phe Asn Arg Gly Glu Cys

FIG.5B

MK-3475

Heavy chain (SEQ ID NO:21)

QVQLVQSGVE VKKPGASVKV SCKASGYTFT NYYMYWVRQA PGQGLEWMGG 50
INPSNGGTNF NEKFKNRVTL TTDSSTTTAY MELKSLQFDD TAVYYCARRD 100
YRFDMGFDYW GQGTTVTVSS ASTKGPSVFP LAPCSRSTSE STAALGCLVK 150
DYFPEPVTVS WNSGALTSGV HTFPAVLQSS GLYSLSSVVT VPSSSLGTKT 200
YTCNVDHKPS NTKVDKRVES KYGPPCPPCP APEFLGGPSV FLFPPKPKDT 250
LMISRTPEVT CVVVDVSQED PEVQFNWYVD GVEVHNAKTK PREEQFNSTY 300
RVVSVLTVLH QDWLNGKEYK CKVSNKGLPS SIEKTISKAK GQPREPQVYT 350
LPPSQEEMTK NQVSLTCLVK GFYPSDIAVE WESNGQPENN YKTTPPVLDS 400
DGSFFLYSRL TVDKSRWQEG NVFSCSVMHE ALHNHYTQKS LSLSLGK 447

Light chain (SEQ ID NO:22)

EIVLTQSPAT LSLSPGERAT LSCRASKGVS TSGYSYLHWY QQKPGQAPRL 50
LIYLASYLES GVPARFSGSG SGTDFTLTIS SLEPEDFAVY YCQHSRDLPL 100
TFGGGTKVEI KRTVAAPSVF IFPPSDEQLK SGTASVVCLL NNFYPREAKV 150
QWKVDNALQS GNSQESVTEQ DSKDSTYSLS STLTLSKADY EKHKVYACEV 200
THQGLSSPVT KSFNRGEC 219

FIG.6

Nivolumab

Heavy chain (SEQ ID NO:23)

```
QVQLVESGGG VVQPGRSLRL DCKASGITFS NSGMHWVRQA PGKGLEWVAV  50
IWYDGSKRYY ADSVKGRFTI SRDNSKNTLF LQMNSLRAED TAVYYCATND  100
DYWGQGTLVT VSSASTKGPS VFPLAPCSRS TSESTAALGC LVKDYFPEPV  150
TVSWNSGALT SGVHTFPAVL QSSGLYSLSS VVTVPSSSLG TKTYTCNVDH  200
KPSNTKVDKR VESKYGPPCP PCPAPEFLGG PSVFLFPPKP KDTLMISRTP  250
EVTCVVVDVS QEDPEVQFNW YVDGVEVHNA KTKPREEQFN STYRVVSVLT  300
VLHQDWLNGK EYKCKVSNKG LPSSIEKTIS KAKGQPREPQ VYTLPPSQEE  350
MTKNQVSLTC LVKGFYPSDI AVEWESNGQP ENNYKTTPPV LDSDGSFFLY  400
SRLTVDKSRW QEGNVFSCSV MHEALHNHYT QKSLSLSLGK             440
```

Light chain (SEQ ID NO:24)

```
EIVLTQSPAT LSLSPGERAT LSCRASQSVS SYLAWYQQKP GQAPRLLIYD  50
ASNRATGIPA RFSGSGSGTD FTLTISSLEP EDFAVYYCQQ SSNWPRTFGQ  100
GTKVEIKRTV AAPSVFIFPP SDEQLKSGTA SVVCLLNNFY PREAKVQWKV  150
DNALQSGNSQ ESVTEQDSKD STYSLSSTLT LSKADYEKHK VYACEVTHQG  200
LSSPVTKSFN RGEC                                         214
```

