

SCORING METHODS FOR ANTI-PD THERAPY ELIGIBILITY AND
COMPOSITIONS FOR PERFORMING SAME

CROSS-REFERENCING

This application is a continuation application of U.S. patent application 16/804,395 filed February 28, 2020, which is a continuation of U.S. patent application 15/422,350, filed February 1, 2017 and issued as U.S. Patent 10,613,092 which claims the benefit of priority to
5 U.S. provisional application 62/317,179, filed April 1, 2016, all applications are incorporated herein by reference.

BACKGROUND

Programmed cell death 1 ligand 1 (PD-L1) expression is implicated in evasion of immune responses involved in many contexts, including suppression of anti-tumor immune
10 activity. PD-L1 expression has been shown in situ on a wide variety of solid tumors including breast, lung, colon, ovarian, melanoma, bladder, liver, salivary, stomach, gliomas, thyroid, thymic epithelial, and head and neck cancers (Brown J A et al., 2003. J. Immunol. 170:1257-66; Dong H et al. 2002. Nat. Med. 8:793-800; Hamanishi J, et al. 2007. Proc. Natl. Acad. Sci. USA 104:3360-65; Strome S E et al. 2003. Cancer Res. 63:6501-5; Inman B A et al. 2007.
15 Cancer 109:1499-505; Konishi J et. al. 2004. Clin. Cancer Res. 10:5094-100; Nakanishi J et. al. 2007. Cancer Immunol. Immunother. 56:1173-82; Nomi T et al. 2007. Clin. Cancer Res. 13:2151-57; Thompson R H et al. 2004. Proc. Natl. Acad. Sci. USA 101:17174-79; Wu C, Zhu Y, Jiang J, Zhao J, Zhang X G, Xu N. 2006. Acta Histochem. 108:19-24). In addition, PD-1 expression can be upregulated on tumor infiltrating lymphocytes (TILs), and this may
20 also contribute to tumor immunosuppression (Blank C et al. 2003. J. Immunol. 171:4574-81).

In ovarian cancer, PD-L1 expression is inversely correlated with intraepithelial, but not stromal, infiltrating CD8 T cells, suggesting that PD-L1 inhibits the intratumor migration of CD8 T cells (Hamanishi J et. al. 2007. Proc. Natl. Acad. Sci. USA 104:3360-65). Translation of PD-L1 mRNA is enhanced by loss of PTEN and the ensuing activation of Akt,
25 a common event in tumorigenesis (Parsa A T et al. 2007. Nat. Med. 13:84-88). Studies relating PD-L1 expression on tumors to disease outcome show that PD-L1 expression strongly correlates with unfavorable prognosis in kidney, ovarian, bladder, breast, gastric, and pancreatic cancer (Hamanishi J et al. 2007. Proc. Natl. Acad. Sci. USA 104:3360-65; Inman B

A et al. 2007. *Cancer* 109:1499-505; Konishi J et. al. 2004. *Clin. Cancer Res.* 10:5094-100; Nakanishi J et. al. 2007. *Cancer Immunol. Immunother.* 56:1173-82; Nomi T et al. 2007. *Clin. Cancer Res.* 13:2151-57; Thompson R H et al. 2004. *Proc. Natl. Acad. Sci. USA* 101:17174-79; Wu C, Zhu Y, Jiang J, Zhao J, Zhang X G, Xu N. 2006. *Acta Histochem.* 108:19-24). In addition, these studies suggest that higher levels of PD-L1 expression on tumors may facilitate advancement of tumor stage and invasion into deeper tissue structures. Studies in animal models demonstrate that PD-L1 on tumors inhibits T cell activation and lysis of tumor cells and in some cases leads to increased tumor-specific T cell death (Dong H et al. 2002. *Nat. Med.* 8:793-800; Hirano F et al. 2005. *Cancer Res.* 65:1089-96).

Non-malignant cells have also been implicated in playing important roles in tumor maintenance and growth. For example, tumor-associated APCs can utilize the PD-1:PD-L pathway to control antitumor T cell responses (Curiel et al. 2003. *Nat. Med.* 9:562-67). In this study, PD-L1 expression on a population of tumor-associated myeloid DCs was shown to be up-regulated by tumor environmental factors.

Given the role PD-1/PD-L1 plays in tumor biology, therapeutic agents that target this molecule have been of significant interest. Indeed, anti-PD-1/PD-L1 therapy (or anti-PD therapy) has generated significant clinical benefits by inducing regression of advanced and metastatic tumors and improving survival. Anti-PD therapy can have durable effects, tolerable toxicity, and is applicable to a broad spectrum of cancer types, especially in solid tumors.

Examples of anti-PD therapeutics currently in use or in development include the following:

Nivolumab, Bristol-Myers Squibb (also known as Opdivo, MDX-1106, BMS-936558, and ONO-4538), was the first mAb targeting PD-1 to show significant clinical activity in unresectable or metastatic melanomas, non-small-cell lung carcinoma (NSCLC), and metastatic renal cell carcinomas.

Pembrolizumab, Merck (also known as Keytruda, lambrolizumab, and MK-3475), is an Anti-PD-1 monoclonal antibody that has shown similar efficacy and safety compared with nivolumab in a phase I clinical trial in advanced melanoma (NCT01295827) and is now an FDA-approved second-line drug for the treatment of melanoma. Pembrolizumab is also effective in patients with advanced NSCLC and has shown promising effects in other solid

tumors, including advanced gastric cancer, advanced bladder cancer, head and neck cancer, classical Hodgkin's lymphoma, and triple-negative breast cancer.

BMS-936559, Bristol-Myers Squibb (also known as MDX-1105) is a fully human IgG4 anti-PD-L1 mAb that inhibits the binding of the PD-L1 ligand to both PD-1 and CD80
5 and has demonstrated therapeutic efficacy in a phase I clinical trial (NCT00729664).

MPDL3280A, Genentech/Roche, is an engineered anti-PD-L1 IgG1 mAb that can inhibit PD-L1 interactions with both PD-1 and B7-1. A study of metastatic urothelial bladder cancer (UBC) demonstrated that MPDL3280A has marked activity in controlling tumor growth. Moreover, owing to the mild side effects, including a lack of renal toxicity, patients
10 with UBC, who are often older and have a higher incidence of renal impairment, are thought to tolerate MPDL3280A better than chemotherapy (NCT01375842). In an expansion phase I trial across multiple cancer types, including NSCLC, melanoma, renal cell carcinoma, and other tumors, responses to MPDL3280A were observed in patients with tumors expressing high levels of PD-L1, especially when PD-L1 was expressed by tumor infiltrating
15 lymphocytes TILs.

Pidilizumab (Medivation/CureTech), MEDI4736 (AstraZeneca), and Avelumab (MSB0010718C; Merck-Sorono) are additional PD-L1 targeting antibody-based therapeutic agents that show promise in the treatment of multiple human cancers.

In addition to developing anti-PD therapeutic agents, work in this area has included
20 performing more detailed analysis of not only the malignant cells in tumor biopsies, but also non-malignant cells to identify patients who may respond to these therapies. For example, some published scoring methods for PD-L1 immunohistochemistry (IHC) staining are either capturing percentage of staining and or staining intensity on tumor cells or the PD-L1 staining and staining intensity on tumor-associated immune cells (Phillips et al. Appl
25 Immunohistochem Mol Morphol. 2015 Sep; 23(8): 541–549) (Garon et al. N Engl J Med. 2015 May 21;372(21):2018-28. doi: 10.1056/NEJMoa1501824. Epub 2015 Apr 19). More and more clinical trials indicated that in some tumor indications PD-L1 staining on both tumor and tumor-associated immune cells is associated with clinical outcome (Rosenberg et al., The Lancet, DOI: dx.doi.org/10.1016/S0140-6736(16)00561-4; Allred D, et al. *Mod*
30 *Pathol.* 1998;11:155-168).

While progress has been made in this area, it is clear that using current scoring methods to capture PD-L1 expression on tumor as well as non-tumor cells (e.g., immune cells) is not practical from an assay development standpoint, as it can double the pathologist's workload and/or lead to confusion with respect to having 2 different cutoffs (one for tumor cells and one for non-tumor cells). There is thus still a need to improve tumor tissue scoring methods for identifying patients that will respond effectively to anti-PD therapy.

SUMMARY

Aspects of the present disclosure provide methods for determining the eligibility of a subject having a malignancy for treatment with an anti-PD therapeutic agent based on a Combined Positive Score (CPS) for a tumor tissue sample from the subject. In certain embodiments, the method includes: determining the number of viable PD-L1 positive tumor cells, the number of viable PD-L1 negative tumor cells, and the number of viable PD-L1 positive mononuclear inflammatory cells (MIC) in a tumor tissue sample from a subject having a malignancy; and calculating a combined positive score (CPS) for the tumor tissue sample using the formula:

$$\text{CPS} = \frac{\text{PD-L1 positive tumor cells} + \text{PD-L1 positive MIC}}{\text{PD-L1 positive tumor cells} + \text{PD-L1 negative tumor cells}} \times 100\%$$

wherein the subject is eligible for treatment with an anti-PD therapeutic agent when the CPS is above a threshold. Compositions and kits for performing the methods are also provided.

BRIEF DESCRIPTION OF THE FIGURES

The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the U.S. Patent and Trademark Office upon request and payment of the necessary fee.

Certain aspects of the following detailed description are best understood when read in conjunction with the accompanying drawings. It is emphasized that, according to common practice, the various features of the drawings are not to scale. On the contrary, the dimensions

of the various features are arbitrarily expanded or reduced for clarity. Included in the drawings are the following figures:

Figure 1 provides an example of rules for evaluating control samples.

Figure 2 provides images of positive (left panel) and negative (right panel) control cell staining.

Figure 3 provides a cartoon of a tissue section with a small staining area.

Figure 4 provides a cartoon of a tissue section with a heterogenous tumor area.

Figure 5 provides images of a stained tissue sections showing different intensities of PD-L1 on tumor cells. Top left panel shows negative staining; top right panel shows weak staining; bottom left panel shows moderate staining; and bottom right panel shows strong staining. Arrows indicate stained cells in the relevant panels.

Figure 6 provides an image of a stained tissue section showing PD-L1 positive inflammatory cells. Arrows indicate positively stained cells.

Figure 7 provides an image of a stained tissue section showing cytoplasmic and membrane staining. Labeled arrows show complete staining, partial staining, cytoplasmic staining, and membrane staining.

Figure 8 provides an image of a stained tissue section showing an edge-staining artifact (example of artifact indicated by arrow).

Figure 9 provides an image of a stained tissue section showing a cytoplasmic blush artifact (example of artifact indicated by arrow).

Figure 10 provides an image of a stained tissue section showing the artifact of staining of necrotic tissue (examples of artifact indicated by arrows).

Figure 11 provides an image of a stained tissue section showing an example of HNSCC with a CPS of 0%. Examples of negative MIC and tumor cells are indicted with arrows.

Figure 12 provides an image of a stained tissue section showing Example of HNSCC with a CPS of 1%. Examples of negative and positive MICs and tumor cells are indicated with arrows.

Figure 13 provides an image of a stained tissue section showing Example of HNSCC with a CPS of 55%. Examples of negative and positive MICs and tumor cells are indicated with arrows.

Figure 14 provides an image of a stained tissue section showing Example of Gastric Carcinoma with a CPS of 0%. Examples of negative MIC and tumor cells are indicted with arrows.

5 Figure 15 provides an image of a stained tissue section showing Example of Gastric Carcinoma with a CPS of 1%. Examples of negative and positive MICs and negative tumor cells are indicted with arrows.

Figure 16 provides an image of a stained tissue section showing Example of Gastric Carcinoma with a CPS of 10%. Examples of negative and positive MICs and tumor cells are indicated with arrows.

10 Figure 17 provides an image of a stained tissue section showing Example of Gastric Carcinoma with a CPS of 40%. Examples of negative and positive MICs and tumor cells are indicated with arrows.

Figure 18 provides an image of a stained tissue section showing Example of Gastric Carcinoma with a CPS of 100%. Example of positive tumor cell is indicated with an arrow.

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DEFINITIONS

Unless defined otherwise herein, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are described.

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All patents and publications, including all sequences disclosed within such patents and publications, referred to herein are expressly incorporated by reference.

Numeric ranges are inclusive of the numbers defining the range. Unless otherwise indicated, nucleic acids are written left to right in 5' to 3' orientation; amino acid sequences are written left to right in amino to carboxy orientation, respectively.

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The headings provided herein are not limitations of the various aspects or embodiments of the invention. Accordingly, the terms defined immediately below are more fully defined by reference to the specification as a whole.

30 Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention

belongs. Singleton, et al., DICTIONARY OF MICROBIOLOGY AND MOLECULAR BIOLOGY, 2D ED., John Wiley and Sons, New York (1994), and Hale & Markham, THE HARPER COLLINS DICTIONARY OF BIOLOGY, Harper Perennial, N.Y. (1991) provide one of skill with the general meaning of many of the terms used herein. Still, certain terms are
5 defined below for the sake of clarity and ease of reference.

The term “PD-L1” refers to the Programmed cell death ligand 1 molecule. PD-L1 may refer to human PD-L1 or homologs in other organisms, depending on the context in which it is used. Human PD-L1 is also known as CD274, B7-H, B7H1, B7-H1, B7 homolog 1, MGC142294, MGC142296, PDCD1L1, PDCD1LG1, PDCD1 ligand 1, PDL1,
10 Programmed cell death 1 ligand 1 and Programmed death ligand 1 and has Uniprot number Q9NZQ7 and NCBI gene ID number 29126. Human PD-L1 is a 290 amino acid type I transmembrane protein encoded by the CD274 gene on human chromosome 9. Mouse PD-L1 has NCBI GenBank ID number ADK70950.1.

A “diagnostic marker” is a specific biochemical in the body which has a particular
15 molecular feature that makes it useful for detecting a disease, measuring the progress of disease or the effects of treatment, or for measuring a process of interest.

The term “epitope” as used herein is defined as small chemical groups on the antigen molecule that is bound to by an antibody. An antigen can have one or more epitopes. In many cases, an epitope is roughly five amino acids or sugars in size. One skilled in the art
20 understands that generally the overall three-dimensional structure or the specific linear sequence of the molecule can be the main criterion of antigenic specificity.

A “subject” of diagnosis or treatment is a plant or animal, including a human. Non-human animals subject to diagnosis or treatment include, for example, livestock and pets.

As used herein, the term “tissue section” refers to a piece of tissue that has been
25 obtained from a subject and mounted on a planar surface, e.g., a microscope slide. The sample may be fixed and/or sectioned as desired. A “tumor tissue sample” or “tumor tissue biopsy sample” includes cells derived from a tumor in a subject, e.g., a human subject having a malignancy. Such tissue samples are sometimes referred to simply as a “biopsy”.

A “cell suspension” is used to refer to any cellular sample in which single cells or very
30 small cell aggregates are suspended in a liquid medium. Cell suspensions are used, e.g., in flow cytometry and other single-cell analysis methods. Cell suspensions can be obtained by

processing a solid tissue sample from a subject, e.g., by treating the sample with enzymes, chemicals, and/or placing them under physically disruptive conditions that disaggregate the cells. Such methods are known in the art.

As used herein, the term “formalin-fixed paraffin embedded (FFPE) tissue section” refers to a piece of tissue, e.g., a biopsy that has been obtained from a subject, fixed in formaldehyde (e.g., 3%-5% formaldehyde in phosphate buffered saline) or Bouin solution, embedded in wax, cut into thin sections, and then mounted on a planar surface, e.g., a microscope slide.

As used herein, the term “resin embedded tissue section” refers to a piece of tissue, e.g. a biopsy that has been obtained from a subject, fixed, (e.g., in 3-5% glutaraldehyde in 0.1M phosphate buffer), dehydrated, infiltrated with epoxy or methacrylate resin, cured, cut into thin sections, and then mounted on a planar surface, e.g., a microscope slide.

As used herein, the term “cryosection” refers to a piece of tissue, e.g. a biopsy that has been obtained from a subject, snap frozen, embedded in optimal cutting temperature embedding material, frozen, cut into thin sections and fixed (e.g. in methanol or paraformaldehyde) and mounted on a planar surface, e.g., a microscope slide.

The term “staining” includes binding a target (e.g., an antigen) in a cellular sample with a target-specific binding agent (e.g., an antibody or a nucleic acid) and then detecting the presence of the target-specific binding agent on the cells of the cellular sample using a detectable label or chromogen. The detectable label can be directly conjugated to the target-specific binding agent (e.g., a primary antibody) or may be conjugated to a secondary reagent that binds specifically to an unlabeled target-specific reagent (e.g., a secondary antibody). In some cases, the target-specific reagent is itself detectable, and thus no additional attached label is needed.

By “fluorophore” or “fluorescent label” is meant any label that absorbs light at a first wavelength (or range of wavelengths) and emits light at a second, different wavelength (or range of wavelengths). In general, the emitted light from a fluorescent labels has a longer wavelength, and therefore lower energy, than the absorbed wavelength. Examples of fluorescent labels include, but are not limited to, phycobiliproteins (e.g., phycoerythrin (PE), phycocyanin (PC), allophycocyanin (APC)), rhodamine, fluorescein, alexa fluor, cascade

blue, tetramethylrhodamine, Texas red, and the like, as well as those listed in greater detail below.

5 A “chromogen” or “chromogenic compound” and the like is a substance that can be converted into a colored compound under specific conditions, e.g., when acted upon by an enzyme or under specific chemical/reaction conditions. Examples of enzyme-substrate combinations include: (i) Horseradish peroxidase (HRP) with hydrogen peroxide as a substrate, where the hydrogen peroxidase oxidizes a dye precursor [e.g., orthophenylene diamine (OPD) or 3,3',5,5'-tetramethyl benzidine hydrochloride (TMB)]; (ii) alkaline phosphatase (AP) with para-Nitrophenyl phosphate as chromogenic substrate; and (iii) β -D-
10 galactosidase (β -D-Gal) with a chromogenic substrate (e.g., p-nitrophenyl- β -D-galactosidase) or fluorogenic substrate (e.g., 4-methylumbelliferyl- β -D-galactosidase). Numerous other enzyme-substrate combinations are available to those skilled in the art. For a general review of these, see U.S. Pat. Nos. 4,275,149 and 4,318,980.

As used herein, the term “target-specific binding agent” means any agent that
15 specifically binds to a target or analyte of interest, e.g., a target of interest that is present in a tissue section as described herein (e.g., a polypeptide or polynucleotide). Examples of target-specific binding agents include antibodies, receptors, and ligands, or target-binding fragments thereof, polynucleotide probes, and the like.

As used herein, the term “multiplexing” refers to using more than one label, stain,
20 and/or chromogen for the simultaneous or sequential detection and measurement of a target in a sample, e.g., a tissue section.

As used herein, the terms “antibody” and “immunoglobulin” are used interchangeably and are well understood by those in the field. Those terms refer to a protein consisting of one or more polypeptides that specifically binds an antigen. One form of antibody constitutes the
25 basic structural unit of an antibody. This form is a tetramer and consists of two identical pairs of antibody chains, each pair having one light and one heavy chain. In each pair, the light and heavy chain variable regions are together responsible for binding to an antigen, and the constant regions are responsible for the antibody effector functions. These terms also include fragments of antibodies which retain specific binding to antigen or target, including, but not
30 limited to, Fab, Fv, scFv, and Fd fragments, chimeric antibodies, humanized antibodies, single-chain antibodies, bi-specific hybrid antibodies, and fusion proteins comprising an

antigen-binding portion of an antibody and a non-antibody protein. See, e.g., Hood et al., "Immunology", Benjamin, N.Y., 2nd ed. (1984); Hunkapiller and Hood, Nature, 323, 15-16 (1986); Lanzavecchia et al., Eur. J. Immunol. 17, 105-111 (1987); Huston et al., Proc. Natl. Acad. Sci. U.S.A., 85, 5879-5883 (1988); and Bird et al., Science, 242, 423-426 (1988) which
5 are hereby incorporated by reference herein in their entirety.

As used herein, the terms "primary antibody" and "secondary antibody" refer to different antibodies, where a primary antibody is a polyclonal or monoclonal antibody from one species (rabbit, mouse, goat, donkey, etc.) that specifically recognizes an antigen (e.g., a biomarker) in a sample (e.g., a human tissue sample) under study, and a secondary antibody is
10 an antibody (usually polyclonal) from a different species that specifically recognizes the primary antibody, e.g., in its Fc region.

Sometimes, the label may be indirectly conjugated with the antibody. The skilled artisan will be aware of various techniques for achieving this. For example, the antibody can be conjugated with biotin and any of the four broad categories of labels mentioned above can
15 be conjugated with avidin, or vice versa. Biotin binds selectively to avidin and thus, the label can be conjugated with the antibody in this indirect manner. Alternatively, to achieve indirect conjugation of the label with the antibody, the antibody may be conjugated with a small hapten and one of the different types of labels mentioned above is conjugated with an anti-hapten antibody. Thus, indirect conjugation of the label with the antibody can be achieved. In
20 some embodiments of the invention one or more secondary antibody molecules may be conjugated with a label-conjugated polymer backbone. Thus, amplification of the signal may be achieved.

Indirectly and directly labeled secondary antibodies are also commercially available. For example, one example of commercially available label-conjugated polymer backbone
25 carrying secondary antibody molecules reagent is EnVision™ reagent (DAKO). A secondary antibody carrying a label aimed for a particular type of detection may be obtained from numerous manufacturers.

The term "specific binding" refers to the ability of a binding agent to preferentially bind to a particular analyte that is present in a homogeneous mixture of different analytes. In
30 certain embodiments, a specific binding interaction will discriminate between desirable and

undesirable analytes in a sample, in some embodiments more than about 10 to 100-fold or more (e.g., more than about 1000- or 10,000-fold).

In certain embodiments, the affinity between a binding agent and analyte when they are specifically bound in a capture agent/analyte complex is characterized by a KD
5 (dissociation constant) of less than 10⁻⁶ M, less than 10⁻⁷ M, less than 10⁻⁸ M, less than 10⁻⁹ M, less than 10⁻¹⁰ M, less than 10⁻¹¹ M, or less than about 10⁻¹² M or less.

A “plurality” contains at least 2 members. In certain cases, a plurality may have at least 10, at least 100, at least 1000, at least 10,000, at least 100,000, at least 10⁶, at least 10⁷, at least 10⁸ or at least 10⁹ or more members.