FIG.7

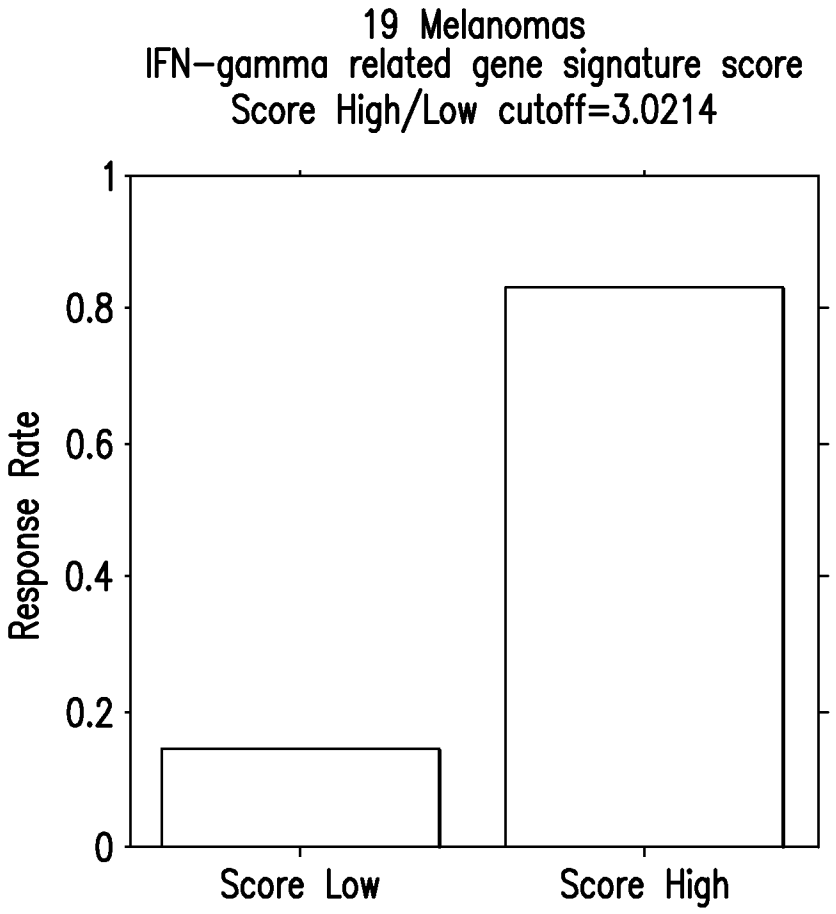
19 Melanomas
IFN-gamma related gene signature score
Score High/Low cutoff=3.0214
Response Rate
1
0.8
0.6
0.4
0.2
0
Score Low
Score High

FIG.8

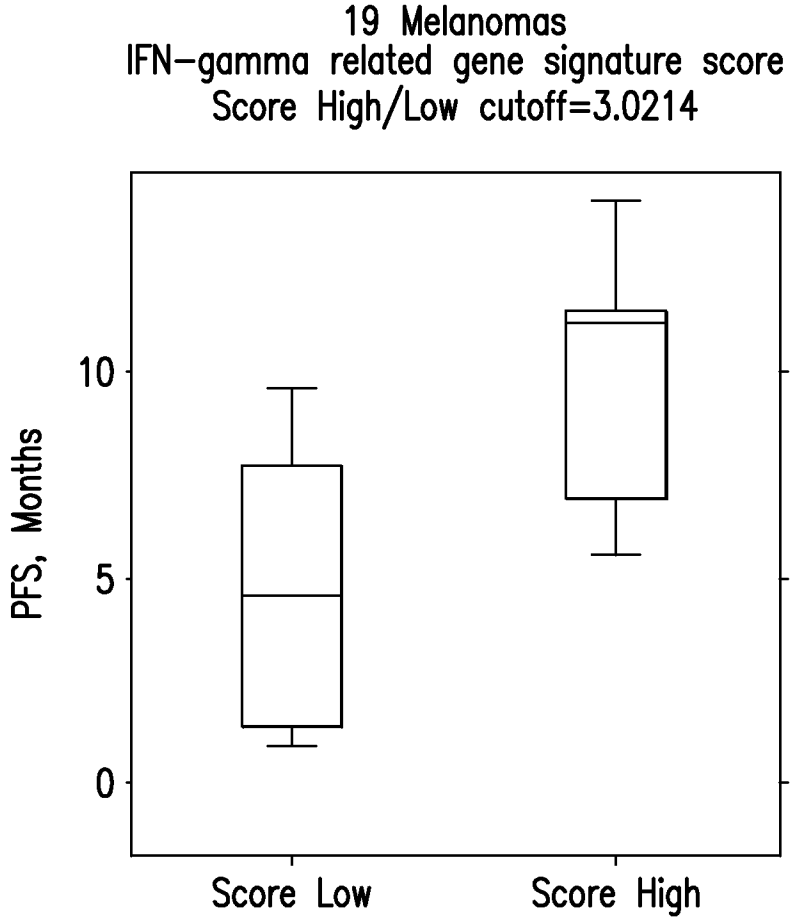
19 Melanomas
IFN-gamma related gene signature score
Score High/Low cutoff=3.0214
PFS, Months
10
5
0
Score Low
Score High

FIG.9

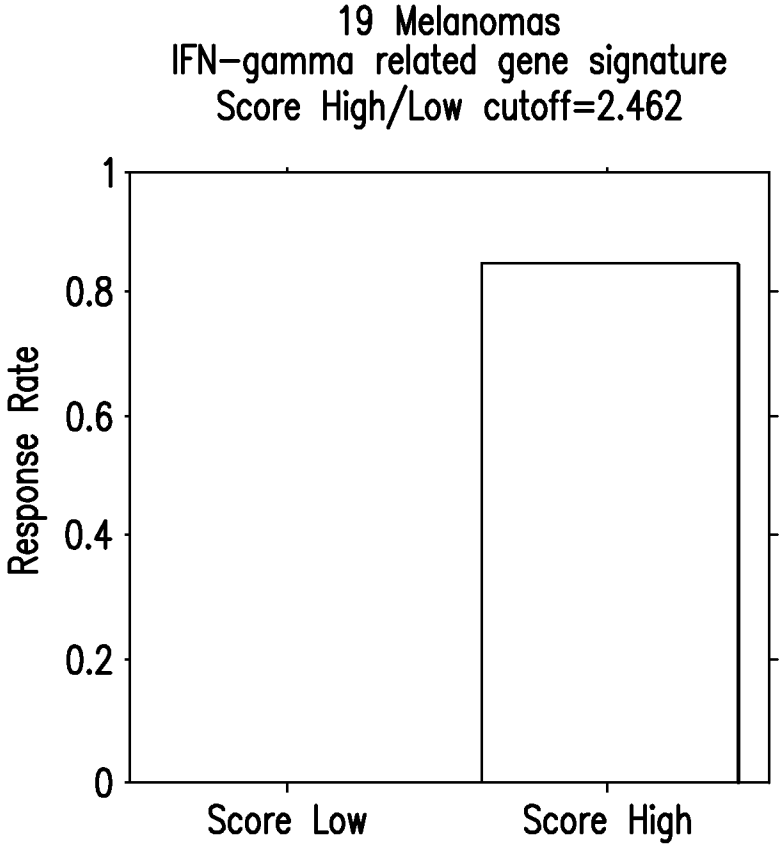
19 Melanomas
IFN-gamma related gene signature
Score High/Low cutoff=2.462
Response Rate
1
0.8
0.6
0.4
0.2
0
Score Low
Score High

FIG.10

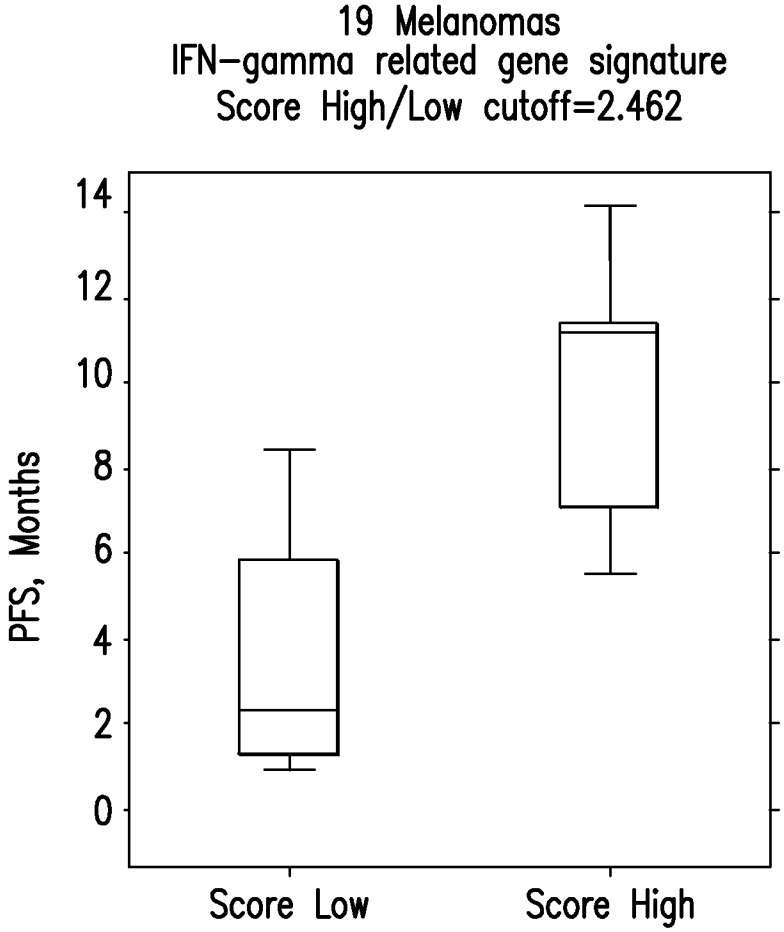
19 Melanomas
IFN-gamma related gene signature
Score High/Low cutoff=2.462
PFS, Months
14
12
10
8
6
4
2
0
Score Low
Score High