10 Other definitions of terms may appear throughout the specification.

DETAILED DESCRIPTION

In order to further illustrate the present invention, the following specific examples are given with the understanding that they are being offered to illustrate the present invention and
15 should not be construed in any way as limiting its scope.

As summarized above, aspects of the present disclosure include methods for determining the eligibility of a subject having a malignancy for treatment with an anti-PD therapeutic agent. In certain embodiments, the method includes determining a Combined Positive Score (CPS) for PD-L1 expression in a tumor sample from a subject. We have found
20 that use of the disclosed CPS dramatically decreases the work load for pathologists in the clinic, is easier to apply, produces higher inter-observer and intra-observer concordances, and identifies subjects eligible for anti-PD therapy who would otherwise be missed using other scoring methods focusing solely on biomarker expression on tumor cells.

The CPS is calculated by: determining the number of viable PD-L1 positive tumor
25 cells, the number of viable PD-L1 negative tumor cells, and the number of viable PD-L1 positive mononuclear inflammatory cells (MIC) in a tumor tissue sample from a subject having a malignancy; and calculating the CPS using the following formula:

$$\text{CPS} = \frac{\text{\# PD-L1 positive tumor cells} + \text{\# PD-L1 positive MIC}}{\text{\# of PD-L1 positive tumor cells} + \text{PD-L1 negative tumor cells}} \times 100\%$$

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Although the score can be calculated as greater than 100%, the maximum CPS is defined as 100%. The specimen is considered PD-L1 positive (and thus the subject eligible for treatment with anti-PD therapy) if CPS is above a threshold level. Thresholds can be determined by a ROC curve for best responder/cut off (threshold) correlation. Threshold (or cut offs/cut points) can thus be any applicable CPS value, including but not limited to: 1%; 5%; 10%; 20%; 30%; 40%; 50%; 60%, etc., and is specific to the drug used. In some embodiments, the threshold is set at 1%. In such embodiments, the specimen is considered PD-L1 positive if CPS $\geq$ 1% (i.e., equal to or above the threshold value). PD-L1 positive cells are viable cells (tumor or mononuclear inflammatory cells) that exhibit membrane staining at any intensity over background.

PD-L1 expression of the specimen is determined by the Combined Positive Score (CPS). In certain embodiments, the tissue sample (or specimen) must contain a minimum number of viable cells for evaluation, e.g., at least 50 viable tumor cells, at least 100 viable tumor cells, at least 150 viable tumor cells, at least 250 viable tumor cells, etc. By viable cells is meant that the cells were viable at the time they were harvested from the subject, and not necessarily viable at the time of staining. For example, where tumor biopsy sample slides are analyzed, the cells are considered viable cells if they are deemed, upon observation and analysis, to have been viable at the time the tumor biopsy was harvested. If the specimen contains the minimum number of viable cells, the specimen is evaluable using the CPS method, e.g., by calculating an eligibility score.

Detecting PD-L1 positive cells in a tumor sample can be done in any convenient manner.

In certain embodiments, the CPS is calculated from a stained tumor tissue biopsy section (e.g., on a slide) or serial tumor tissue biopsy sections by immunohistochemistry (IHC) staining, in-situ hybridization (ISH; e.g., fluorescence-in-situ-hybridization, or FISH), histological stain, and combinations thereof. In certain embodiments, a tumor tissue biopsy section is analyzed by IHC to calculate the CPS. In certain of these embodiments, the percentage of viable PD-L1 positive and negative tumor cells and PD-L1 positive mononuclear inflammatory cells (MIC) is determined within the tumor nests and the adjacent supporting stroma. In such embodiments, cells are positive for PD-L1 staining if they display partial or complete membrane staining relative to all viable tumor cells present in the sample.

It is noted here that patients with advanced-stage disease of certain tumor types (e.g., non small cell lung carcinoma (NSCLC)) are often diagnosed on a small biopsy or cytology specimen obtained through a minimally invasive procedure. These small biopsy or cytology specimens are often the only samples available for testing. The methods described herein are especially attractive for cytology specimens such as these, i.e., those in which the context of the tissue architecture is lost and it is very challenging to distinguish tumor from immune cells. Such samples include fine needle aspirates (where a thin needle is inserted into an area of abnormal-appearing tissue) or body fluid for sampling of cells. It can be the case that using CPS or a variation thereof is the only reliable method to analyze these specimens.

In other embodiments, the CPS is calculated from a tumor tissue sample that is not a fixed section on a slide. For example, in certain embodiments, the CPS is calculated using flow cytometric analysis of a cell suspension from the tumor tissue sample. In these embodiments, the tumor tissue cell suspension can be stained with a detectable PD-L1 binding agent (e.g., a fluorescently labeled antibody) and analyzed on a flow cytometer for counting the number of tumor cells and MIC cells expressing PD-L1. Tumor cells and MIC in the sample can be distinguished using any convenient flow cytometric parameter, e.g., forward scatter (FS), side scatter (SS), or by the expression of one or more additional markers using corresponding detectable binding agents for the one or more additional markers, e.g., markers specific for MIC or tumor cells. In other embodiments, the cells in the tumor tissue sample can be analyzed on a cell-by-cell basis for mRNA expression of PD-L1 and any other desired target, e.g., using single-cell nucleic acid sequencing methods for gene expression profiling (e.g., next generation sequencing methods).

As noted above, certain embodiments of the disclosed methods include staining the tumor tissue biopsy section for at least one (or multiple) additional target(s) or with a stain. Where multiple targets are assessed, e.g., a cell type-specific marker or a second tumor cell marker, the staining process may be done in a multiplex fashion, i.e., all desired markers are assessed on the same tissue sample simultaneously (e.g., using detectably distinguishable target-specific binding agents). In other embodiments, the multiple targets are detected on separate tissue samples, e.g., serial tissue sections derived from the same tumor biopsy from the subject. In embodiments where an additional stain is employed, the stain can be a histological stain, including but not limited to hematoxylin and eosin (H&E stain), which is

the most commonly used light microscopy stain in histology and histopathology.

Hematoxylin, a basic dye, stains nuclei blue due to an affinity to nucleic acids in the cell nucleus; eosin, an acidic dye, stains the cytoplasm pink. Another commonly performed histochemical technique is the Perls Prussian blue reaction, used to demonstrate iron deposits in diseases like hemochromatosis. In some embodiments, the tissue sample is stained with a viability stain or dye to enhance the identification of viable cells. For example, a tissue sample analyzed by flow cytometry can be contacted with a viability dye prior to analysis, e.g., propidium iodide. Any convenient viability stain may be employed, with many examples known in the art. Further, there are many other staining techniques known in those of skill in the art that can be used to selectively stain cells and cellular components that find use in the present disclosure, and as such no limitation in this regard is intended.

The staining of a target (e.g., PD-L1) in cells from a tumor tissue biopsy is generally done by contacting the cells with one or more detectable target-specific binding agents under suitable conditions to allow for binding of the target-specific binding agent to its desired target (while minimizing non-target binding). As noted above, the term “target-specific binding agent” means any agent that specifically binds to a target or analyte of interest, e.g., a target of interest that is present in a tissue section as described herein (e.g., a polypeptide or polynucleotide). In some embodiments, the target-specific binding agent is an antibody (or target-binding fragments thereof), e.g., as used in IHC and flow cytometry. Staining may be performed with primary and secondary antibodies or without using secondary antibodies (e.g., where the primary antibody is detectably labeled). Non limiting examples of anti-PD-L1 antibodies include, but are not limited to, clone 22C3 (Merck & Co.), clone 28-8 (Bristol-Myers Squibb), and clones SP263 or SP142 (Spring Biosciences). In certain other embodiments, the target-specific binding agent is a nucleic acid or nucleic acid binding agent, e.g., as employed in in situ hybridization (ISH) reactions. For example, the target binding reagent can be a DNA, RNA, DNA/RNA hybrid molecule, peptide nucleic acid (PNA), and the like. No limitation in the metes and bounds of a target-specific binding agent that finds use in the subject disclosure is intended.

The target-specific binding agent (or any secondary reagent used to detect the target-specific binding agent) may be attached to any suitable detectable label (or chromogen) or enzyme capable of producing a detectable label. Thus, in certain embodiments, the first or

second label is produced by an enzymatic reaction, e.g., by the activity of horseradish peroxidase, alkaline phosphatase, and the like. Any convenient enzymatic label/chromogen deposition system can be employed (e.g., as used in standard IHC methods), and as such, no limitation in this regard is intended. In some embodiments, the detectable label is a
5 fluorescent tag.

In some embodiments, the staining reagents used may include a target-specific antibody (e.g., a PD-L1 specific antibody). Where an additional target is to be detected, the staining reagents used may include one or more additional antibodies that each bind to a different antigen. For example, a set of antibodies may include a first antibody that binds to a
10 first antigen (e.g., PD-L1), a second antibody that binds to a second antigen, a third antibody that binds to a third antigen and, optionally a fourth antibody that binds to a fourth antigen and/or further antibodies that bind to further antigens. In some embodiments, the antibody/antibodies used are primary antibodies that are detected by use of a secondary antibody (or other reagent). The staining steps thus may be done by incubating the cells of
15 the tissue sample, e.g., a tissue section or cell suspension, with the primary antibody/antibodies and then, after the primary antibody has bound to the desired target in/on the cells, incubating the cells with the labeled secondary antibody/antibodies (e.g., as is done in standard IHC protocols). In some multiplex embodiments, each of the primary antibodies for each different target is from a different species (e.g., goat, rabbit, mouse, camel, chicken,
20 donkey, etc.) and the corresponding secondary antibodies specific for each different primary antibody are distinguishably labeled from each other.

In some multiplex embodiments, the first and second (and subsequent) targets being detected are different from each other, e.g., are different proteins or polynucleotides (e.g., different genes). However, in some multiplex embodiments, there may be some overlap. For
25 example, in certain cases, a first target-specific binding agent may bind to the same target as a second target-specific binding agent but at a different epitope or site.

As noted above, the tissue sample (e.g., tissue section or cell suspension) can be assessed for targets other than PD-L1. In certain embodiments, the additional target is one that identifies tumor cells. Examples include, but are not limited to: cytokeratin markers
30 including pan-CK cocktails, (e.g., AE1/AE3, CAM5.2/AE1) for adenocarcinomas and CK903 (34βE12) for squamous cell carcinomas. In certain embodiments, the additional target is one

that identifies MIC. Examples include, but are not limited to: CD3, CD5, CD4, CD7, CD8, CD20, and other markers of MIC.

5 In certain embodiments, the sample being analyzed is a tissue section, e.g., a formalin fixed and paraffin embedded (FFPE) tissue section. In alternative embodiments, the tissue section has been fixed in a different way, including tissue sections that have been fixed in, e.g., acrolein, glyoxal, smium tetroxide, arbodiimide, mercuric chloride, zinc salts, picric acid, potassium dichromate, ethanol, methanol, acetone, and/or acetic acid.

10 In embodiments in which a tissue section is analyzed by microscopy, the method further comprises comparing the relative location of the detected first (or any subsequent) label on the tissue section(s). This can be done, for example, by overlaying multiple images of the slide or series of slides that were collected during the analysis (e.g., for different labels). For example, one or more images collected for the labels of a first tissue section (or first label) can be overlaid onto one or more images collected for a second adjacent tissue section (or second, distinguishable label).

15 In certain embodiments, the images may be overlaid and analyzed to identify the boundaries of individual cells or regions in the tissue section, and/or subcellular features in individual cells, in the image. Computer-implemented methods for segmenting images of cells and tissues are known in the art and range from relatively simple thresholding techniques (see, e.g., Korde et al. Anal Quant Cytol Histol. 2009 31: 83–89 and Tuominen et al. Breast Cancer Res. 2010 12: R56), to more sophisticated methods, such as, for instance, adaptive attention windows defined by the maximum cell size (Ko et al. J Digit Imaging. 2009 22: 259–274) or gradient flow tracking (Li, et al. J Microsc. 2008 231: 47–58). Some suitable image segmentation methods may be reviewed in Ko et al. (J Digit Imaging. 2009 22: 259-74) and Ong et al. (Comput Biol Med. 1996 26:269-79). Next the data that corresponds to each of the
25 individual parameters that have been defined by the segmenting are integrated to provide, for each cell, values that indicate which markers are associated with the cell. In certain cases, a cell may be identified as being malignant, non-malignant, infiltrating non-malignant, etc., as a result of this analysis. This data may allow one to potentially type the cells in the sample. As such, this method may comprise displaying an image of the sample, in which the cells are
30 color-coded by their type.

In certain embodiments, the tissue section may be a section of a tissue biopsy obtained from a patient, e.g., a patient having a malignancy. Biopsies of interest include both tumor and non-neoplastic biopsies of skin (melanomas, carcinomas, etc.), soft tissue, bone, breast, colon, liver, kidney, adrenal, gastrointestinal, pancreatic, gall bladder, salivary gland, cervical, 5 ovary, uterus, testis, prostate, lung, thymus, thyroid, parathyroid, pituitary (adenomas, etc.), brain, spinal cord, ocular, nerve, and skeletal muscle, etc. In certain embodiments, the subject from which the biopsy is obtained has a malignancy is selected from: gastric cancer, esophageal carcinomas, head and neck cancer (e.g., head and neck squamous cell carcinoma, or HNSCC), renal cell carcinoma, urothelial/bladder carcinoma, ovarian carcinoma, myeloma, 10 melanoma, lung cancer, classical Hodgkin's lymphoma, and breast cancer (e.g., triple negative breast cancer, hormone receptor positive (ER and/or PR) and Her2 positive breast cancer), small cell lung cancer, salivary gland carcinoma, vulvar carcinoma, thyroid carcinoma, anal canal carcinoma, biliary carcinoma, mesothelioma, cervical carcinoma, and neuroendocrine carcinoma.

15 In some embodiments, the method may involve obtaining one or more image as described above (e.g., an electronic form of which may have been forwarded from a remote location) and may be analyzed by a doctor or other medical professional to calculate the CPS. In other embodiments, the tissue sections are assessed in real time, i.e., not from a stored image of the slide (or other form of stored data). In some embodiments, a slide or image of 20 the slide, as described above, is assessed and a CPS is calculated in an automated fashion *in silico*, e.g., without the slide(s) or image(s) of the slide being assessed by a human. In such embodiments, the slide(s)/image(s) are analyzed by a computer that has been programmed to analyze the staining pattern and identify the PD-L1 positive and negative tumor cells as well as the PD-L1 positive MICs. In other embodiments, the image of the slide or slides is 25 annotated by a slide imaging device such that cells of each different cell type (e.g., PD-L1 positive tumor, PD-L1 negative tumor, and PD-L1 positive MIC) are readily identifiable by a user, e.g., by color coding of the cells or regions in the image.

In any embodiment, data can be forwarded to a "remote location," where "remote location" means a location other than the location at which the image is examined. For 30 example, a remote location could be another location (e.g., office, lab, etc.) in the same city, another location in a different city, another location in a different state, another location in a

different country, etc. As such, when one item is indicated as being “remote” from another, what is meant is that the two items can be in the same room but be separated, or at least in different rooms or different buildings, and can be at least one mile, ten miles, or at least one hundred miles apart. “Communicating” information references transmitting the data
5 representing that information as electrical signals over a suitable communication channel (e.g., a private or public network). “Forwarding” an item refers to any means of getting that item from one location to the next, whether by physically transporting that item or otherwise (where that is possible) and includes, at least in the case of data, physically transporting a medium carrying the data or communicating the data. Examples of communicating media
10 include radio or infra-red transmission channels as well as a network connection to another computer or networked device, and the internet or include email transmissions and information recorded on websites and the like. In certain embodiments, the image may be analyzed by an MD or other qualified medical professional, and a report based on the results of the analysis of the image may be forwarded to the patient from which the sample was
15 obtained.

Specific Embodiments

The specific embodiments below are intended for illustration purposes only and are not intended to be limiting.

20 The purpose of these embodiments is to provide guidelines for evaluating PD-L1 expression on formalin-fixed, paraffin-embedded (FFPE) tumor tissue section with Dako’s PD-L1 IHC 22C3 pharmDx kit (SK006). This immunohistochemical (IHC) assay is performed using the Dako Autostainer Link 48 automated staining system.

The embodiments below are described with respect to the use of the PD-L1 IHC 22C3
25 pharmDx kit, which is a qualitative immunohistochemical assay using Monoclonal Mouse Anti-PD-L1, Clone 22C3. This kit is intended for use in the detection of PD-L1 protein in formalin-fixed, paraffin-embedded (FFPE) tumor tissue using EnVision FLEX visualization system on Autostainer Link 48. Here, PD-L1 protein expression is used to determine a Combined Positive Score (CPS; as describe above). In these embodiments, the specimen is
30 considered PD-L1 positive if the $CPS \geq 1\%$, where PD-L1 positivity is defined as a viable cell

exhibiting membrane staining with the 22C3 antibody at any intensity (as compared to positive and negative controls, as described below).

PD-L1 IHC 22C3 pharmDx is indicated as an aid in identifying patients having a malignancy for treatment with KEYTRUDA® (pembrolizumab) (e.g., patients with gastric carcinoma or HNSCC).

Definitions

Word / Acronym	Definition of Word / Word(s) for Acronym
CPS	Combined Positive Score
H&E	Hemotaxylin and eosin
MCF-7	PD-L1-negative control cell line
MIC	Mononuclear inflammatory cells
NCI-H226	PD-L1-positive control cell line
NCR	Negative Control Reagent
PD-L1	Programmed Death Ligand 1

Clinical Interpretation Guidelines for PD-L1 IHC 22C3 pharmDx in TumorTissue

Specimen Criteria

A hemotaxylin and eosin (H&E) stained section is recommended for the evaluation of an acceptable tumor tissue sample. PD-L1 IHC 22C3 pharmDx and the H&E staining is performed on serial sections from the same paraffin block of the specimen to confirm:

1. The histological diagnosis of the cancer/malignancy.
2. The specimen contains a minimum of 100 viable tumor cells to determine the percentage of positive cells. For patient specimens with less than 100 viable tumor cells, tissue from a deeper level of the block, or potentially another block, could present sufficient number of viable tumor cells for PD-L1 IHC 22C3 pharmDx testing.
3. The specimen has been properly fixed and prepared for IHC analysis. Well-preserved and well-stained areas of the specimen are used to make a determination of the percentage of cells present (e.g, PD-L1 positive tumor cells).

Evaluating Controls

Deviations in the recommended procedures for tissue fixation, processing and embedding in the user's laboratory may produce significant variability in results. Therefore,
5 the following controls can be included in each staining run.

Quality Control Summary		
Control Type	Reagents Used in Testing	Purpose of Testing
Control Slide supplied by Dako	Primary Antibody, *Negative Control Reagent & Detection System	Controls staining procedure only
Positive Control: Tissue or cells containing target antigen to be detected. The ideal control is weakly positive staining tissue, which may be more sensitive in detecting reagent degradation.	Primary Antibody, Negative Control Reagent & Detection System	Controls all steps of the analysis. Validates reagents and procedures used for PD-L1 staining.
Negative Control: Tissues or cells expected to be negative (could be located in patient tissue or positive control tissue).	Primary Antibody, Negative Control Reagent & Detection System	Detection of unintended antibody cross-reactivity to cells/cellular components.
Patient tissue slide	Negative Control Reagent & same Detection System as used with the Primary Antibody	Detection of non-specific background staining.

*From the same species as the primary antibody, but not directed against the same target antigen. To detect non-specific antibody binding, e.g. binding of Fc portion of antibody by the tissue.

All control tissues must meet acceptance criteria to proceed with evaluation of PD-L1 staining on gastric carcinoma tissues. (See Figure 1 for an example of the Rules to follow when evaluating controls.)

5 ***PD-L1 IHC 22C3 pharmDx Control Cell Line Slide***

Examine the PD-L1 IHC 22C3 pharmDx control cell line slide to determine that reagents are functioning properly. Each slide contains sections of cell pellets with positive and negative PD-L1 expression. Assess the percentage of positive cells and the staining intensity. If any staining of the control cell line slide is not satisfactory, all results with the
10 specimens should be considered invalid. Control cell line slide is not used as an aid in interpretation of patient results.

Evaluate the overall staining intensity using the following guide:

0	Negative
1+	Weak Intensity
2+	Moderate Intensity
3+	Strong Intensity

Figure 2 shows representative PD-L1 positive (left panel) and negative (right panel)
15 control cell line staining. The PD-L1 positive cell line NCI-H226 and the negative control cell line is MCF-7. As seen in this figure, at least 70% of the PD-L1 positive control cells contain cell membrane staining of at least 2+ average staining intensity and any non-specific staining is < 1+ staining intensity. For the PD-L1 negative cell pellet, the majority of cells demonstrate no staining. (The presence of 10 or fewer cells with distinct cell membrane
20 staining in the negative control cell sample is generally acceptable). Any non-specific staining is < 1+ staining intensity.

User-Provided Control Tissue Slides

A user-provided positive control tissue slide is examined after the control cell line
25 slides described above. This slide verifies that the fixation method and epitope retrieval process are effective. The ideal positive control tissue provides a complete dynamic representation of weak to moderate cell membrane staining of tumor cells and/or cell

membrane/cytoplasmic staining of tumor-associated mononuclear inflammatory cells (MICs). Use intact cells for interpretation of staining results because necrotic or degenerated cells often stain non-specifically. Non-specific staining should be $\leq 1+$.

5 A user-provided negative control tissue slide is also examined to verify the specificity of the labeling of the target antigen by the primary antibody. The ideal negative control tissue demonstrates no staining on tumor cells and MICs. The absence of specific staining in the negative control tissue slide confirms the lack of kit cross-reactivity to cells/cellular components. The variety of different cell types present in most tissue sections offers internal negative control sites; this should be verified by the user.

10 If staining of positive and/or negative control tissues are not satisfactory, the results with the patient specimen should be considered invalid.

In addition, patient specimens are stained with a Negative Control Reagent (NCR) from the PD-L1 IHC 22C3 pharmDx kit. Specimens stained with the NCR should have 0 specific staining and $\leq 1+$ non-specific staining. Staining occurring in the cytoplasm of tumor cells of the specimen treated with the NCR should be considered non-specific staining.

Example of Scoring Guidelines

For evaluation of tissue section staining and scoring (e.g., using IHC), an objective of 10-20x magnification is used. Partial or complete cell membrane staining of tumor cells that is perceived distinct from cytoplasmic staining is considered positive, as is cell membrane/cytoplasmic staining of MICs within the tumor nests and the adjacent supporting stroma. Adjacent MICs are defined as being within the same 20x field as the tumor. However, MICs that are NOT directly associated with the response against the tumor should be excluded. For example, in some cases, MICs are in the 20x field of view but can clearly be directly linked to non-tumor related causes. Such MICs should be excluded from scoring.

Refer to the table below for details in evaluating PD-L1 positivity.

Element	Included in Scoring	Excluded from Scoring
Tumor cells	Convincing partial or complete membrane staining (at any intensity) of viable carcinoma	Tumor cell cytoplasmic staining

	tumor cells	
Immune cells	Membrane and/or cytoplasmic* staining (at any intensity) of mononuclear inflammatory cells (MIC) within tumor nests and adjacent supporting stroma**, such as: ○ Lymphocytes (lymphocyte aggregates) ○ Monocytes ○ Macrophages	• Immune cells not infiltrating nor adjacent to tumor • Normal cells adjacent to tumor cells • Stromal cells (fibroblasts) • Necrotic cells and/or cellular debris that may stain PD-L1 positive • Plasma cells • Neutrophils
Morphology Patterns	• N/A	• Tissue damage/immune cells not responsive to tumor (e.g., chronic inflammation)

*Lymphocytes often present indistinctive staining of membrane and cytoplasm due to a high nuclear to cytoplasmic ratio. Therefore, membrane and/or cytoplasmic staining in lymphocytes shall be included in the score.

**Adjacent MICs are defined as being within the same 20x field as the tumor. However, MICs that are NOT directly associated with the response against the tumor should be excluded.

Examples for Simple Calculations of CPS

Example 1: Calculate CPS for a specimen containing 100 total tumor cells, 80 positive tumor cells, and 50 positive adjacent MICs.

$$\text{CPS} = \frac{80 \text{ positive tumor cells} + 50 \text{ positive MICs}}{100 \text{ total tumor cells}} \times 100\%$$

In this case the CPS = 100% (although the score is greater than 100%, the maximum CPS is defined as 100%). The specimen is thus scored as PD-L1 Positive, and as such the subject is considered as eligible for anti-PD therapy.

Example 2: Calculate CPS for a specimen containing 1000 total tumor cells, 0 positive
5 tumor cells, 10 positive adjacent MICs.

$$\text{CPS} = \frac{0 \text{ positive tumor cells} + 10 \text{ positive MICs}}{1000 \text{ total tumor cells}} \times 100\%$$

10

In this case the CPS = 1%. The specimen is thus scored as PD-L1 Positive (the threshold is 1% in this example), and as such the subject is considered as eligible for anti-PD therapy.

Example 3: Calculate CPS for a specimen containing 1000 total tumor cells, 5 positive
tumor cells, and 0 positive adjacent MICs.

15

$$\text{CPS} = \frac{5 \text{ positive tumor cells} + 0 \text{ positive MICs}}{1000 \text{ total tumor cells}} \times 100\%$$

20 In this case the CPS = 0.5%. The specimen is thus scored as PD-L1 Negative, and as such the subject is not considered as eligible for anti-PD therapy.

Examples of More Complex Tissue Stains

Example 1: Calculation of Combined Positive Score in a Small Tumor Area with
25 Staining

Figure 3 shows a cartoon of a tissue section with a small staining area. At lower magnification (left), only about 10% of the tissue area is stained (90% of the tissue area is without staining). To calculate the CPS, the stained area is evaluated at a higher magnification (right) to estimate the number of PD-L1 positive and negative tumor cells and
30 mononuclear inflammatory cells that are PD-L1 positive. In this area, 50 out of 100 tumor cells are PD-L1 positive, and there are 34 mononuclear inflammatory cells that are PD-L1 positive. The CPS score of the stained area is thus:

$$\frac{50 \text{ positive tumor cells} + 34 \text{ positive MICs}}{100 \text{ tumor cells}} \times 100 = 84\%$$

5 Because only 10% of the sample was stained, the CPS score is adjusted by multiplying the CPS of the stained area with the percentage of the tissue that was stained (in this case, 10%). The CPS score for the entire tissue sample is thus 10% x 84% = 8.4%. This specimen is thus above the threshold of 1% and is considered PD-L1 positive (and thus the subject is eligible for anti-PD therapy).

10 Example 2: Calculation of Combined Positive Score in a Heterogeneous Tumor Area

Figure 4 shows a cartoon of a tissue section with a heterogenous tumor area. At lower magnifications (upper left), the tissue sample is visually divided into sections. A CPS for each section is determined by observing them at higher magnification (lower right). CPS for each of the four respective sections is as follows (clockwise from top left: 81%, 29%, 100%,
15 and 53%. To get an overall CPS, these four scores are averaged, giving an overall CPS of 65%. This specimen is thus above the threshold of 1% and is considered PD-L1 positive (and thus the subject is eligible for anti-PD therapy).

Examples of Images for Interpretation of PD-L1 IHC 22C3 pharmDx Staining

20 Figures 5 through 18 provide examples of images of tissue sections stained with the PD-L1 IHC 22C3 pharmDx kit.

Figure 5 provides an image of a stained tissue section showing different intensities of PD-L1 on tumor cells.

25 Figure 6 provides an image of a stained tissue section showing PD-L1 positive inflammatory cells.

Figure 7 provides an image of a stained tissue section showing cytoplasmic and membrane staining. Labeled arrows show complete staining, partial staining, cytoplasmic staining, and membrane staining.

30 Figure 8 provides an image of a stained tissue section showing an edge-staining artifact (example of artifact indicated by arrow).

Figure 9 provides an image of a stained tissue section showing a cytoplasmic blush artifact (example of artifact indicated by arrow).

Figure 10 provides an image of a stained tissue section showing the artifact of staining of necrotic tissue (examples of artifact indicated by arrows).

Figure 11 provides an image of a stained tissue section showing an example of HNSCC with a CPS of 0%. Examples of negative MIC and tumor cells are indicted with
5 arrows.

Figure 12 provides an image of a stained tissue section showing Example of HNSCC with a CPS of 1%. Examples of negative and positive MICs and tumor cells are indicated with arrows.

Figure 13 provides an image of a stained tissue section showing Example of HNSCC
10 with a CPS of 55%. Examples of negative and positive MICs and tumor cells are indicated with arrows.

Figure 14 provides an image of a stained tissue section showing Example of Gastric Carcinoma with a CPS of 0%. Examples of negative MIC and tumor cells are indicted with arrows.

Figure 15 provides an image of a stained tissue section showing Example of Gastric Carcinoma with a CPS of 1%. Examples of negative and positive MICs and negative tumor cells are indicted with arrows.

Figure 16 provides an image of a stained tissue section showing Example of Gastric Carcinoma with a CPS of 10%. Examples of negative and positive MICs and tumor cells are
20 indicated with arrows.

Figure 17 provides an image of a stained tissue section showing Example of Gastric Carcinoma with a CPS of 40%. Examples of negative and positive MICs and tumor cells are indicated with arrows.

Figure 18 provides an image of a stained tissue section showing Example of Gastric
25 Carcinoma with a CPS of 100%. Example of positive tumor cell is indicated with an arrow.

Kits

Also provided by this disclosure are kits that provide reagents for analyzing a tissue section(s) for determining the eligibility of a subject having a malignancy for treatment with
30 an anti-PD therapeutic agent according to the methods described herein.

For example, a kit may contain a detectable PD-L1 specific binding agent and instructions for determining and/or calculating a combined positive score (CPS). The instructions can thus include descriptions for sample analysis, for interpreting the results of the test, and/or for calculating a CPS score to determine the eligibility of a subject having a malignancy for treatment with an anti-PD therapeutic agent, e.g., an anti-PD-L1 antibody therapy. Such instructions for practicing the subject methods and interpreting the results are generally recorded on a suitable recording medium. For example, the instructions may be printed on a substrate, such as paper or plastic, etc. As such, the instructions may be present in the kits as a package insert, in the labeling of the container of the kit or components thereof (i.e., associated with the packaging or subpackaging), etc. In other embodiments, the instructions are present as an electronic storage data file present on a suitable computer readable storage medium, e.g., CD-ROM, diskette, USB drive, Cloud storage location, etc. In yet other embodiments, the actual instructions are not present in the kit, but means for obtaining the instructions from a remote source, e.g., via the internet, are provided. An example of this embodiment is a kit that includes a web address where the instructions can be viewed and/or from which the instructions can be downloaded. As with the instructions, this means for obtaining the instructions is recorded on a suitable substrate.

The kit can further include any one or more of the following: a positive control sample, a negative control sample, a detectable binding agent for a marker specific for mononuclear inflammatory cells (MIC), e.g., CD3, CD5, CD4, CD7, CD8, CD20, and other markers of MIC, one or more reagents for performing an immunohistochemistry (IHC) staining reaction on a tumor tissue sample from a subject.

The various components of the kit may be present in separate containers or certain compatible components may be pre-combined into a single container, as desired.

EXAMPLES

Example 1

The main challenge around interpretation of tumor tissue stains for PD-L1 (e.g., TPS and MIDS scores, described in detail below) is to distinguish tumor from immune cells. This is especially true in gastric carcinoma since tumor and immune cells are of similar size and

therefore very challenging to differentiate. Importantly, recent studies indicate that in certain tumor types expression of PD-L1 in immune as well as in tumor cells may be a good predictor of efficacy of anti-PD-1 immunotherapy (e.g., like pembrolizumab).

To address the challenges around companion diagnostic assay development timelines
5 and scoring interpretation, we propose below a Combined Positive Score (CPS).

CPS for use in Gastric Carcinoma

Samples Analyzed

Specimens from subjects having gastric carcinoma patients are analyzed. These
10 samples can be from patients prior to treatment, during treatment, or after an initial round of treatment with a therapeutic agent, e.g., pembrolizumab.

Tissue specimen preparation and staining

Specimens for analysis are formalin-fixed and paraffin-embedded (FFPE) tissue
15 sections. Sections are cut to a desired thickness (e.g., 4 um thickness), placed on charged slides, and dried in an oven for one hour. For example, human tissue sections can be dried at 56-60°C. The cut sections can be stored in the dark at 2-8°C and stained with the PD-L1 IHC assay within 6 months.

The IHC staining for PD-L1 expression is performed using the Dako Autostainer Link
20 48 platform (Dako AS480) and an automated staining protocol validated for the PD-L1 IHC 22C3 pharmDx assay. Deparaffinization, rehydration and target retrieval of the tissue sections is performed in the PT Link (Dako PT100) using a 3-in-1 procedure. Following incubation with the monoclonal mouse anti-human PD-L1 antibody (clone 22C3) or the Negative Control Reagent (NCR) (mouse IgG isotype control), specimens are incubated with
25 anti-mouse linker antibody specific to the host species of the primary antibody, and then incubated with a ready-to-use visualization reagent consisting of secondary antibody molecules and horseradish peroxidase molecules coupled to a dextran polymer backbone. The enzymatic conversion of the subsequently added 3,3'-diaminobenzidine tetrahydrochloride (DAB) chromogen followed by DAB enhancer results in precipitation of a visible reaction
30 product at the site of antigen. The specimens are then counterstained with Hematoxylin and coverslipped. Results are interpreted by direct observation using a light microscope. Assay

performance is optimized for sensitivity with minimum non-specific staining by adjusting primary antibody concentration and reagent incubation times.

Staining interpretation

5 Hematoxylin and Eosin stain (H&E) is used by the pathologist to confirm diagnosis and situate the tumor. Hematoxylin counterstain on the PD-L1 slide is used to estimate the number of cells. Tumor cells are typically larger than mononuclear inflammatory cells.

Initially, PD-L1 expression is determined and interpreted as follows:

1. The Tumor Proportion Score (TPS): the percentage of tumor cells expressing PD-
10 L1 on the cell membrane. Linear partial or complete cell membrane staining is interpreted as positive for PD-L1.

2. Mononuclear Inflammatory Density Score (MIDS): the ratio of the number of PD-
L1 expressing mononuclear inflammatory cells infiltrating or adjacent to the tumor compared to the total number of tumor cells. The MIDS is recorded at a scale from 0 to 4 with (0 =
15 None; 1 = Present, but less than one MIC for every 100 tumor cells (< 1%; a negative result); 2 = At least one MIC for every 100 tumor cells, but less than one MIC per 10 tumor cells (1-9%; a positive result); 3 = At least one MIC for every 10 tumor cells, but fewer MIC's than tumor cells (10-99%; a positive result); 4= At least as many MIC's as tumor cells ($\geq 100\%$; a positive result).

20 3. Combined Positive Score (CPS): the ratio of the number of PD-L1 positive tumor cells and PD-L1 positive mononuclear inflammatory cells (MIC) within the tumor nests and the adjacent supporting stroma (numerator) compared to the total number tumor cells (denominator; i.e., the number of PD-L1 positive and PD-L1 negative tumor cells). (See detailed description above for further details.) PD-L1 expression at any intensity is
25 considered positive, i.e., weak (1+), moderate (2+), or strong (3+).

A cut-off is the score that differentiates diagnostically positive from diagnostically negative. The cut-off can be set to achieve the following goals:

1. Identify all responders (i.e., no false negatives); and
2. Minimize the number of non-responders (i.e., false positives).

30 It is expected that setting the cut-off at $\geq 1\%$ for a positive result for the scores above, CPS will identify subjects who are responsive to anti-PD therapy that are missed by the TPS

and MIDS analyses described above. The CPS scoring method is very reproducible between pathologists and therefore an excellent scoring algorithm to interpret PD-L1 expression to identify subjects eligible for anti-PD therapy.

5 ***CPS Used in Other Cancers***

The CPS approach is generally applicable to any cancers expressing PD-L1 in either tumor and/or immune cells. For example, CPS can be used to analyze triple negative breast carcinoma using a similar cut-off value as described above.

10

EXEMPLARY EMBODIMENTS

Non-limiting examples of embodiments of certain aspects of the subject disclosure are provided below.

1. A method for determining the eligibility of a subject having a malignancy for treatment with an anti-PD therapeutic agent, the method comprising: determining the number of viable PD-L1 positive tumor cells, the number of viable PD-L1 negative tumor cells, and the number of viable PD-L1 positive mononuclear inflammatory cells (MIC) in a tumor tissue sample from a subject having a malignancy; calculating a combined positive score (CPS) for the tumor tissue sample using the formula:

20

$$\text{CPS} = \frac{\text{PD-L1 positive tumor cells} + \text{PD-L1 positive MIC}}{\text{PD-L1 positive tumor cells} + \text{PD-L1 negative tumor cells}} \times 100\%$$

wherein the subject is eligible for treatment with an anti-PD therapeutic agent when the CPS is above a threshold.

2. The method of embodiment 1, wherein the threshold is from about 10% to about 1%.

3. The method of embodiment 1 or 2, wherein the threshold is 1%.

4. The method of any one of embodiments 1 to 3, wherein the tumor tissue sample is a tissue section of a tumor biopsy.

5. The method of embodiment 4, wherein PD-L1 is detected by immunohistochemistry (IHC) staining.

6. The method of embodiment 4 or 5, wherein the tumor tissue section is a formalin fixed and embedded in paraffin wax (FFPE) tumor tissue section.
7. The method of any one of embodiments 4 to 6, wherein the tissue section is stained.
- 5 8. The method of embodiment 7, wherein the stain is a hematoxylin and eosin (H&E) stain.
9. The method of any one of embodiments 4 to 8, wherein the viable PD-L1 positive tumor cells, the number of viable PD-L1 negative tumor cells, and the number of viable PD-L1 positive MIC are counted in the tumor nests and the adjacent supporting stroma
- 10 of the tumor tissue sample.
10. The method of any one of embodiments 1 to 3, wherein the tumor tissue sample is a cell suspension.
11. The method of embodiment 10, wherein the number of viable PD-L1 positive tumor cells, the number of viable PD-L1 negative tumor cells, and the number of viable PD-
- 15 L1 positive MIC in the tumor tissue sample are determining by flow cytometry.
12. The method of any one of embodiments 1 to 11, wherein one or more additional marker is detected in the tumor tissue sample.
13. The method of embodiment 12, wherein the marker is specific for MIC.
14. The method of embodiment 13, wherein the marker is selected from the group
- 20 consisting of: CD3, CD5, CD4, CD7, CD8, and CD20.
15. The method of any one of embodiments 12 to 14, wherein PD-L1 and the one or more additional marker are detected simultaneously.
16. The method of any one of embodiments 12 to 14, wherein PD-L1 and the one or more additional marker are detected sequentially.
- 25 17. The method of any one of embodiments 1 to 16, wherein the method further comprises, prior to the determining step, contacting the tumor tissue sample with a detectable anti-PD-L1 binding agent.
18. The method of embodiment 17, wherein the detectable anti-PD-L1 binding agent comprises an anti-PD-L1 antibody or binding fragment thereof.
- 30 19. The method of any one of embodiments 1 to 3, wherein the number of viable PD-L1 positive tumor cells, the number of viable PD-L1 negative tumor cells, and the number

of viable PD-L1 positive MIC in the tumor tissue sample are determined by single cell sequencing.

20. The method of any preceding embodiment, wherein the malignancy is selected from the group consisting of: gastric cancer, head and neck cancer, renal cell carcinoma, urothelial/bladder carcinoma, ovarian carcinoma, myeloma, melanoma, lung cancer, squamous cell carcinoma, classical Hodgkin's lymphoma, and breast cancer (e.g., triple negative breast cancer, hormone receptor positive (ER and/or PR) and Her2 positive breast cancer), small cell lung cancer, salivary gland carcinoma, vulvar carcinoma, thyroid carcinoma, anal canal carcinoma, biliary carcinoma, mesothelioma, cervical carcinoma, and neuroendocrine carcinoma.

21. The method of embodiment 18, wherein the malignancy is gastric cancer.

22. The method of embodiment 18, wherein the malignancy is head and neck cancer.

23. The method of embodiment 18, wherein the head and neck cancer is head and neck squamous cell carcinoma (HNSCC).

24. The method of any preceding embodiment, wherein the anti-PD therapeutic agent inhibits the anti-cell death activity of PD-1/PD-L1.

25. The method of any preceding embodiment, wherein the anti-PD therapeutic agent comprises an anti-PD or anti-PD-L1 specific antibody or binding fragment thereof.

26. The method of embodiment 25, wherein the anti-PD therapeutic agent is selected from the group consisting of: Avelumab (MSB0010178C), Nivolumab, Pembrolizumab, BMS-936559, MPDL3280A, Pidilizumab, and MEDI4736.

27. The method of embodiment 23, wherein the anti-PD therapeutic agent is Avelumab.

28. The method of any preceding embodiment, wherein the subject is a human.

29. A kit for determining the eligibility of a subject having a malignancy for treatment with an anti-PD therapeutic agent, comprising: a detectable PD-L1 specific binding agent; and instructions for calculating a combined positive score (CPS).

30. The kit of embodiment 29, further comprising a positive and negative control sample.

31. The kit of embodiment 29 or 30, further comprising a detectable binding agent for a marker specific for mononuclear inflammatory cells (MIC).

32. The kit of embodiment 31, wherein the marker specific for MIC is selected from the group consisting of: CD3, CD5, CD4, CD7, CD8, and CD20.

5 33. The kit of any one of embodiments 29 to 32, further comprising one or more reagents for performing an immunohistochemistry (IHC) staining reaction on a tumor tissue sample from a subject.

A method of treatment is provided. In some embodiments, the method comprises:
determining the number of viable PD-L1 positive tumor cells, the number of viable
10 PD-L1 negative tumor cells, and the number of viable PD-L1 positive mononuclear inflammatory cells (MIC) in a tumor tissue sample from a subject having a malignancy;
calculating a combined positive score (CPS) for the tumor tissue sample using the formula:

PD-L1 positive tumor cells + PD-L1 positive MIC
CPS = ----- X
15 100%
PD-L1 positive tumor cells + PD-L1 negative tumor cells
and treating the subject with an anti-PD therapeutic agent if the CPS is above a threshold.

20 It will also be recognized by those skilled in the art that, while the invention has been described above in terms of preferred embodiments, it is not limited thereto. Various features and aspects of the above described invention may be used individually or jointly. Further, although the invention has been described in the context of its implementation in a particular environment, and for particular applications those skilled in the art will recognize that its
25 usefulness is not limited thereto and that the present invention can be beneficially utilized in any number of environments and implementations. Accordingly, the claims set forth below should be construed in view of the full breadth and spirit of the invention as disclosed herein.

What is claimed is:

1. A method for determining the eligibility of a subject having a malignancy for treatment with an anti-PD therapeutic agent, the method comprising:
determining the number of viable PD-L1 positive tumor cells, the number of viable PD-L1 negative tumor cells, and the number of viable PD-L1 positive mononuclear inflammatory cells (MIC) in a tumor tissue sample from a subject having a malignancy;
calculating a combined positive score (CPS) for the tumor tissue sample using the formula:

$$\text{CPS} = \frac{\text{PD-L1 positive tumor cells} + \text{PD-L1 positive MIC}}{\text{PD-L1 positive tumor cells} + \text{PD-L1 negative tumor cells}} \times 100\%$$

wherein the subject is eligible for treatment with an anti-PD therapeutic agent when the CPS is above a threshold.

2. The method of claim 1, wherein the threshold is from about 10% to about 1%.
3. The method of claim 1, wherein the threshold is 1%.
4. The method of claim 1, wherein the tumor tissue sample is a tissue section of a tumor biopsy.
5. The method of claim 4, wherein PD-L1 is detected by immunohistochemistry (IHC) staining.
6. The method of claim 4, wherein the tumor tissue section is a formalin fixed and embedded in paraffin wax (FFPE) tumor tissue section.
7. The method of claim 4, wherein the tissue section is stained.