FIG.11

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 14/70232

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 filed or furnished:

   a. (means)

   ☐ on paper

   ☒ in electronic form

   b. (time)

   ☒ in the international application as filed

   ☐ together with the international application in electronic form

   ☐ subsequently to this Authority for the purposes of search

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

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 14/70232

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.: 7, 11
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:

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 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.:

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.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 14/70232

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C12Q 1/68 (2015.01)
CPC - C12Q 1/68, C12Q 1/6886, C12Q 2600/106
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)
IPC (8)- C12Q 1/68 (2015.01)
CPC - C12Q 1/68, C12Q 1/6886, C12Q 2600/106

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
CPC - C12Q 1/6883, C12Q 2600/158

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PatBase, Google Patent, Google Scholar
Search Terms: PD-1, RNA, marker or biomarker, gene signature, IFN-gamma or interfereon-gamma or IFNG, CCL4 or CCL5 or CCR5 or CD2 or CD86 or CIITA or CXCL10 or CXCL11 or CXCL9 or GZMA or HLA-DRA or IDO1 or IFNG or KLRK1 or PRF1 or STAT1

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2012/018538 A2 (CARVEN et al.) 09 February 2012 (09.02.2012) claims 1, 8, 12-13, 15-16; para. [0040], [0042], [0097], [00122], [00129], [00161], [00172], [00205], [00211]; Fig. 4A	1-6, 8-10, 12-15
Y	US 2013/0034540 A1 (MULE et al.) 07 February 2013 (07.02.2013) para. [0010]-[0013], [0026], [0062], [0068]	1-6, 8-10, 12-15
Y	WO 2013/043569 A1 (ROLLAND et al.) 28 March 2013 (28.03.2013) para. [0012], [0044]	10
Y	SPRANGER et al. Rational combinations of immunotherapeutics that target discrete pathways. J. Immunother. Cancer. September 2013, Vol. 1, No. 16, pg. 1-14. Especially pg. 9, col. 2, para. 2; pg. 5, col.1, para. 1; pg. 5, col. 2, para. 1	6, 9, 10/9, 14
Y	WRANGLE et al. Alterations of immune response of non-small cell lung cancer with Azacytidine. Oncotarget. November 2013, Vol. 4, No. 11, pg. 2067-2079. Especially abstract; pg. 2070, col. 2, para. 2	6, 9, 10/9, 14
Y	WADDELL et al. Dissecting Interferon-Induced Transcriptional Programs in Human Peripheral Blood Cells. PLoS ONE. 22 March 2010, Vol. 5, No. 3, pg. 1-13. Especially pg. 4, col. 1, para. 1	6, 9, 10/9, 14
Y	MATSUSHITA et al. Strong HLA-DR antigen expression on cancer cells relates to better prognosis of colorectal cancer patients: Possible involvement of c-myc suppression by interferon-gamma in situ. Cancer Sci. January 2006, Vol. 97, No. 1, pg. 57-63. Especially abstract	6, 9, 10/9, 14

☐ Further documents are listed in the continuation of Box C. ☐

* 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
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Date of the actual completion of the international search	Date of mailing of the international search report
24 February 2015 (24.02.2015)	12 MAR 2015
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

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