8. The method of claim 7, wherein the stain is a hematoxylin and eosin (H&E) stain.
9. The method of claim 4, wherein the viable PD-L1 positive tumor cells, the number of viable PD-L1 negative tumor cells, and the number of viable PD-L1 positive MIC are counted in the tumor nests and the adjacent supporting stroma of the tumor tissue sample.
10. The method of claim 1, wherein the tumor tissue sample is a cell suspension.
11. The method of claim 10, wherein the number of viable PD-L1 positive tumor cells, the number of viable PD-L1 negative tumor cells, and the number of viable PD-L1 positive MIC in the tumor tissue sample are determining by flow cytometry.
12. The method of claim 1, wherein one or more additional marker is detected in the tumor tissue sample.
13. The method of claim 12, wherein the marker is specific for MIC or is selected from the group consisting of: CD3, CD5, CD4, CD7, CD8, and CD20.
14. The method of claim 1, wherein the method further comprises, prior to the determining step, contacting the tumor tissue sample with an anti-PD-L1 antibody or binding fragment thereof.
15. The method of claim 1, wherein the number of viable PD-L1 positive tumor cells, the number of viable PD-L1 negative tumor cells, and the number of viable PD-L1 positive MIC in the tumor tissue sample are determined by single cell sequencing.
16. The method of claim 1, wherein the malignancy is selected from the group consisting of: gastric cancer, head and neck cancer, renal cell carcinoma, urothelial/bladder carcinoma, ovarian carcinoma, myeloma, melanoma, lung cancer, squamous cell carcinoma, classical Hodgkin's lymphoma, and breast cancer (e.g., triple negative breast cancer, hormone receptor positive (ER and/or PR) and Her2 positive breast cancer), small cell lung cancer, salivary

gland carcinoma, vulvar carcinoma, thyroid carcinoma, anal canal carcinoma, biliary carcinoma, mesothelioma, cervical carcinoma, and neuroendocrine carcinoma.

17. The method of claim 1, wherein the anti-PD therapeutic agent inhibits the anti-cell death activity of PD-1/PD-L1.

18. The method of claim 1, wherein the anti-PD therapeutic agent comprises an anti-PD or anti-PD-L1 specific antibody or binding fragment thereof.

19. The method of claim 1, wherein the anti-PD therapeutic agent is selected from the group consisting of: Avelumab (MSB0010178C), Nivolumab, Pembrolizumab, BMS-936559, MPDL3280A, Pidilizumab, and MEDI4736.

20. A kit for determining the eligibility of a subject having a malignancy for treatment with an anti-PD therapeutic agent, comprising:

a detectable PD-L1 specific binding agent; and
instructions for calculating a combined positive score (CPS).

SCORING METHODS FOR ANTI-PD THERAPY ELIGIBILITY AND
COMPOSITIONS FOR PERFORMING SAME

ABSTRACT

Aspects of the present disclosure provide methods for determining the eligibility of a subject having a malignancy for treatment with an anti-PD therapeutic agent based on a Combined Positive Score (CPS) for a tumor tissue sample from the subject. Compositions and
5 kits or performing the disclosed methods are also provided.

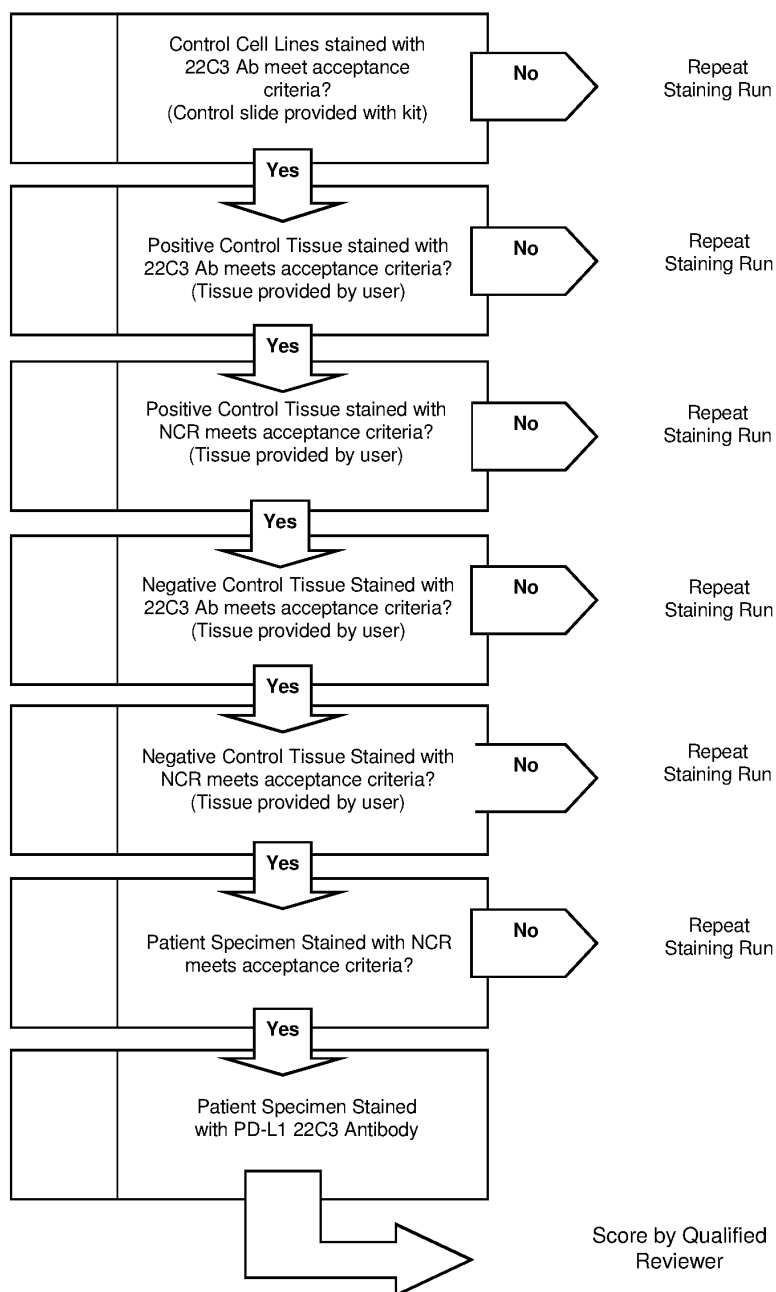


FIG. 1

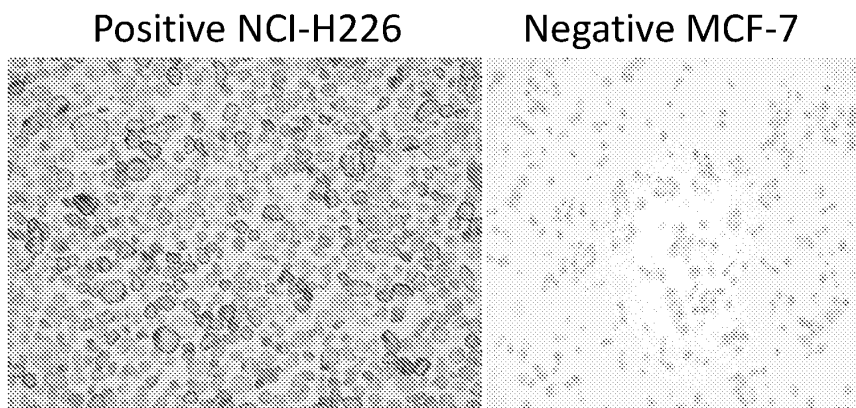


FIG. 2

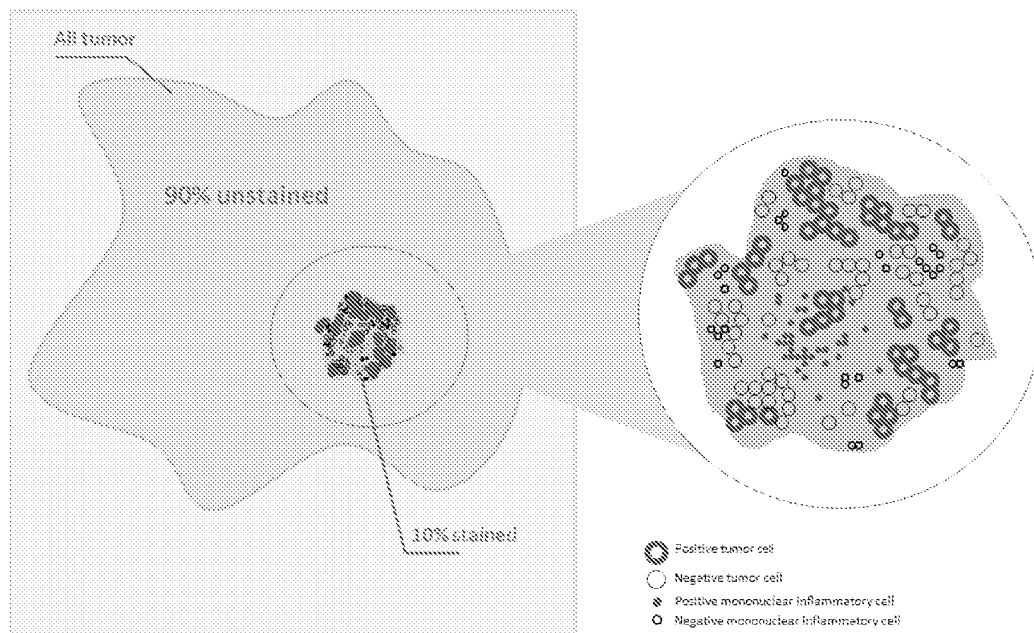


FIG. 3

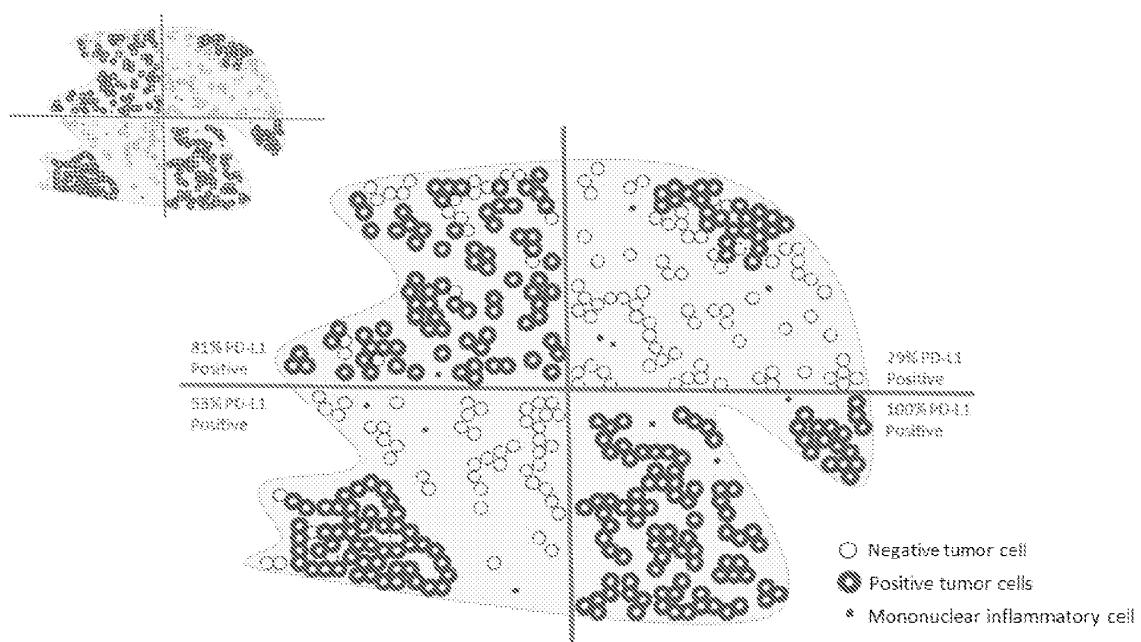


FIG. 4

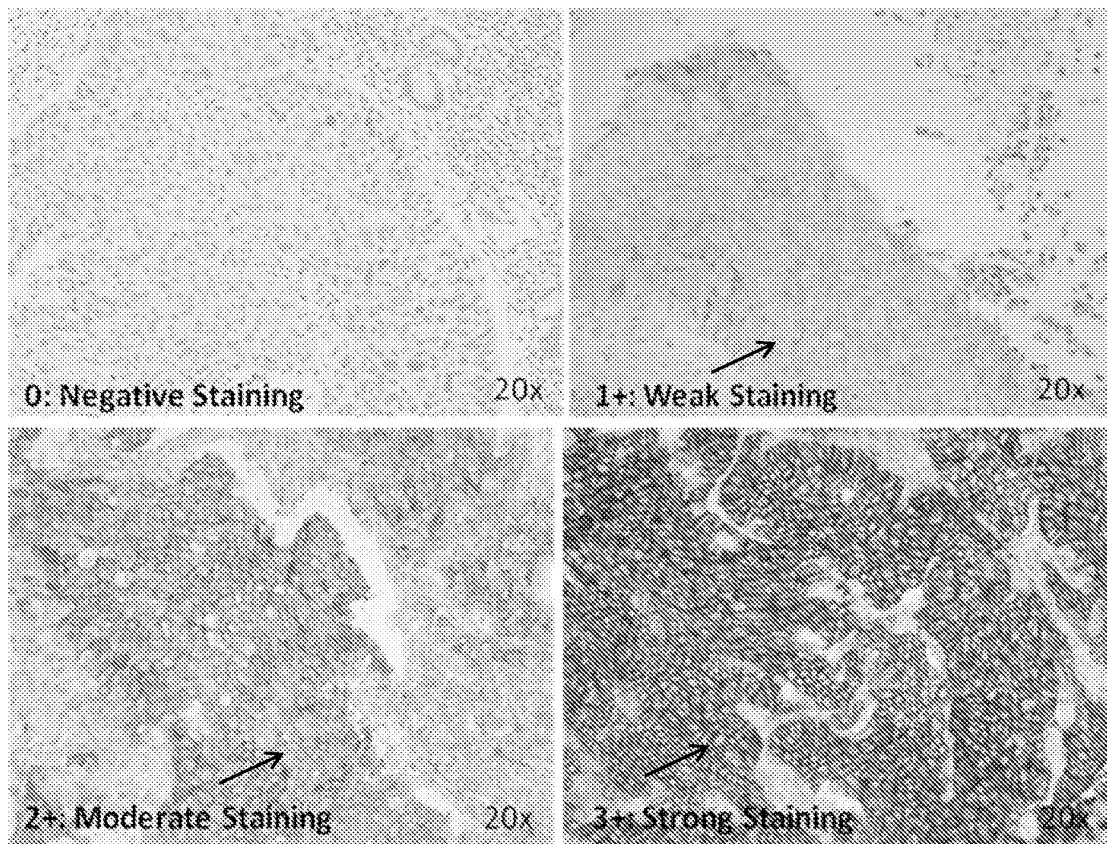


FIG. 5

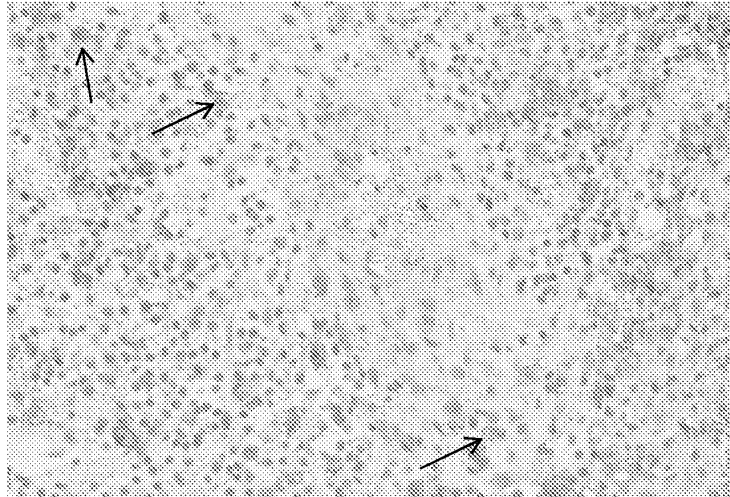


FIG. 6

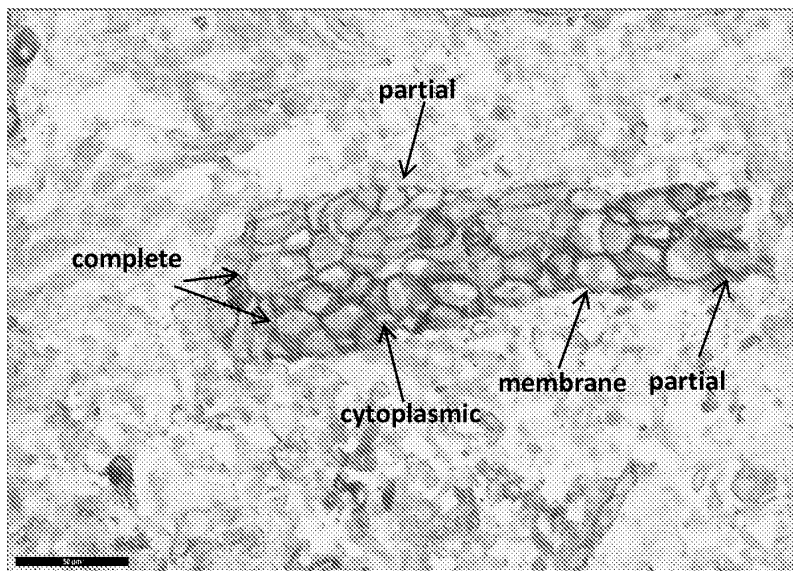


FIG. 7

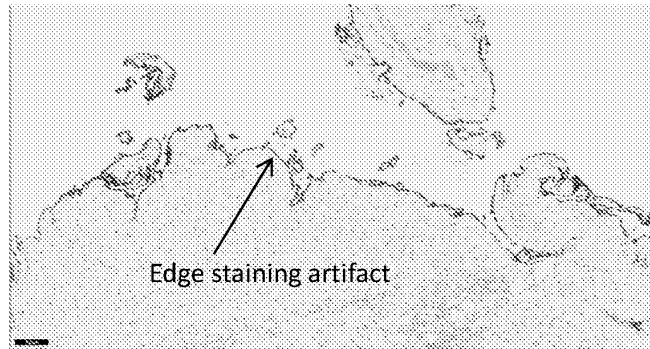


FIG. 8

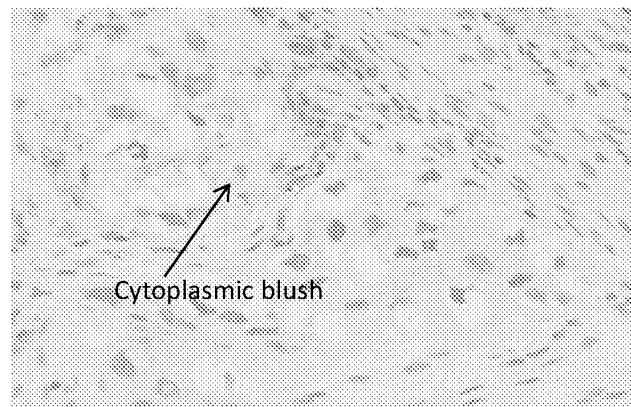


FIG. 9

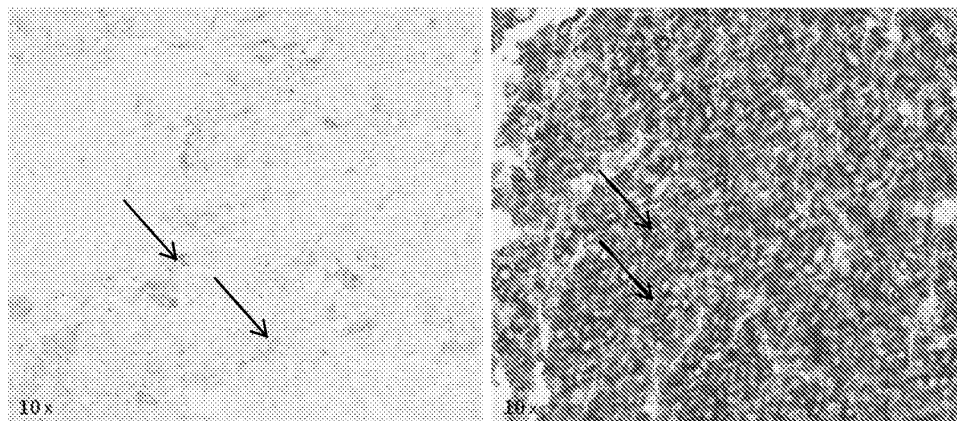


FIG. 10

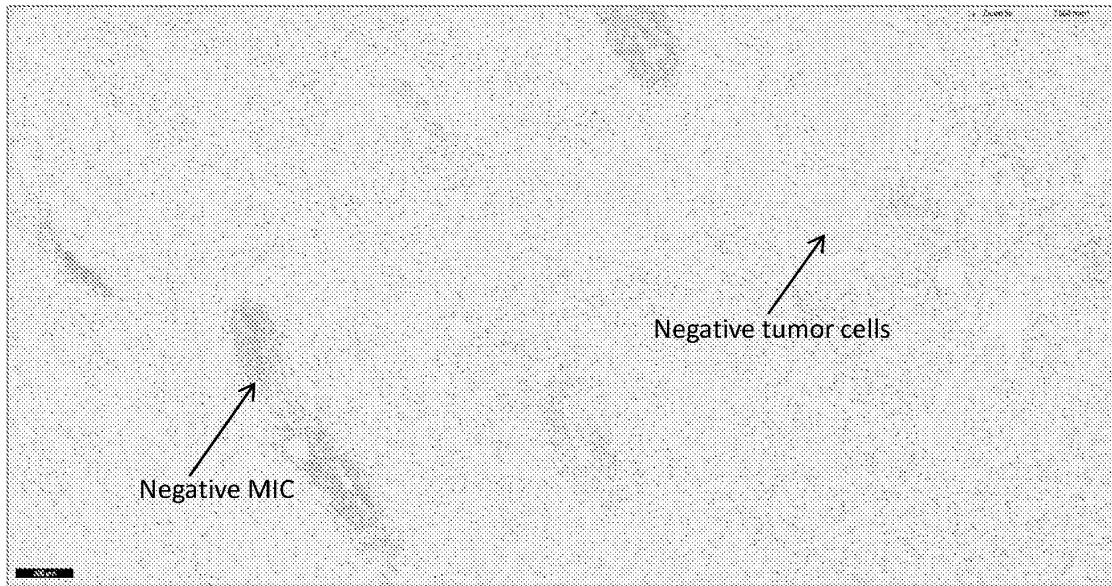


FIG. 11

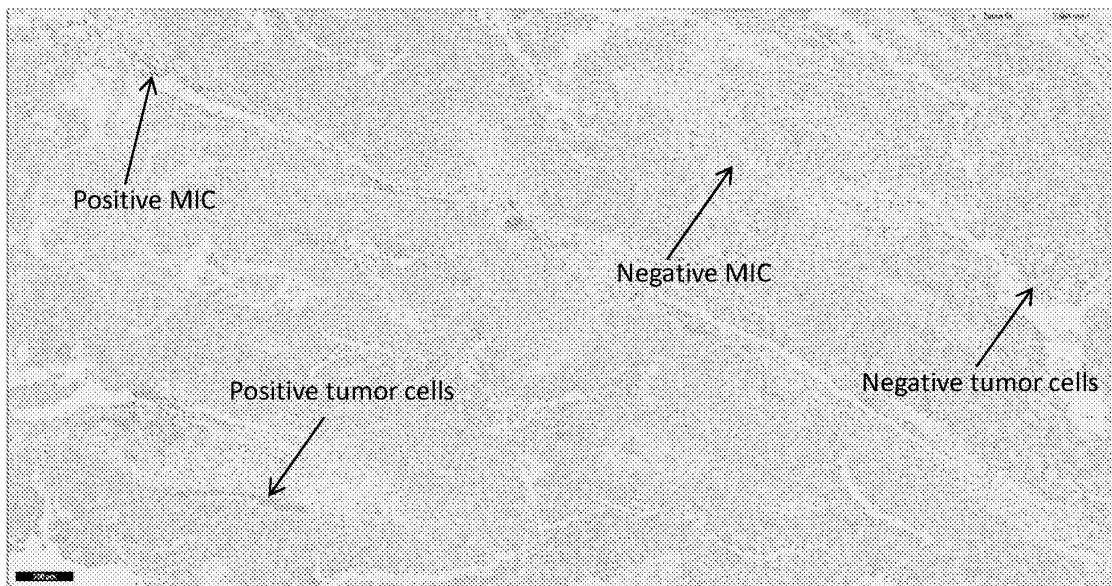


FIG. 12

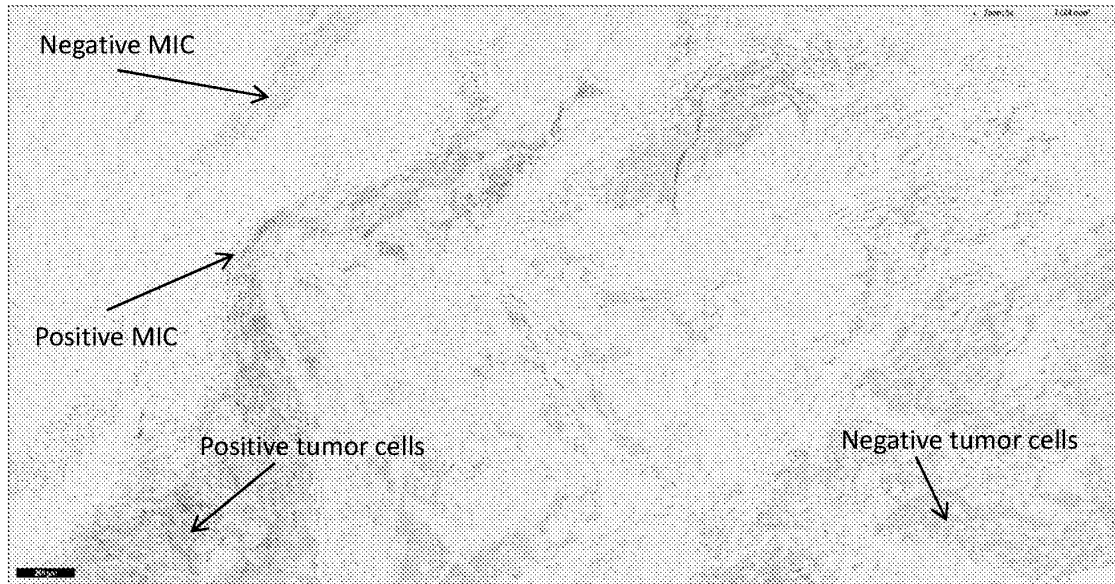


FIG. 13

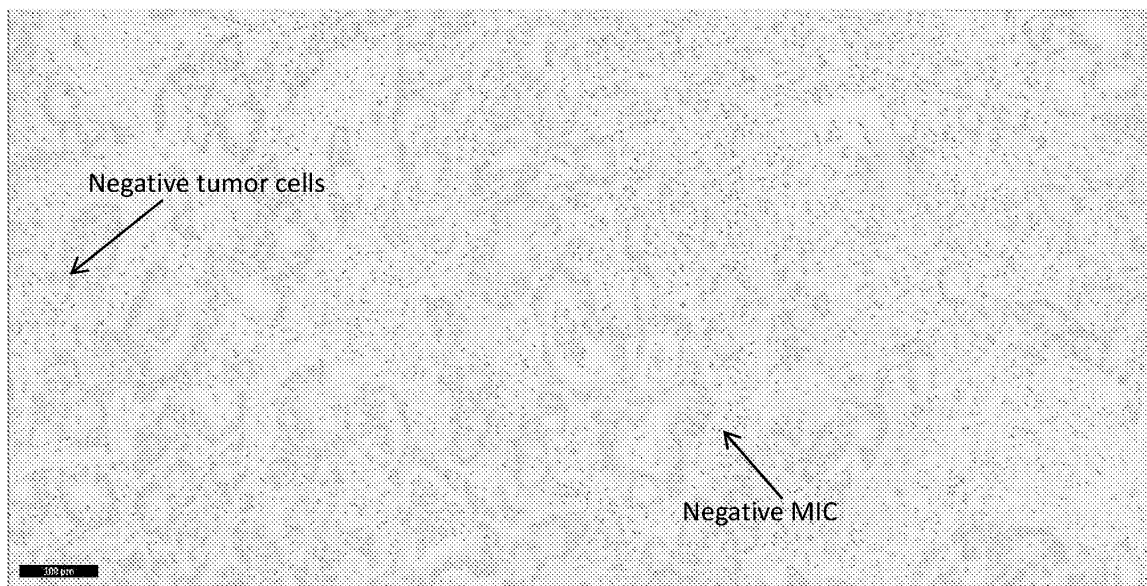


FIG. 14

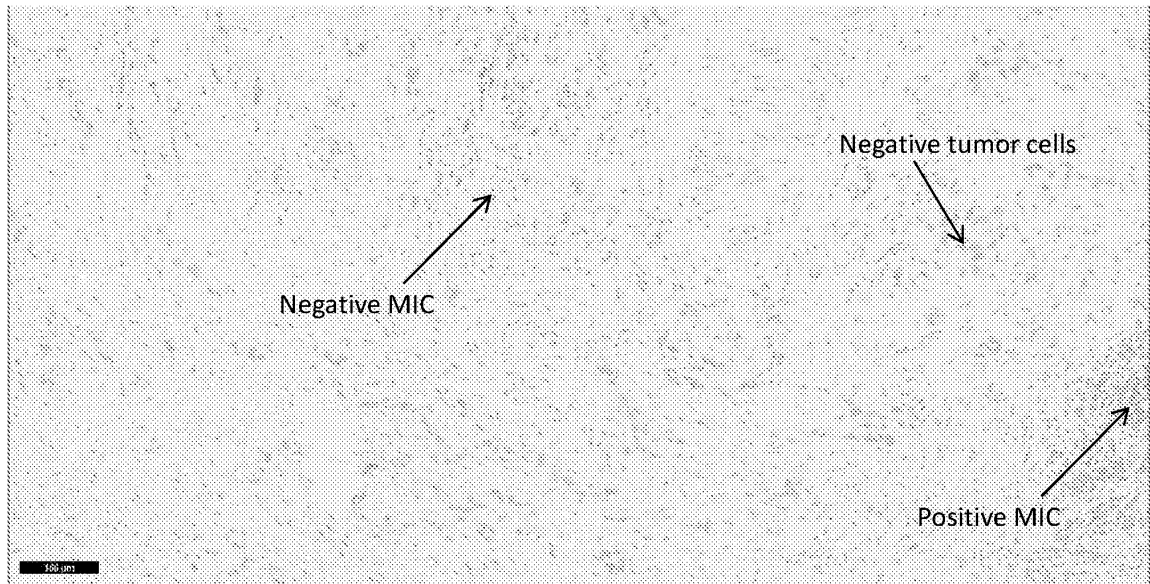


FIG. 15

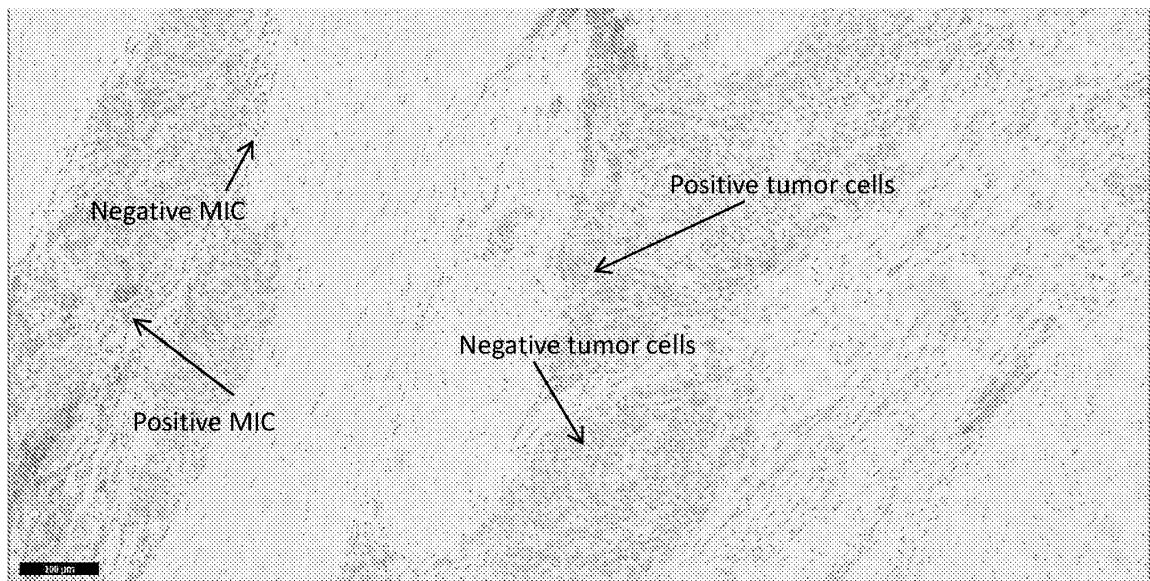


FIG. 16

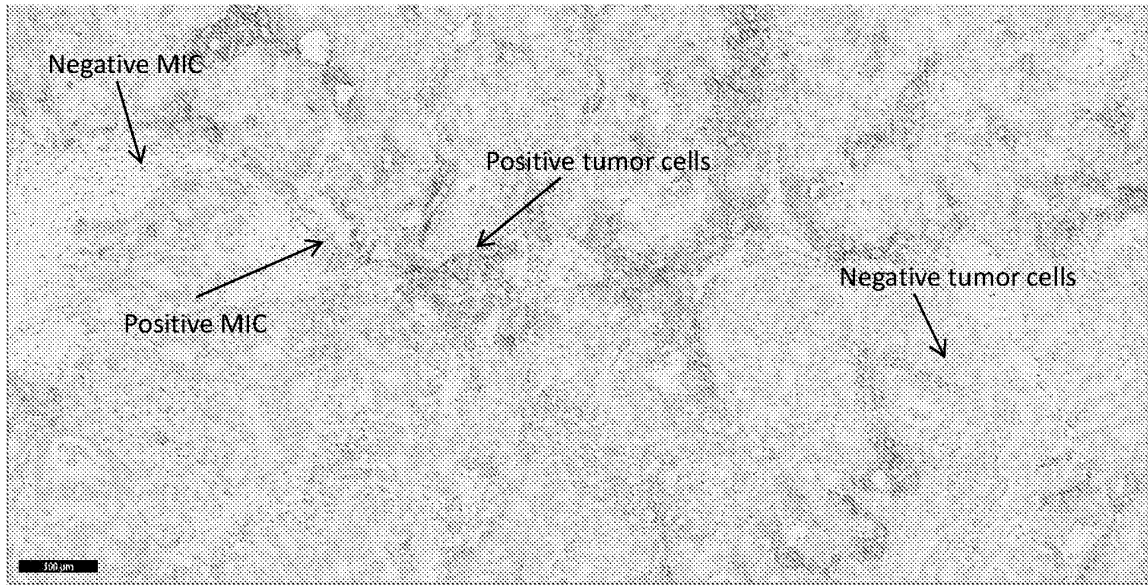


FIG. 17

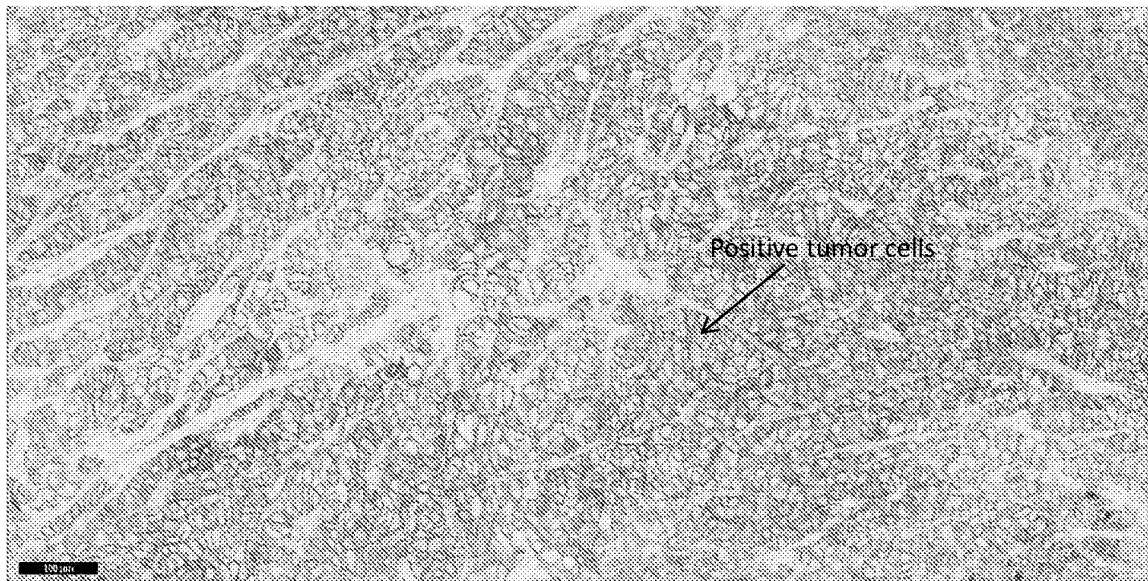


FIG. 18

THIRD-PARTY SUBMISSION UNDER 37 CFR 1.290 CONCISE DESCRIPTION OF RELEVANCE		
Application Number		18098198
U.S. PATENTS		
Cite No	Patent Number	Concise Description of Relevance
U.S. PATENT APPLICATION PUBLICATION		
Cite No	Publication Number	Concise Description of Relevance
1	20160009805	See Attached

2	20160084839	See Attached
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FOREIGN PATENT DOCUMENTS		
CiteNo	Foreign Document Number	Concise Description of Relevance
1	2014/151006	See Attached
2	2015/181343	See Attached

NON-PATENT PUBLICATIONS		
Cite No	Reference	Concise Description of Relevance

1	Differential Activity of Nivolumab Pembrolizumab and MPDL3280A according to the Tumor Expression of Programmed Death Ligand PD L1	See Attached
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2	Clinical outcomes in patients with advanced gastric cancer treated with pembrolizumab MK-3475 in KEYNOTE 012	See Attached
---	--	--------------

3	PD L1 IHC 22C3 pharmDx	See Attached
---	------------------------	--------------

4	Pembrolizumab for the Treatment of Non Small Cell Lung Cancer	See Attached
---	---	--------------

5	Development of an Automated PD L1 Immunohistochemistry IHC Assay for Non Small Cell Lung Cancer	See Attached
---	---	--------------

6	PD L1 expression in nonclear cell renal cell carcinoma	See Attached
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Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

THIRD-PARTY SUBMISSION UNDER 37 CFR 1.290	Application Number	18098198

U.S. PATENTS						
Cite No	Patent Number	Kind Code ¹	Issue Date (YYYY-MM-DD)	First Named Inventor		
U.S. PATENT APPLICATION PUBLICATIONS						
Cite No	Publication Number	Kind Code ¹	Publication Date (YYYY-MM-DD)	First Named Inventor		
1	20160084839	A1	2016-03-24	Dolled-Filhart et al		
2	20160009805	A1	2016-01-14	Kowanetz et al		
FOREIGN PATENTS AND PUBLISHED FOREIGN PATENT APPLICATIONS						
Cite No	Foreign Document Number ³	Country Code ²	Kind Code ¹	Publication Date (YYYY-MM-DD)	Applicant, Patentee or First Named Inventor	T ⁵
1	2015/181343	WO	A2	2015-12-03	Nitta et al	☐
2	2014/151006	WO	A2	2014-09-25	Chen et al	☐
NON-PATENT PUBLICATIONS (e.g., journal article, Office action)						

THIRD-PARTY SUBMISSION UNDER 37 CFR 1.290	Application Number	18098198

Cite No	Author (if any), title of the publication, page(s) being submitted, publication date, publisher (where available), place of publication (where available).	T ⁵	E ⁶
1	PD L1 expression in nonclear cell renal cell carcinoma	☐	☒
2	Development of an Automated PD L1 Immunohistochemistry IHC Assay for Non Small Cell Lung Cancer	☐	☒
3	Pembrolizumab for the Treatment of Non Small Cell Lung Cancer	☐	☒

THIRD-PARTY SUBMISSION UNDER 37 CFR 1.290	Application Number	18098198

4	PD L1 IHC 22C3 pharmDx	☐	☒
5	Clinical outcomes in patients with advanced gastric cancer treated with pembrolizumab MK-3475 in KEYNOTE 012	☐	☒
6	Differential Activity of Nivolumab Pembrolizumab and MPDL3280A according to the Tumor Expression of Programmed Death Ligand PD L1	☐	☒

STATEMENTS
The party making the submission is not an individual who has a duty to disclose information with respect to the above-identified application under 37 CFR 1.56. This submission complies with the requirements of 35 U.S.C. 122(e) and 37 CFR 1.290.

THIRD-PARTY SUBMISSION UNDER 37 CFR 1.290	Application Number	18098198

☒ The fee set forth in 37 CFR 1.290(f) has been submitted herewith.			
☐ The fee set forth in 37 CFR 1.290(f) is not required because this submission lists three or fewer total items and, to the knowledge of the person signing the statement after making reasonable inquiry, this submission is the first and the only submission under 35 U.S.C. 122(e) filed in the above-identified application by the party making the submission or by a party in privity with the party.			
☐ This resubmission is being made responsive to a notification of non-compliance issued for an earlier filed third-party submission. The corrections in this resubmission are limited to addressing the non-compliance. As such, the party making this resubmission: (1) requests that the Office apply the previously-paid fee set forth in 37 CFR 1.290(f), or (2) states that no fee is required to accompany this resubmission as the undersigned is again making the fee exemption statement set forth in 37 CFR 1.290(g).			
Signature	/Maganbhai Bhanderi/		
Name/Print	Bhanderi Maganbhai	Registration Number (if applicable)	
Examiner Signature		Date Considered	
*EXAMINER: Signature indicates all documents listed above have been considered, except for citations through which a line is drawn. Draw line through citation if not considered. Include a copy of this form with next communication to applicant. 1. If known, enter kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16. See MPEP 901.04(a). 2. Enter the country or patent office that issued the document, by two-letter code under WIPO standard ST.3. See MPEP 1851. 3. For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. 4. If known, enter the kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16. See MPEP 901.04(a). 5. Check mark indicates translation attached. 6. Check mark indicates evidence of publication attached.			

Application No. 18/098,198

CONCISE DESCRIPTION OF RELEVANCE

OF THE THIRD PARTY PREISSUANCE SUBMISSION UNDER 37 C.F.R. § 1.290

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application No.: 18/098,198
Application Type: Utility
Applicant: Agilent Technologies, Inc.; Merck Sharp & Dohme LLC
Inventors: Kulangara et al.
Filing Date: January 18, 2023
Publication No.: US 2023-0152326 A1
Publication Date: May 18, 2023
Title: SCORING METHODS FOR ANTI-PD THERAPY ELIGIBILITY
AND COMPOSITIONS FOR PERFORMING SAME
Art Unit: 1642
Confirmation No.: 9732
Examiner: DOCKET CENTRAL

Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450

THIRD PARTY PREISSUANCE SUBMISSION UNDER 37 C.F.R. § 1.290

The Examiner is respectfully requested to consider the relevance of the documents submitted herewith with respect to the patentability of the pending claims in the captioned patent application in view of the enclosed concise description of the asserted relevance of each document.

Document list begins on page 2 of this document.

Concise Description of Relevance begins on page 4 of this document.

Third Party Submission – Document List

Applicant: Agilent Technologies, Inc.; Merck Sharp & Dohme LLC
Application No.: 18/098,198
Application Type: Utility
Filing Date: January 18, 2023
Publication No.: US 2023-0152326 A1
Publication Date: May 18, 2023

Published Foreign Patent Applications

Publication 1: WO 2015/181343 A2; Published Dec. 03, 2015 (**Nitta et al.**)

Publication 2: WO 2014/151006 A2; Published Sep. 25, 2014 (**Chen et al.**)

U.S. Patent Application Publication

Publication 3: US 2016/084839 A1; Published Mar. 24, 2016 (**Dolled-Filhart et al.**)

Publication 4: US 2016/0009805 A1; Published Jan. 14, 2016 (**Kowanetz et al.**)

Non-Patent Publications

Publication 5: “PD-L1 expression in nonclear-cell renal cell carcinoma”, Annals of Oncology 25: 2178–2184, 2014 doi:10.1093/annonc/mdu445; Published Sep. 05 2014 (**Choueiri et al.**)

Publication 6: “Development of an Automated PD-L1 Immunohistochemistry (IHC) Assay for Non–Small Cell Lung Cancer”, Appl Immunohistochem Mol Morphol 2015; 23:541–549; Published Sept 2015 (**Phillips et al.**)

Publication 7: “Pembrolizumab for the Treatment of Non–Small-Cell Lung Cancer”, N Engl J Med 2015; 372:2018-28; DOI: 10.1056/NEJMoa1501824; Published April 19, 2015 (**Garon et al.**)

Publication 8: Label for Dako, PD-L1 IHC 22C3 pharmDx; SK006; 50 tests for use with Autostainer Link 48, Published September 2015.

Publication 9: “Clinical outcomes in patients with advanced gastric cancer treated with pembrolizumab (MK-3475) in KEYNOTE-012”, Annals of Oncology 26 (Supplement 7): vii12, 2015 doi:10.1093/annonc/mdv406.3; Published 2015 (**Muro**)

Publication 10: “Differential Activity of Nivolumab, Pembrolizumab and MPDL3280A according to the Tumor Expression of Programmed Death-Ligand-1 (PD-L1): Sensitivity Analysis of Trials in Melanoma, Lung and Genitourinary Cancers”, PLoS ONE 10(6): e0130142. doi :10.1371/journal.pone.0130142; Published June 18, 2015 (**Carbognin et al.**)

Third Party Submission – Concise Description of Relevance

Applicant: Agilent Technologies, Inc.; Merck Sharp & Dohme LLC
Application No.: 18/098,198
Application Type: Utility
Filing Date: January 18, 2023
Publication No.: US 2023-0152326 A1
Publication Date: May 18, 2023

Claims 1, 2-6 and 20: Publication 1 (Nitta et al.), Publication 2 (Chen et al.), Publication 3 (Dolled-Filhart et al.), Publication 4 (Kowanetz et al.), Publication 5 (Choueiri et al.), Publication 6 (Phillips et al.), Publication 7 (Garon et al.) and Publication 8 (Dako label)

The general inventive concept underlying the set of claims of the application is that Combined Positive Score (CPS) for a tumor tissue sample from the subject, above a threshold value indicates a response to anti- PD therapeutic agent.

Publication 1 describes methods for detecting and scoring PD-L1 expression in tumor tissues (para [0001]) and assays for improved scoring of tumor tissues stained with PD-L1 (para [0005]) wherein a positive scoring is predictive of improved outcomes for PD-L1 inhibitor treatment therapies and patients having tumors that are scored positive for PD-L1 are administering a therapy based on PD-L1 (para [0056]). It is further disclosed that a positive result is determined by calculating the percentage of PD-L1 positive tumor cells and determining if that percentage is above the threshold for positivity or a predetermined cut-off (para [0045]) or by calculating the number of PD-L1 positive tumor cells divided by the total number of cells (e.g., number of tumor plus immune cells) and determining if that value is above the threshold for positivity (para [0046]). **Publication 1** further states that a positive result can also be determined by calculating the sum of the percentage of PD-L1 positive tumor cells and PD-L1 positive immune cells and determining if that value is above the threshold for positivity (e.g., value greater than 40, value greater than 50, value greater than 60, etc.) (para [0047]). Another method of scoring PD-1 expression is reported in para [0048] wherein a positive result is determined by calculating the number of PD-L1 positive tumor cells divided by the number of PD-L1 negative tumor cells and determining if that value is above the threshold for positivity (e.g., value greater than 1.5, value greater than 1.8, value greater than 2, etc.). **Publication 1** mentions that tumor samples suitable for histochemical or cytochemical

analysis including Immunohistochemistry (IHC) and the samples are fresh frozen or formalin-fixed paraffin-embedded (FFPE)) samples (para [0010], [0016], [0029]).

Publication 2 discloses methods identifying an individual with a disease or disorder who is more likely to respond to treatment with a PD- L1 axis binding antagonist, the method comprising: determining the presence of a PD-L1 biomarker in a sample from the individual (para [0006]), wherein PD-L1 biomarker is detected as a weak staining intensity in the sample tissue by protein expression using immunohistochemistry (para [0026], [0222] and [0246]). The sample tissue obtained from the individual is generally a tumor tissue obtained from biopsy (para [0235]) and is selected from the group consisting of tumor cells, tumor infiltrating cells, stromal cells and any combinations thereof (paras [0026], [0052] and [0197]). It is further reported that the tissue sample is formalin fixed and paraffin embedded (para [0206]). **Publication 2** specifically discloses in Example 1 (paras [0419] and [0420]) that a PD-L1 IHC scoring system was developed to measure PD-L1 specific signal in tumor cells and tumor infiltrating immune cells, wherein the tumor cell staining is expressed as the percent of all tumor cells showing membranous staining of any intensity; infiltrating immune cell staining is defined as the percent of the total tumor area occupied by immune cells that show staining of any intensity; the total tumor area encompasses the malignant cells as well as tumor-associated stroma, including areas of immune infiltrates immediately adjacent to and contiguous with the main tumor mass.

Publication 3 describes quantitation of PD-L1 expression in tissue samples by immuno-histochemical (IHC) assay and identification of PD-L1 expression levels that correlate with response of cancer patients to therapy with PD-1 antagonists (para [0001]). It is further explained that quantifying PD-L1 expression using a PD-L1 expression score is useful in selecting patients who are more likely to respond to treatment with anti-PD-1 therapy (para [0009]). It is reported that if the PD-L1 expression score is at or above a threshold score that is correlated with response or better response to treatment with a PD-1 antagonist, the physician selects a chemotherapeutic regimen that includes at least the PD-1 antagonist and if the reported PD-L1 expression score is below a threshold score that is correlated with no response or poor response to treatment with a PD-1 antagonist, the physician selects a regimen that does not include any PD-1 antagonist (para [0118]). It is further stated that scoring includes estimation across all of the viable tumor cells and stained mononuclear inflammatory cells in all of the examined tumor nests (para [0011]). The scoring is assigned by estimating, across all of the viable tumor cells and stained mononuclear inflammatory cells in all of the examined

tumor nests, the percentage of cells that have at least partial membrane staining of any intensity, and the resulting percentage is assigned to the tissue section as the score (para [0012]). **Publication 3** also discloses that the tissue section is preferably a formalin-fixed, paraffin-embedded tissue section (para [0021]).

Publication 4 describes that the presence of PD-L1 in biological sample (preferably formalin fixed paraffin embedded tissue) is detected by immunohistochemistry (IHC) using antibodies (para [0016], [0017]). Publication 4 mentions that expression level of PD-L1 in the sample, indicates that subject is likely to respond to treatment with anti-cancer therapy, wherein the sample is a tumor sample comprising tumor infiltrating immune cells, tumor cells, stromal cells or any combination thereof. The sample is preferably a fixed tissue which is formalin fixed paraffin embedded tissue (FFPE) (para [0017]). **Publication 4** further mentions that tissue stained with anti-PD antibodies can be scored for the presence of PD-L1 positive expression using different cut-offs, which are used for patient selection, for example for selection of patients who are likely to respond to therapy comprising PD-1 axis binding antagonist (para [0373]). It is further disclosed that the scoring for PD-L1 expression is based on percentage of tumor area that is covered with PD-L1 positive tumor infiltrating immune cells of any intensity (para [0375]). **Publication 4** further discloses that when tissue from a tumor is examined using IHC, staining includes determining or assessing in tumor-infiltrating immune cells, including intratumoral or peritumoral immune cells wherein, the presence of PD-L1 is detected by IHC in >0% of the sample, in at least 1% of the sample, in at least 5% of the sample, in at least 10% of the sample, ..., in at least 60% of the sample, in at least 65% of the sample, ... (para [0317]).

Publication 5 discloses PD-L1 expression in nonclear-cell RCC (non-ccRCC) evaluated in formalin-fixed paraffin-embedded (FFPE) specimens. PD-L1 expression was evaluated by immunohistochemistry in both tumor cell membrane and tumor-infiltrating mononuclear cells (TIMC), wherein PD-L1 tumor positivity was defined as $\geq 5\%$ tumor cell membrane staining. For PD-L1 expression in TIMC, a combined score based on the extent of infiltrate and percentage of positive cells was used (background and methods). In this study, PD-L1 expression and its association with clinical outcome in a large series of patients with non-ccRCC (page 2179, right column) was studied, wherein the percentage of PD-L1-positive TIMC was evaluated and an adjusted score representing PD-L1 expression was calculated multiplying the percentage of TIMC that stained positive for PD-L1 and the extent of mononuclear cell infiltration (page 2180, left column). PD-L1 tumor positivity was defined as

≥5% tumor cell membrane staining and for PD-L1 expression in TIMCs, any score greater than zero was considered positive (page 2180). The study concluded that PD-L1 is a promising predictive biomarker of response to agents that target the PD1/PD-L1 axis as patients with PD-L1+ tumors have numerically higher response to agents that target the PD-L1/PD-1 axis than PD-L1 negative tumors (page 2182, right column).

Publication 6 discloses study investigating PD-L1 as a potential biomarker to predict clinical response to nivolumab in clinical settings, wherein an automated PD-L1 immunohistochemistry (IHC) assay was used to detect cell surface PD-L1 in formalin-fixed paraffin-embedded human tumor tissue specimens in small NSCLC cohorts (see Abstract). The PD-L1 positivity was measured by the overall fluorescent signals in tumor tissue compartment above the expression threshold, with specimen considered to be PD-L1 positive if determined to be greater than or equal to the threshold tumor score (page 541, right column). The estimated number of viable tumor cells in the tumor space was used as the denominator for computation of the score (page 544, right column).

Publication 7 discloses efficacy of programmed cell death 1 (PD-1) inhibition with Pembrolizumab in patients with advanced non-small-cell lung cancer. The study was carried out to validate an expression level of the PD-1 ligand 1 (PD-L1) that is associated with the likelihood of clinical benefit, wherein PD-L1 expression was evaluated in tumor samples using immunohistochemical analysis, with results reported as the percentage of neoplastic cells with staining for membranous PD-L1 (proportion score PD-L1 expression in at least 50% of tumor cells correlated with improved efficacy of pembrolizumab) (see Abstract). PD-L1 positivity was defined as membranous staining in at least 1% of cells (neoplastic and intercalated mononuclear inflammatory cells) within tumor nests or a distinctive staining pattern caused by the infiltration of mononuclear inflammatory cells in the stroma that formed a banding pattern adjacent to tumor nests (page 2020, left column). It was concluded that a proportion score of at least 50% was associated with a higher response rate and longer progression-free and overall survival than was a proportion score of less than 50% in both previously untreated patients and previously treated patients, which indicates that this is a subgroup of patients in whom the PD-L1 pathway can be successfully targeted (page 2025, left para).

Publication 8 discloses *in-vitro* use of PD-L1 IHC 22C3 pharmDx, a qualitative immunohistochemical assay for use in the detection of PD-L1 in formalin-fixed, paraffin-embedded (FFPE) non-small cell lung cancer (NSCLC) tissue, specifically for patients

undergoing treatment with Pembrolizumab. It is further mentioned that PD-L1 protein expression is determined by using Tumor Proportion Score (TPS), which is the percentage of viable tumor cells showing partial or complete membrane staining, wherein the specimen is considered PD-L1 positive if $TPS \geq 50\%$ of the viable tumor cells exhibiting staining (see page 1/12, intended use).

Claims 7-10: Publication 1 (Nitta et al.), Publication 2 (Chen et al.), Publication 3 (Dolled-Filhart et al.), Publication 4 (Kowanetz et al.), Publication 5 (Choueiri et al.), Publication 6 (Phillips et al.), Publication 7 (Garon et al.) and Publication 8 (Dako label)

Publication 1 discloses multiplex assays for improved scoring of tumor tissues stained with PD-L1 (para [0005]). Figure 1 (para [0007]) specifically shows staining of a NSCLC tumor sample. The disclosed assays feature staining of PD-L1 as well as staining of one or more of tumor and immune cell markers (para [0022]), wherein the methods of staining include immunohistochemistry (IHC) (para [0029]) and the counterstain comprises hematoxyline and/or eosin (para [0033]).

Publication 2 mentions that PD-L1 biomarker is detected in the sample tissue as a staining intensity, using immunohistochemistry (para [0026], [0222]), wherein the sample tissue obtained from the individual is generally a tumor tissue obtained from biopsy (para [0235]) and is selected from the group consisting of tumor cells, tumor infiltrating cells, stromal cells and any combinations thereof (paras [0026], [0052] and [0197]). The staining is carried out using hematoxylin or H&E (paras [0455], [0467], [0479], [0489] and [0493]).

Publication 3 relates to the quantitation of PD-L1 expression in animal tissue samples by immunohistochemical (IHC) assay, wherein the process for scoring the expression of a PD-L1 protein in a tumor sample comprises obtaining a tissue section from the tumor that has been stained with an antibody that binds to the PD-L1 protein (anti-PD-L1 Ab), examining each tumor nest in the tissue section for staining, and assigning to the tissue section a score (para [0010]). The scoring process is assigned by estimating, across all of the viable tumor cells and stained mononuclear inflammatory cells in all of the examined tumor nests and further comprises examining the stroma surrounding each tumor nest for the presence or absence of a band of anti-membrane-stained cells within the stroma (paras [0012] and [0013]). The staining process uses hematoxylin (para [0175]).

Publication 4 mentions that expression level of PD-L1 is detected in a sample, using IHC, wherein the sample is a tumor sample comprising tumor infiltrating immune cells, tumor cells, stromal cells or any combination thereof (para [0017] and [0304]) and the tumor-infiltrating immune cells include intratumoral immune cells, peritumoral immune cells or any combinations thereof, and other tumor stroma cells (para [0318]). The tissue section from NSCLC patients, used in IHC, were stained with hematoxylin or with H&E, as shown in Figures 10, 11, 12 and 17 (paras [0090] to [0092], [0098] to [0100] and [0376]).

Publication 5 discloses that PD-L1 tumor positivity was defined as $\geq 5\%$ tumor cell membrane staining and the extent of tumor-infiltrating mononuclear cells (TIMC) (i.e. lymphocytes and macrophages) was assessed in hematoxylin and eosin-stained slides (page 2180, left column).

Publication 6 discloses that PD-L1 IHC assay utilizes hematoxylin counterstain (page 542, right column).

Publication 7 discloses that PD-L1 expression was assessed in tumor samples using immunohistochemical analysis, with results reported as the percentage of neoplastic cells with staining for membranous PD-L1 (proportion score), wherein counterstain used is hematoxylin (see Figure 1). PD-L1 positivity was defined as membranous staining in at least 1% of cells (neoplastic and intercalated mononuclear inflammatory cells) within tumor nests or a distinctive staining pattern caused by the infiltration of mononuclear inflammatory cells in the stroma that formed a banding pattern adjacent to tumor nests (page 2018, See: methods).

Publication 8 discloses *in-vitro* use of PD-L1 IHC 22C3 pharmDx, a qualitative immunohistochemical assay for use in the detection of PD-L1 protein in formalin-fixed, paraffin-embedded (FFPE) non-small cell lung cancer (NSCLC) tissue, specifically for patients undergoing treatment with Pembrolizumab. The assay uses hematoxylin as one of the materials (see page 2/12 and 4/12 under counterstain) and stain comprising hematoxylin and eosin (H&E) is recommended for the first evaluation of the patient specimen (page 6/12). **Publication 8** further discusses that controls should be fresh biopsy/surgical specimens of the same tumor indication as the patient specimen, fixed, processed and embedded, wherein the positive tissue controls give weak to moderate positive staining and are indicative of correctly prepared tissues and proper staining techniques (page 5/12).

Claims 11-19: Publication 2 (Chen et al.), Publication 3 (Dolled-Filhart et al.), Publication 4 (Kowanetz et al.), Publication 6 (Phillips et al.) Publication 7 (Garon et al.), Publication 8 (Dako label), Publication 9 (Muro) and Publication 10 (Carbognin et al.)

Publication 2 discloses that the sample tissue is obtained from the individual suffering from cancer or malignancy selected from non-small cell lung cancer, small cell lung cancer, renal cell cancer, colorectal cancer, ovarian cancer, breast cancer, pancreatic cancer, gastric carcinoma, bladder cancer, esophageal cancer, mesothelioma, melanoma, head and neck cancer, thyroid cancer, sarcoma, prostate cancer, glioblastoma, cervical cancer, thymic carcinoma, leukemia, lymphomas, myelomas, mycoses fungoids, merkel cell cancer, and other hematologic malignancies (para [0208]) and is contacted with anti-PD-L1 antibodies (para [0217]).

Publication 3 relates to process for scoring the expression of a PD-L1 protein in a tumor sample (para [0010]), the process comprising obtaining a tissue section from the tumor that has been stained with an antibody that binds to the PD-L1 protein (anti-PD-L1 Ab) (para [0017]). It is further mentioned that the tissue section of a tumor sample is obtained from a patient diagnosed with non-small cell lung carcinoma (NSCLC), head and neck squamous carcinoma, transitional cell carcinoma of the bladder (TCC) (para [0014]), melanoma or breast cancer ([0015]), gastric cancer, bladder cancer, Hodgkins lymphoma, non-Hodgkins lymphoma, melanoma, anal canal squamous cell carcinoma, esophageal squamous cell carcinoma or adenocarcinoma, estrogen receptor (ER) positive HER2 negative breast cancer, HER2 positive breast cancer, triple negative breast cancer, ovarian epithelial, cervical squamous cell cancer, Vulvar Squamous cell carcinoma, Small cell lung cancer, mesothelioma (malignant pleural esothelioma), thyroid cancer, small-cell lung cancer, cervical cancer, ovarian cancer, liver cancer, bladder cancer, (para [0017] and [0327]). It is further disclosed that PD-1 antagonist includes antibodies selected from BMS-936558, (nivolumab), MPDL3280A, CT-011, ONO-4538, BMS 936559, MEDI4736, AMP-224, and an anti-PD-1 antibody comprising the heavy and light chain amino acid sequences shown in FIGS. 9 and 10 (para [0072] and claim 20).

Publication 4 describes that the presence of PD-L1 in biological sample is detected by immunohistochemistry (IHC) using PD-L1 binding antagonists or an antibody that binds to the same epitope; the antibody is selected from MSB0010718C (avelumab), MDX 1106 (nivolumab), MK-3475 (pembrolizumab), BMS-936559, MPDL3280A (atezolizumab), CT-011 (pidilizumab), and MEDI4736 (durvalumab (paras [0010], [0016], [0017] and [0337]). It

is further mentioned that PD-L1 is overexpressed in many cancers, including a wide variety of solid tumors, such as gastrointestinal cancer, head and neck cancer, kidney cancer, endometrial or uterine carcinoma, ovarian cancer, non-small cell lung cancer (NSCLC) (including adenocarcinoma of the lung and squamous carcinoma of the lung), carcinoma, lymphoma (including Hodgkin's and non-Hodgkin's lymphoma), breast cancer, small-cell lung cancer, bladder cancer, salivary gland carcinoma, vulval cancer, thyroid cancer, cervical cancer, squamous cell cancer, or various other types (paras [0003], [0017] and [0327]).

Publication 6 discloses Nivolumab as a potential biomarker to predict clinical response in non-small cell lung cancer (NSCLC) (See abstract). The specimen was considered to be PD-L1 positive if determined to be greater than or equal to the threshold tumor score, or PD-L1 negative if determined to be less than the threshold tumor score (page 544, right column).

Publication 7 discloses efficacy of programmed cell death 1 (PD-1) inhibition with Pembrolizumab in patients with advanced non-small-cell lung cancer (see Abstract). It is further reported that a proportion score of at least 50% was associated with a higher response rate and longer progression-free and overall survival than was a proportion score of less than 50% in both previously untreated patients and previously treated patients (page 2025, left column).

Publication 8 discloses *in-vitro* use of PD-L1 IHC 22C3 pharmDx, a qualitative immunohistochemical assay using monoclonal mouse Anti-PD-L1, Clone 22C3, for use in the detection of PD-L1 protein in formalin-fixed, paraffin-embedded (FFPE) non-small cell lung cancer (NSCLC) tissue, specifically for patients undergoing treatment with Pembrolizumab.

Publication 9 discloses Pembrolizumab, an anti-PD-1 monoclonal antibody, shows antitumor activity in patients with advanced gastric cancer (AGC). Archival tumor samples from patients with recurrent or metastatic adenocarcinoma of the stomach or gastroesophageal junction were screened for PD-L1 expression using a prototype IHC assay, wherein patients with distinctive stromal or 1% or more tumor nest cell PD-L1 staining were eligible.

Publication 10 discloses an analysis to determine the differential activity of nivolumab, pembrolizumab and MPDL3280A in terms of overall response rate (ORR), according to tumor PD-L1 expression, for the treatment of advanced melanoma, NSCLC and genitourinary cancers, wherein PD-L1 IHC positivity on tumor cells was defined by a 1% or 5% threshold

and a cut off of 50% was adopted to define the IHC PD-L1 positivity (see Abstract and page 11). It was concluded that the checkpoint inhibitors targeting PD-1 (nivolumab and pembrolizumab) and its ligand PD-L1 (MPDL3280A) provide a significant differential effect in terms of activity according to PD-L1 expression on tumor cells status, which translates into a higher overall response rate for PD-L1 positive advanced tumors in comparison to PD-L1 negative tumors (pages 9 and 11).



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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
18/098,198	01/18/2023	Karina Kulangara	20160057-04/6363.133884C2	9732
22878	7590	03/20/2025	EXAMINER	
Agilent Technologies, Inc. Global IP Operations 5301 Stevens Creek Blvd, 1A-PB Santa Clara, CA 95051			XIAO, YAN	
			ART UNIT	PAPER NUMBER
			1642	
			NOTIFICATION DATE	DELIVERY MODE
			03/20/2025	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

Agilentdocketing@cpaglobal.com
ipopsadmin@agilent.foundationip.com

Notice of Pre-AIA or AIA Status

1. The present application, filed on or after March 16, 2013, is being examined under the first inventor to file provisions of the AIA.

DETAILED ACTION

2. Claims 1-20 are pending in the application and are currently subject to the following restriction and election requirement.

Election/Restrictions

3. Restriction to one of the following inventions is required under 35 U.S.C. 121:

Group I, claims 1-19, drawn to a method for determining the eligibility of a subject having a malignancy for treatment with an anti-PD therapeutic agent, the method comprising: determining the number of viable PD-L1 positive tumor cells, the number of viable PD-L1 negative tumor cells, and the number of viable PD-L1 positive mononuclear inflammatory cells (MIC) in a tumor tissue sample from a subject having a malignancy; classified, for example, G01N 33/574.

Group II, claim 20, drawn to a kit for determining the eligibility of a subject having a malignancy for treatment with an anti-PD therapeutic agent, comprising: a detectable PD-Li specific binding agent; and instructions for calculating a combined positive score (CPS); classified, for example, G01N 33/574.

4. The inventions are distinct, each from the other because of the following reasons: Inventions of Group II and Group I are related as product and process of use. The inventions can be shown to be distinct if either or both of the following can be shown: (1) the process for using the product as claimed can be practiced with another materially different product or (2) the product as claimed can be used in a materially different process of using that product. See MPEP § 806.05(h). In the instant case, the antibody of the Group II can be used to purify a protein to which the antibody binds.

5. Restriction for examination purposes as indicated is proper because all these inventions listed in this action are independent or distinct for the reasons given above and there would be a serious search and examination burden if restriction were not required because one or more of the following reasons apply:

(a) the inventions have acquired a separate status in the art in view of their different classification;

(b) the inventions have acquired a separate status in the art due to their recognized divergent subject matter;

(c) the inventions require a different field of search (for example, searching different classes/subclasses or electronic resources, or employing different search queries).

6. **Applicant is advised that the reply to this requirement to be complete must include (i) an election of a invention to be examined even though the requirement may be traversed (37 CFR 1.143) and (ii) identification of the claims encompassing the elected invention.**

The election of an invention may be made with or without traverse. To reserve a right to petition, the election must be made with traverse. If the reply does not distinctly and specifically point out supposed errors in the restriction requirement, the election shall be treated as an election without traverse. Traversal must be presented at the time of election in order to be considered timely. Failure to timely traverse the requirement will result in the loss of right to petition under 37 CFR 1.144. If claims are added after the election, applicant must indicate which of these claims are readable on the elected invention.

If claims are added after the election, applicant must indicate which of these claims are readable upon the elected invention.

Should applicant traverse on the ground that the inventions are not patentably distinct, applicant should submit evidence or identify such evidence now of record showing the inventions to be obvious variants or clearly admit on the record that this is the case. In either instance, if the examiner finds one of the inventions unpatentable over

the prior art, the evidence or admission may be used in a rejection under 35 U.S.C. 103 of the other invention.

7. This application contains claims generic to the following disclosed patentably distinct species of the inventions of Group I:

1) This application contains claims directed to the following patentably distinct species of cancer. Applicant is required to select one species of cancer from gastric cancer, head and neck cancer, renal cell carcinoma, urothelial/bladder carcinoma, ovarian carcinoma, myeloma, melanoma, lung cancer, squamous cell carcinoma, classical Hodgkin's lymphoma, and breast cancer, small cell lung cancer, salivary gland carcinoma, vulvar carcinoma, thyroid carcinoma, anal canal carcinoma, biliary carcinoma, mesothelioma, cervical carcinoma, or neuroendocrine carcinoma.

2) This application contains claims directed to the following patentably distinct species of anti-PD therapeutic agent. Applicant is required to select one species of anti-PD therapeutic agent from Avelumab (MSB0010178C), Nivolumab, Pembrolizumab, BMS- 936559, MPDL3280A, Pidilizumab, or MEDI4736.

8. The species are independent or distinct because each species is a materially and manipulatively distinct process comprising the use of structurally and/or functionally different products. In addition, these species are not obvious variants of each other based on the current record.

Applicant is required under 35 U.S.C. 121 to elect a single disclosed species, or a single grouping of patentably indistinct species, for prosecution on the merits to which the claims shall be restricted if no generic claim is finally held to be allowable.

There is a search and/or examination burden for the patentably distinct species as set forth above because at least the following reason(s) apply:

Because each member of cancer, or anti-PD therapeutic agent is structurally and/or functionally different from the others, the use of each different species of the invention of Group I is a materially and manipulatively different process. In addition, these species are not obvious variants of each other based on the current record. Accordingly,

the examination of claims directed to any one species of invention would require a unique search that is not required for examination of any of the other species of invention, because the search of any one member of cancer, or anti-PD therapeutic agent will not provide adequate information regarding any other. Moreover, the search necessary to examine claims directed to any one species of invention is not the same, nor is it coextensive with the search necessary to examine claims directed to any other. Since having to perform more than one search would constitute a serious burden, it is proper to restrict these species of invention and require Applicant to elect only one. See MPEP § 809.

9. **Applicant is advised that the reply to this requirement to be complete must include (i) an election of a species or a grouping of patentably indistinct species to be examined even though the requirement may be traversed (37 CFR 1.143) and (ii) identification of the claims encompassing the elected species or grouping of patentably indistinct species**, including any claims subsequently added. An argument that a claim is allowable or that all claims are generic is considered nonresponsive unless accompanied by an election.

The election may be made with or without traverse. To preserve a right to petition, the election must be made with traverse. If the reply does not distinctly and specifically point out supposed errors in the election of species requirement, the election shall be treated as an election without traverse. Traversal must be presented at the time of election in order to be considered timely. Failure to timely traverse the requirement will result in the loss of right to petition under 37 CFR 1.144. If claims are added after the election, applicant must indicate which of these claims are readable on the elected species or grouping of patentably indistinct species.

Should applicant traverse on the ground that the species, or groupings of patentably indistinct species from which election is required, are not patentably distinct, applicant should submit evidence or identify such evidence now of record showing them to be obvious variants or clearly admit on the record that this is the case. In either instance, if the examiner finds one of the species unpatentable over the prior art, the

evidence or admission may be used in a rejection under 35 U.S.C. 103 of the other species.

Upon the allowance of a generic claim, applicant will be entitled to consideration of claims to additional species which depend from or otherwise require all the limitations of an allowable generic claim as provided by 37 CFR 1.141.

10. Applicant is reminded that upon the cancellation of claims to a non-elected invention, the inventorship must be amended in compliance with 37 CFR 1.48(b) if one or more of the currently named inventors is no longer an inventor of at least one claim remaining in the application. Any amendment of inventorship must be accompanied by a request under 37 CFR 1.48(b) and by the fee required under 37 CFR 1.17(i).

11. The examiner has required restriction between product and process claims. Where applicant elects claims directed to the product, and the product claims are subsequently found allowable, withdrawn process claims that depend from or otherwise require all the limitations of the allowable product claim will be considered for rejoinder. All claims directed to a nonelected process invention must require all the limitations of an allowable product claim for that process invention to be rejoined.

In the event of rejoinder, the requirement for restriction between the product claims and the rejoined process claims will be withdrawn, and the rejoined process claims will be fully examined for patentability in accordance with 37 CFR 1.104. Thus, to be allowable, the rejoined claims must meet all criteria for patentability including the requirements of 35 U.S.C. 101, 102, 103 and 112. Until all claims to the elected product are found allowable, an otherwise proper restriction requirement between product claims and process claims may be maintained. Withdrawn process claims that are not commensurate in scope with an allowable product claim will not be rejoined. See MPEP § 821.04(b). Additionally, in order to retain the right to rejoinder in accordance with the above policy, applicant is advised that the process claims should be amended during prosecution to require the limitations of the product claims. **Failure to do so may result in a loss of the right to rejoinder.** Further, note that the prohibition against double

patenting rejections of 35 U.S.C. 121 does not apply where the restriction requirement is withdrawn by the examiner before the patent issues. See MPEP § 804.01.

Conclusion

12. Any inquiry concerning this communication or earlier communications from the examiner should be directed to YAN XIAO whose telephone number is (571)270-3578. The examiner can normally be reached M-F 8-5 EST.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Samira Jean-Louis can be reached on 571-270-3503. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of published or unpublished applications may be obtained from Patent Center. Unpublished application information in Patent Center is available to registered users. To file and manage patent submissions in Patent Center, visit: <https://patentcenter.uspto.gov>. Visit <https://www.uspto.gov/patents/apply/patent-center> for more information about Patent Center and <https://www.uspto.gov/patents/docx> for information about filing in DOCX format. For additional questions, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/YAN XIAO/
Primary Examiner, Art Unit 1642

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

<i>In re</i> Application of: Karina Kulangara et al.	Group Art Unit: 1642
Serial No.: 18/098,198	Examiner: XIAO, YAN
Filing Date: January 18, 2023	Attorney Docket No.: 20160057-04/ AGLT.P0027US
Title: SCORING METHODS FOR ANTI-PD THERAPY ELIGIBILITY AND COMPOSITIONS FOR PERFORMING SAME	Confirmation No.: 9732

RESPONSE TO RESTRICTION REQUIREMENT DATED MARCH 20, 2025

Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

Commissioner:

This paper is submitted in response to the Restriction Requirement dated March 20, 2025, for which a response is due May 20, 2025. No fees are believed to be due. However, if any fees are due for any reason relating to the enclosed materials, the Commissioner is authorized to deduct said fees from Parker Highlander PLLC Account No.: 50-5902/AGLT.P0027US.

Amendments to the Claims begin on page 2.

Remarks begin on page 5.

AMENDMENT TO THE CLAIMS

This listing of claims will replace all prior versions, and listings, of claims in the application.

1. (Original) A method for determining the eligibility of a subject having a malignancy for treatment with an anti-PD therapeutic agent, the method comprising:

determining the number of viable PD-L1 positive tumor cells, the number of viable PD-L1 negative tumor cells, and the number of viable PD-L1 positive mononuclear inflammatory cells (MIC) in a tumor tissue sample from a subject having a malignancy;

calculating a combined positive score (CPS) for the tumor tissue sample using the formula:

$$\text{CPS} = \frac{\text{PD-L1 positive tumor cells} + \text{PD-L1 positive MIC}}{\text{PD-L1 positive tumor cells} + \text{PD-L1 negative tumor cells}} \times 100\%$$

wherein the subject is eligible for treatment with an anti-PD therapeutic agent when the CPS is above a threshold.

2. (Original) The method of claim 1, wherein the threshold is from about 10% to about 1%.
3. (Original) The method of claim 1, wherein the threshold is 1%.
4. (Original) The method of claim 1, wherein the tumor tissue sample is a tissue section of a tumor biopsy.
5. (Original) The method of claim 4, wherein PD-L1 is detected by immunohistochemistry (IHC) staining.
6. (Original) The method of claim 4, wherein the tumor tissue section is a formalin fixed and embedded in paraffin wax (FFPE) tumor tissue section.
7. (Original) The method of claim 4, wherein the tissue section is stained.

8. (Original) The method of claim 7, wherein the stain is a hematoxylin and eosin (H&E) stain.
9. (Original) The method of claim 4, wherein the viable PD-L1 positive tumor cells, the number of viable PD-L1 negative tumor cells, and the number of viable PD-L1 positive MIC are counted in the tumor nests and the adjacent supporting stroma of the tumor tissue sample.
10. (Original) The method of claim 1, wherein the tumor tissue sample is a cell suspension.
11. (Original) The method of claim 10, wherein the number of viable PD-L1 positive tumor cells, the number of viable PD-L1 negative tumor cells, and the number of viable PD-L1 positive MIC in the tumor tissue sample are determining by flow cytometry.
12. (Original) The method of claim 1, wherein one or more additional marker is detected in the tumor tissue sample.
13. (Original) The method of claim 12, wherein the marker is specific for MIC or is selected from the group consisting of: CD3, CD5, CD4, CD7, CD8, and CD20.
14. (Original) The method of claim 1, wherein the method further comprises, prior to the determining step, contacting the tumor tissue sample with an anti-PD-L1 antibody or binding fragment thereof.
15. (Original) The method of claim 1, wherein the number of viable PD-L1 positive tumor cells, the number of viable PD-L1 negative tumor cells, and the number of viable PD-L1 positive MIC in the tumor tissue sample are determined by single cell sequencing.
16. (Original) The method of claim 1, wherein the malignancy is selected from the group consisting of: gastric cancer, head and neck cancer, renal cell carcinoma, urothelial/bladder carcinoma, ovarian carcinoma, myeloma, melanoma, lung cancer, squamous cell carcinoma, classical Hodgkin's lymphoma, and breast cancer (e.g., triple negative breast cancer, hormone receptor positive (ER and/or PR) and Her2 positive breast cancer), small cell lung cancer, salivary gland carcinoma, vulvar carcinoma, thyroid carcinoma, anal canal carcinoma, biliary carcinoma, mesothelioma, cervical carcinoma, and neuroendocrine carcinoma.

17. (Original) The method of claim 1, wherein the anti-PD therapeutic agent inhibits the anti-cell death activity of PD-1/PD-L1.
18. (Original) The method of claim 1, wherein the anti-PD therapeutic agent comprises an anti-PD or anti-PD-L1 specific antibody or binding fragment thereof.
19. (Original) The method of claim 1, wherein the anti-PD therapeutic agent is selected from the group consisting of: Avelumab (MSB0010178C), Nivolumab, Pembrolizumab, BMS-936559, MPDL3280A, Pidilizumab, and MEDI4736.
20. (Withdrawn) A kit for determining the eligibility of a subject having a malignancy for treatment with an anti-PD therapeutic agent, comprising:
a detectable PD-L1 specific binding agent; and
instructions for calculating a combined positive score (CPS).

REMARKS

A. Status of the Claims

Claims 1-20 were pending at the time of this Restriction Requirement. No claims have been added, canceled, or amended herein. Claim 20 has been marked as withdrawn in view of the below election. Claims 1-19 are pending and under consideration.

B. Elections

In response to the restriction requirement imposed by the Examiner, Applicants elect, without traverse, the Group I claims. In response to the Examiners invitation to elect a species, Applicants elect, for initial search and examination, gastric cancer as the species of cancer and pembrolizumab as the anti-PD therapeutic agent. Claims 1-19 are believed to relate to the elected subject matter. Rejoinder of the unelected subject matter is respectfully requested upon an indication that the elected subject matter is otherwise allowable.

C. Conclusion

The examiner is invited to contact the undersigned attorney with any questions, comments, or suggestions relating to the referenced patent application.

Respectfully submitted,

/Amanda S.J. Schnepf/

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Date: May 5, 2025



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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
18/098,198	01/18/2023	Karina Kulangara	20160057-04/6363.133884C2	9732
22878	7590	06/02/2025	EXAMINER	
Agilent Technologies, Inc.			XIAO, YAN	
Global IP Operations				
5301 Stevens Creek Blvd, 1A-PB			ART UNIT	
Santa Clara, CA 95051			PAPER NUMBER	
			1642	
			NOTIFICATION DATE	
			DELIVERY MODE	
			06/02/2025	
			ELECTRONIC	

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

Agilentdocketing@cpaglobal.com
ipopsadmin@agilent.foundationip.com

Notice of Pre-AIA or AIA Status

1. The present application, filed on or after March 16, 2013, is being examined under the first inventor to file provisions of the AIA.

DETAILED ACTION

2. The election without traverse filed 05/05/2025 in response to the Office Action of 03/20/2025 is acknowledged and has been entered.

Applicant has elected invention Group I, claims 1-19, drawn to a method for determining the eligibility of a subject having a malignancy for treatment with an anti-PD therapeutic agent, the method comprising: determining the number of viable PD-L1 positive tumor cells, the number of viable PD-L1 negative tumor cells, and the number of viable PD-L1 positive mononuclear inflammatory cells (MIC) in a tumor tissue sample from a subject having a malignancy.

Additionally, the Applicant has elected species of the Group I, gastric cancer as the species of cancer and pembrolizumab as the anti-PD therapeutic agent.

3. Claims 1-20 are pending in the application. Claim 20 has been withdrawn from further consideration pursuant to 37 CFR 1.142(b) as being drawn to a nonelected species, there being no allowable generic or linking claim. Election was made **without** traverse in the reply filed on 05/05/2025.

4. Claims 1-19 are currently under prosecution.

Priority

5. Applicant's claim under 35 U.S.C. §§ 119(e) and 120 for benefit of the earlier filing date of application, is acknowledged.

Claim Rejections - 35 USC § 112

6. The following is a quotation of 35 U.S.C. 112(b):

(B) CONCLUSION.—The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the inventor or a joint inventor regards as the invention.

7. The following is a quotation of 35 U.S.C. 112 (pre-AIA), second paragraph:

The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

8. Claims 2 and 16 is rejected under 35 U.S.C. 112(b) or 35 U.S.C. 112 (pre-AIA), second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which the inventor or a joint inventor, or for pre-AIA the applicant regards as the invention.

Claim 2 is indefinite because the claim 2 recites "about".

M.P.E.P. § 2173.05(b) III. A. states:

In determining the range encompassed by the term "about", one must consider the context of the term as it is used in the specification and claims of the application. *Ortho-McNeil Pharm., Inc. v. Caraco Pharm. Labs., Ltd.*, 476 F.3d 1321, 1326, 81 USPQ2d 1427, 1432 (Fed. Cir. 2007). In *W.L. Gore & Associates, Inc. v. Garlock, Inc.*, 721 F.2d 1540, 220 USPQ 303 (Fed. Cir. 1983), the court held that a limitation defining the stretch rate of a plastic as "exceeding about 10% per second" is definite because infringement could clearly be assessed through the use of a stopwatch. However, in another case, the court held that claims reciting "at least about" were invalid for indefiniteness where there was close prior art and there was nothing in the specification, prosecution history, or the prior art to provide any indication as to what range of specific activity is covered by the term "about." *Amgen, Inc. v. Chugai Pharmaceutical Co.*, 927 F.2d 1200, 18 USPQ2d 1016 (Fed. Cir. 1991).

In this case, there is nothing in the specification to provide any indication as to what range of specific activity is covered by the term "about."

Regarding claim 16, the phrase "e.g.," renders the claim indefinite because it is unclear whether the limitation(s) following the phrase are part of the claimed invention. See MPEP § 2173.05(d).

9. The following is a quotation of 35 U.S.C. 112(a):

(a) IN GENERAL.—The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it

is most nearly connected, to make and use the same, and shall set forth the best mode contemplated by the inventor or joint inventor of carrying out the invention.

10. The following is a quotation of 35 U.S.C. 112 (pre-AIA), first paragraph:

The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

11. Claims 1-13 and 15-17 are rejected under 35 U.S.C. 112(a) or 35 U.S.C. 112 (pre-AIA), first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor or a joint inventor, or for pre-AIA the inventor(s), at the time the application was filed, had possession of the claimed invention.

This is a “written description” rejection.

The considerations that are made in determining whether a claimed invention is supported by an adequate written description are outlined by the published Guidelines for Examination of Patent Applications Under the 35 U.S.C. 112, para. 1, “Written Description” Requirement (Federal Register; Vol. 66, No. 4, January 5, 2001; The 2015 Written Description Workshop materials; hereinafter “Guidelines”).

These guidelines state that rejection of a claim for lack of written description, where the claim recites the language of an original claim should be rare. Nevertheless, these guidelines further state, “the issue of a lack of written description may arise even for an original claim when an aspect of the claimed invention has not been described with sufficient particularity such that one skilled in the art would recognize that the applicant has possession of the claimed invention” (*Id.* at 1105). The “Guidelines” continue:

The claimed invention as a whole may not be adequately described if the claims require an essential or critical feature which is not adequately described in the specification and which is not conventional in the art or known to one of ordinary skill in the art. This problem may arise where an invention is described solely in terms of a method of its making coupled with its function and there is no described or art-recognized correlation or relationship between the structure of the invention and its function. A lack of adequate written description issue also arises if the knowledge and level of skill in the art would not permit

one skilled in the art to immediately envisage the product claimed from the disclosed process.

With further regard to the proposition that, as *original* claims, the claims themselves provide *in haec verba* support sufficient to satisfy the written description requirement, the Federal Circuit has explained that *in ipsius verbis* support for the claims in the specification does not *per se* establish compliance with the written description requirement:

Even if a claim is supported by the specification, the language of the specification, to the extent possible, must describe the claimed invention so that one skilled in the art can recognize what is claimed. The appearance of mere indistinct words in a specification or a claim, even an original claim, does not necessarily satisfy that requirement. The disclosure must allow one skilled in the art to visualize or recognize the identity of the subject matter purportedly described. *Eli Lilly*, 119 F.3d at 1568, 43 USPQ2d at 1406.

Regents of the University of California v. Eli Lilly & Co., 119 F.3d 1559, 43 USPQ2d 1398 (Fed. Cir. 1997). *See also*: *University of Rochester v. G.D. Searle & Co.*, 69 USPQ2d 1886 1892 (CA FC 2004).

Thus, an original claim may provide written description for itself, but it must still be an adequate written description, which establishes that the inventor was in possession of the invention.

The claims are herein drawn to a method for determining the eligibility of a subject having a malignancy for treatment with an anti-PD therapeutic agent.

In this instance, the claims are directed to a genus of anti-PD therapeutic agents for treating cancer.

A genus of anti-PD therapeutic agents may include, for example, a polypeptide, an antibody, a nucleic acid, or small molecule.

Although the specification teaches the anti-PD therapeutic agent includes an anti-PD1 or an anti-PD-L1 specific antibody (e.g., Avelumab (MSB0010178C), Nivolumab, Pembrolizumab, BMS-936559, MPDL3280A, Pidilizumab, or MED14736; see [0007-0011]), it is not representative of the claimed a genus of anti-PD therapeutic agents; this is because the claimed anti-PD therapeutic agents, for example, a polypeptide, an antibody, a nucleic acid, or small molecule have markedly different structures. The artisan

would not expect that any given anti-PD therapeutic agent would have activity for treating cancer.

There is no correlation between any one particularly identifying structural feature that is shared by at least a substantial number of the members of the claimed a genus of anti-PD therapeutic agents; because each anti-PD therapeutic agent is structurally and functionally different. Although the artisan could potentially screen anti-PD therapeutic agents, it cannot be predicted whether or not one will be successful. The written description provision set forth under 35 USC 112(a) is severable from its enablement provision, so that written description requirement cannot be met by describing how one might make the invention – rather the invention must be described in such clear and particular terms so as to reasonably convey to the skilled artisan that applicant had possession of the claimed invention as of the filing date of the application (i.e., the earlier effective US filing date sought).

The skilled artisan could not immediately envision, recognize or distinguish at least a substantial number of the members of the claimed genus of anti-PD therapeutic agents.

The specification therefore would not reasonably convey to the skilled artisan Applicant's possession of the claimed invention as of the filing date of the application.

Notably, the Federal Circuit has decided that a patentee of a biotechnological invention cannot necessarily claim a genus after only describing a limited number of species because there may be unpredictability in the results obtained from species other than those specifically enumerated. See *Noelle v. Lederman*, 69 USPQ2d 1508 1514 (CA FC 2004) (citing *Enzo Biochem II*, 323 F.3d at 965; *Regents*, 119 F.3d at 1568).

Furthermore, Applicant is reminded that “generalized language may not suffice if it does not convey the detailed identity of an invention.” *University of Rochester v. G.D. Searle Co.*, 69 USPQ2d 1886 1892 (CAFC 2004).

In this instance, there is no language that adequately describes with any of the requisite clarity or particularity the claimed a genus of anti-PD therapeutic agents would have activity for treating cancer. A description of what a material does, rather than of what it is, does not suffice to describe the claimed invention.

While the written description requirement can be satisfied without an actual reduction to practice, the disclosure of a catalog of potentially effective substances that

might be found to be useful in practicing the claimed invention does not fulfill the written description requirement.

Recognizing that the claims are drawn to an anti-PD therapeutic agent would have activity for treating cancer, it is aptly noted that the Federal Circuit has decided that a generic statement that defines a genus of substances by *only* their functional activity, does not provide an adequate written description of the genus. See *The Reagents of the University of California v. Eli Lilly*, 43 USPQ2d 1398 (CAFC 1997). The Court indicated that while applicants are not required to disclose every species encompassed by a genus, the description of a genus is achieved by the recitation of a precise definition of a representative number of members of the genus, such as by reciting the structure, formula, chemical name, or physical properties of those members, rather than by merely reciting a wish for, or even a plan for obtaining a genus of molecules having a particular functional property. The recitation of a functional property alone, which must be shared by the members of the genus, is merely descriptive of what the members of genus must be capable of doing, not of the substance and structure of the members.

Finally, Guidelines states, “[p]ossession may be shown in a variety of ways including description of an actual reduction to practice, or by showing the invention was ‘ready for patenting’ such as by disclosure of drawings or structural chemical formulas that show that the invention was complete, or by describing distinguishing identifying characteristics sufficient to show that the applicant was in possession of the claimed invention” (*Id.* at 1104). “Guidelines” further states, “[f]or inventions in an unpredictable art, adequate written description of a genus which embraces widely variant species *cannot* be achieved by disclosing only one species within the genus” (*Id.* at 1106); accordingly, it follows that an adequate written description of a genus cannot be achieved in the absence of a disclosure of at least one species within the genus. Moreover, because the claims encompass a genus of anti-PD therapeutic agents would have activity for treating cancer, but which otherwise vary materially, structurally and/or functionally, an adequate written description of the claimed invention must include sufficient description of at least a representative number of species by actual reduction to practice, reduction to drawings, or by disclosure of relevant, identifying characteristics sufficient to show that Applicant was in possession of the claimed genus. In this instance, factual

evidence of an actual reduction to practice has not been disclosed by Applicant in the specification; Applicant has not shown the invention was “ready for patenting” by disclosure of drawings or structural chemical formulas that show that the invention was complete; and Applicant has not described distinguishing identifying characteristics sufficient to show that Applicant was in possession of the claimed invention at the time the application was filed.

Double Patenting

12. The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the “right to exclude” granted by a patent and to prevent possible harassment by multiple assignees. A nonstatutory double patenting rejection is appropriate where the claims at issue are not identical, but at least one examined application claim is not patentably distinct from the reference claim(s) because the examined application claim is either anticipated by, or would have been obvious over, the reference claim(s). See, e.g., *In re Berg*, 140 F.3d 1428, 46 USPQ2d 1226 (Fed. Cir. 1998); *In re Goodman*, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi*, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van Ornum*, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); *In re Vogel*, 422 F.2d 438, 164 USPQ 619 (CCPA 1970); and *In re Thorington*, 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) or 1.321(d) may be used to overcome an actual or provisional rejection based on a nonstatutory double patenting ground provided the reference application or patent either is shown to be commonly owned with this application, or claims an invention made as a result of activities undertaken within the scope of a joint research agreement. A terminal disclaimer must be signed in compliance with 37 CFR 1.321(b).

The USPTO internet Web site contains terminal disclaimer forms which may be used. Please visit <http://www.uspto.gov/forms/>. The filing date of the application will determine what form should be used. A web-based eTerminal Disclaimer may be filled out completely online using web-screens. An eTerminal Disclaimer that meets all requirements is auto-processed and approved immediately upon submission. For more

information about eTerminal Disclaimers, refer to

<http://www.uspto.gov/patents/process/file/efs/guidance/eTD-info-I.jsp>.

13. Claims 1-19 are rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 1-20 of U.S. Patent No. 10,613,092. Although the conflicting claims are not identical, they are not patentably distinct from each other because for the following reasons:

Claims 1-19 are herein drawn to a method for determining the eligibility of a subject having a malignancy for treatment with an anti-PD therapeutic agent, the method comprising: determining the number of viable PD-L1 positive tumor cells, the number of viable PD-L1 negative tumor cells, and the number of viable PD-L1 positive mononuclear inflammatory cells (MIC) in a tumor tissue sample from a subject having a malignancy; and calculating a combined positive score (CPS) for the tumor tissue sample using the formula:

$$CPS = \frac{PD-L1 \text{ positive tumor cells} + PD-L1 \text{ positive MIC}}{PD-L1 \text{ positive tumor cells} + PD-L1 \text{ positive tumor cells}} \times 100\%$$

wherein the subject is eligible for treatment with said anti-PD therapeutic agent when the CPS is above a threshold.

Claims 1-20 of U.S. Patent No. 10,613,092 are drawn to a method for determining the eligibility of a subject having a malignancy for treatment with an anti-PD therapeutic agent known to have an anti-PD-1/PD-L1 therapeutic effect, the method comprising: determining the number of viable PD-L1 positive tumor cells, the number of viable PD-L1 negative tumor cells, and the number of viable PD-L1 positive mononuclear inflammatory cells (MIC) in a tumor tissue sample from a subject having a malignancy; and calculating a combined positive score (CPS) for the tumor tissue sample using the formula:

$$CPS = \frac{PD-L1 \text{ positive tumor cells} + PD-L1 \text{ positive MIC}}{PD-L1 \text{ positive tumor cells} + PD-L1 \text{ negative tumor cells}} \times 100\%$$

wherein the subject is eligible for treatment with said anti-PD therapeutic agent known to have an anti-PD-1/PD-L1 therapeutic effect when the CPS is above a threshold.

It is noted that 35 USC § 121 does not provide protection for continuation or continuation in-part applications, and this application is a continuation of U.S. Patent No. 10,613,092. See 92 USPQ2d 1289 *Amgen Inc. v. F. Hoffmann-La Roche Ltd.* (Fed. Cir. 2009) and *Pfizer, Inc. v. Teva Pharmaceuticals USA, Inc.* 518 F.3d 1353 (Fed. Cir. 2008).

14. Claims 1-19 are rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 1-22 of U.S. Patent No. 11,585,817. Although the conflicting claims are not identical, they are not patentably distinct from each other because for the following reasons:

Claims 1-19 are herein drawn to a method for determining the eligibility of a subject having a malignancy for treatment with an anti-PD therapeutic agent, the method comprising: determining the number of viable PD-L1 positive tumor cells, the number of viable PD-L1 negative tumor cells, and the number of viable PD-L1 positive mononuclear inflammatory cells (MIC) in a tumor tissue sample from a subject having a malignancy; and calculating a combined positive score (CPS) for the tumor tissue sample using the formula:

$$\text{CPS} = \frac{\text{PD-L1 positive tumor cells} + \text{PD-L1 positive MIC}}{\text{PD-L1 positive tumor cells} + \text{PD-L1 positive tumor cells}} \times 100\%$$

wherein the subject is eligible for treatment with said anti-PD therapeutic agent when the CPS is above a threshold.

Claims 1-22 of U.S. Patent No. 11,585,817 are drawn to a method for determining the eligibility of a subject having a malignancy for treatment with an anti-PD therapeutic agent, the method comprising: determining the number of viable PD-L1 positive tumor cells, the number of viable PD-L1 negative tumor cells, and the number of viable PD-L1 positive mononuclear inflammatory cells (MIC) in a tumor tissue sample from a subject

having a malignancy; and calculating a combined positive score (CPS) for the tumor tissue sample using the formula:

$$\text{CPS} = \frac{\text{PD-L1 positive tumor cells} + \text{PD-L1 positive MIC}}{\text{total number of viable tumor cells}} \times 100\%$$

wherein the subject is eligible for treatment with said anti-PD therapeutic agent when the CPS is above a threshold from about 60 to about 1.

It is noted that 35 USC § 121 does not provide protection for continuation or continuation in-part applications, and this application is a continuation of U.S. Patent No. 11,585,817. See 92 USPQ2d 1289 *Amgen Inc. v. F. Hoffmann-La Roche Ltd.* (Fed. Cir. 2009) and *Pfizer, Inc. v. Teva Pharmaceuticals USA, Inc.* 518 F.3d 1353 (Fed. Cir. 2008).

Conclusion

15. No claim is allowed.

16. Any inquiry concerning this communication or earlier communications from the examiner should be directed to YAN XIAO whose telephone number is (571)270-3578. The examiner can normally be reached M-F 8-5 EST.

Examiner interviews are available via telephone, in-person, and video conferencing using a USPTO supplied web-based collaboration tool. To schedule an interview, applicant is encouraged to use the USPTO Automated Interview Request (AIR) at <http://www.uspto.gov/interviewpractice>.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Samira Jean-Louis can be reached on 571-270-3503. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of published or unpublished applications may be obtained from Patent Center. Unpublished application information in Patent Center is available to registered users. To file and manage patent submissions in Patent Center,

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Art Unit: 1642

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visit: <https://patentcenter.uspto.gov>. Visit <https://www.uspto.gov/patents/apply/patent-center> for more information about Patent Center and <https://www.uspto.gov/patents/docx> for information about filing in DOCX format. For additional questions, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/YAN XIAO/
Primary Examiner, Art Unit 1642

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

<i>In re</i> Application of: Karina Kulangara et al.	Group Art Unit: 1642
Serial No.: 18/098,198	Examiner: XIAO, YAN
Filing Date: January 18, 2023	Attorney Docket No.: 20160057-04/ AGLT.P0027US
Title: SCORING METHODS FOR ANTI-PD THERAPY ELIGIBILITY AND COMPOSITIONS FOR PERFORMING SAME	Confirmation No.: 9732

AMENDMEND AND RESPONSE TO OFFICE ACTION DATED JUNE 2, 2025

Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

Commissioner:

This paper is submitted in response to the Office Action dated June 2, 2025, for which a response is due September 2, 2025. No fees are believed to be due. However, if any fees are due for any reason relating to the enclosed materials, the Commissioner is authorized to deduct said fees from Parker Highlander PLLC Account No.: 50-5902/AGLT.P0027US.

Amendments to the Claims begin on page 2.

Remarks begin on page 5.

AMENDMENT TO THE CLAIMS

This listing of claims will replace all prior versions, and listings, of claims in the application.

1. (Currently amended) A method for determining the eligibility of a subject having a malignancy for treatment with an anti-PD therapeutic agent, the method comprising:

determining the number of viable PD-L1 positive tumor cells, the number of viable PD-L1 negative tumor cells, and the number of viable PD-L1 positive mononuclear inflammatory cells (MIC) in a tumor tissue sample from a subject having a malignancy;

calculating a combined positive score (CPS) for the tumor tissue sample using the formula:

$$\text{CPS} = \frac{\text{PD-L1 positive tumor cells} + \text{PD-L1 positive MIC}}{\text{PD-L1 positive tumor cells} + \text{PD-L1 negative tumor cells}} \times 100\%$$

wherein the subject is eligible for treatment with [[an]] said anti-PD therapeutic agent when the CPS is above a threshold, wherein said anti-PD therapeutic agent comprises an anti-PD or anti-PD-L1 specific antibody or binding fragment thereof.

2. (Currently amended) The method of claim 1, wherein the threshold is from ~~about~~ 10% to ~~about~~ 1%.

3. (Original) The method of claim 1, wherein the threshold is 1%.

4. (Original) The method of claim 1, wherein the tumor tissue sample is a tissue section of a tumor biopsy.

5. (Original) The method of claim 4, wherein PD-L1 is detected by immunohistochemistry (IHC) staining.

6. (Original) The method of claim 4, wherein the tumor tissue section is a formalin fixed and embedded in paraffin wax (FFPE) tumor tissue section.

7. (Original) The method of claim 4, wherein the tissue section is stained.
8. (Original) The method of claim 7, wherein the stain is a hematoxylin and eosin (H&E) stain.
9. (Original) The method of claim 4, wherein the viable PD-L1 positive tumor cells, the number of viable PD-L1 negative tumor cells, and the number of viable PD-L1 positive MIC are counted in the tumor nests and the adjacent supporting stroma of the tumor tissue sample.
10. (Original) The method of claim 1, wherein the tumor tissue sample is a cell suspension.
11. (Original) The method of claim 10, wherein the number of viable PD-L1 positive tumor cells, the number of viable PD-L1 negative tumor cells, and the number of viable PD-L1 positive MIC in the tumor tissue sample are determining by flow cytometry.
12. (Original) The method of claim 1, wherein one or more additional marker is detected in the tumor tissue sample.
13. (Original) The method of claim 12, wherein the marker is specific for MIC or is selected from the group consisting of: CD3, CD5, CD4, CD7, CD8, and CD20.
14. (Original) The method of claim 1, wherein the method further comprises, prior to the determining step, contacting the tumor tissue sample with an anti-PD-L1 antibody or binding fragment thereof.
15. (Original) The method of claim 1, wherein the number of viable PD-L1 positive tumor cells, the number of viable PD-L1 negative tumor cells, and the number of viable PD-L1 positive MIC in the tumor tissue sample are determined by single cell sequencing.
16. (Currently amended) The method of claim 1, wherein the malignancy is selected from the group consisting of: gastric cancer, head and neck cancer, renal cell carcinoma, urothelial/bladder carcinoma, ovarian carcinoma, myeloma, melanoma, lung cancer, squamous cell carcinoma, classical Hodgkin's lymphoma, and breast cancer (e.g., ~~triple negative breast cancer, hormone receptor positive (ER and/or PR) and Her2 positive breast cancer~~), small cell lung cancer, salivary

gland carcinoma, vulvar carcinoma, thyroid carcinoma, anal canal carcinoma, biliary carcinoma, mesothelioma, cervical carcinoma, and neuroendocrine carcinoma.

17. (Original) The method of claim 1, wherein the anti-PD therapeutic agent inhibits the anti-cell death activity of PD-1/PD-L1.

18. (Canceled)

19. (Original) The method of claim 1, wherein the anti-PD therapeutic agent is selected from the group consisting of: Avelumab (MSB0010178C), Nivolumab, Pembrolizumab, BMS-936559, MPDL3280A, Pidilizumab, and MEDI4736.

20. (Canceled)

21. (New) The method of claim 16, wherein the breast cancer is triple negative breast cancer, hormone receptor positive breast cancer, estrogen receptor positive breast cancer, progesterone receptor positive breast cancer, or Her2 positive breast cancer .

22. (New) The method of claim 1, further comprising administering said anti-PD therapeutic agent to the subject when the CPS is above the threshold.

23. (New) The method of claim 22, wherein the threshold is greater than or equal to 1%.

REMARKS

A. Status of the Claims

Claims 1-20 were pending at the time of this Office Action, with claim 20 being withdrawn from consideration. Claims 1, 2, and 16 are amended herein. Claims 21-23 are added herein. Support can be found throughout the original claims and the specification as filed. Support for new Claim 21 can be found, for example, at Paragraphs [0080] and [0162] of the application as published and original Claim 16. Support for new Claim 22 can be found, for example, at Paragraphs [0014], [0065], [0086], [0123], [0124], [0142], [0143], [0171]-[0178] of the application as published and original Claims 1 and 20. Support for new Claim 23 can be found, for example, at paragraph [0065] of the application as published. Claims 18 and 20 are canceled herein. Claims 1-17, 19, and 21-23 are pending and under consideration.

B. Rejections Under 35 U.S.C. § 112(b)

The Action rejects claims 2 and 16 as allegedly indefinite due to the recitation of the terms “about” and “e.g.”. Applicant has amended the claims to address these concerns. As such, withdrawal of the rejections is respectfully requested.

C. Rejections Under 35 U.S.C. § 112(a), written description

The Action rejects claims 1-13 and 15-17 as allegedly failing to comply with the written description requirement. Without opining on the merits of the rejection, and in the interest of compact prosecution, Applicant has amended claim 1 to recite the limitations of previous claim 18, which was not subject to this rejection. As such, this rejection is believed to be moot.

D. Double Patenting Rejections

The Action rejects claims 1-19 on the ground of nonstatutory double patenting as being allegedly unpatentable over claims 1-20 of U.S. Pat. 10,613,092 and over claims 1-22 of U.S. Pat.

11,585,817. Without opining on the merits of the rejection, and in the interest of compact prosecution, Applicant has filed a terminal disclaimer relative to these patents. As such, this rejection is believed to be moot.

E. Conclusion

The examiner is invited to contact the undersigned attorney with any questions, comments, or suggestions relating to the referenced patent application.

Respectfully submitted,

/Amanda S.J. Schnepf/

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Date: August 26, 2025



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NOTICE OF ALLOWANCE AND FEE(S) DUE

22878 7590 09/24/2025
Agilent Technologies, Inc.
Global IP Operations
5301 Stevens Creek Blvd, 1A-PB
Santa Clara, CA 95051

EXAMINER

XIAO, YAN

ART UNIT PAPER NUMBER

1642

DATE MAILED: 09/24/2025

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
18/098,198	01/18/2023	Karina Kulangara	20160057-04/6363.133884C2	9732

TITLE OF INVENTION: SCORING METHODS FOR ANTI-PD THERAPY ELIGIBILITY AND COMPOSITIONS FOR PERFORMING SAME

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	UNDISCOUNTED	\$1290	\$0.00	\$0.00	\$1290	12/24/2025

THE APPLICATION IDENTIFIED ABOVE HAS BEEN EXAMINED AND IS ALLOWED FOR ISSUANCE AS A PATENT. PROSECUTION ON THE MERITS IS CLOSED. THIS NOTICE OF ALLOWANCE IS NOT A GRANT OF PATENT RIGHTS. THIS APPLICATION IS SUBJECT TO WITHDRAWAL FROM ISSUE AT THE INITIATIVE OF THE OFFICE OR UPON PETITION BY THE APPLICANT. SEE 37 CFR 1.313 AND MPEP 1308.

THE ISSUE FEE AND PUBLICATION FEE (IF REQUIRED) MUST BE PAID WITHIN THREE MONTHS FROM THE MAILING DATE OF THIS NOTICE OR THIS APPLICATION SHALL BE REGARDED AS ABANDONED. THIS STATUTORY PERIOD CANNOT BE EXTENDED. SEE 35 U.S.C. 151. THE ISSUE FEE DUE INDICATED ABOVE DOES NOT REFLECT A CREDIT FOR ANY PREVIOUSLY PAID ISSUE FEE IN THIS APPLICATION. IF AN ISSUE FEE HAS PREVIOUSLY BEEN PAID IN THIS APPLICATION (AS SHOWN ABOVE), THE RETURN OF PART B OF THIS FORM WILL BE CONSIDERED A REQUEST TO REAPPLY THE PREVIOUSLY PAID ISSUE FEE TOWARD THE ISSUE FEE NOW DUE.

HOW TO REPLY TO THIS NOTICE:

I. Review the ENTITY STATUS shown above. If the ENTITY STATUS is shown as SMALL or MICRO, verify whether entitlement to that entity status still applies.

If the ENTITY STATUS is the same as shown above, pay the TOTAL FEE(S) DUE shown above.

If the ENTITY STATUS is changed from that shown above, on PART B - FEE(S) TRANSMITTAL, complete section number 5 titled "Change in Entity Status (from status indicated above)".

For purposes of this notice, small entity fees are 40% the amount of undiscounted fees, and micro entity fees are 20% the amount of undiscounted fees.

II. PART B - FEE(S) TRANSMITTAL, or its equivalent, must be completed and returned to the United States Patent and Trademark Office (USPTO) with your ISSUE FEE and PUBLICATION FEE (if required). If you are charging the fee(s) to your deposit account, section "4b" of Part B - Fee(s) Transmittal should be completed. If an equivalent of Part B is filed, a request to reapply a previously paid issue fee must be clearly made, and delays in processing may occur due to the difficulty in recognizing the paper as an equivalent of Part B.

III. All communications regarding this application must give the application number. Please direct all communications prior to issuance to Mail Stop ISSUE FEE unless advised to the contrary.

IMPORTANT REMINDER: Maintenance fees are due in utility patents issuing on applications filed on or after Dec. 12, 1980. It is patentee's responsibility to ensure timely payment of maintenance fees when due. More information is available at www.uspto.gov/PatentMaintenanceFees.

PART B - FEE(S) TRANSMITTAL

Complete and send this form, together with applicable fee(s), by mail or fax, or via the USPTO patent electronic filing system.

By mail, send to: Mail Stop ISSUE FEE
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

By fax, send to: (571)-273-2885

INSTRUCTIONS: This form should be used for transmitting the ISSUE FEE and PUBLICATION FEE (if required). Blocks 1 through 5 should be completed where appropriate. All further correspondence will be mailed to the current correspondence address as indicated unless corrected below or directed otherwise in Block 1, by (a) specifying a new correspondence address; and/or (b) indicating a separate "FEE ADDRESS" for maintenance fee notifications. **Because electronic patent issuance may occur shortly after issue fee payment, any desired continuing application should preferably be filed prior to payment of this issue fee in order not to jeopardize copendency.**

CURRENT CORRESPONDENCE ADDRESS (Note: Use Block 1 for any change of address)

22878 7590 09/24/2025
Agilent Technologies, Inc.
Global IP Operations
5301 Stevens Creek Blvd, 1A-PB
Santa Clara, CA 95051

Note: A certificate of mailing can only be used for domestic mailings of the Fee(s) Transmittal. This certificate cannot be used for any other accompanying papers. Each additional paper, such as an assignment or formal drawing, must have its own certificate of mailing or transmission.

Certificate of Mailing or Transmission

I hereby certify that this Fee(s) Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Mail Stop ISSUE FEE address above, or being transmitted to the USPTO via the USPTO patent electronic filing system or by facsimile to (571) 273-2885, on the date below.

(Typed or printed name)
(Signature)
(Date)

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
18/098,198	01/18/2023	Karina Kulangara	20160057-04/6363.133884C2	9732

TITLE OF INVENTION: SCORING METHODS FOR ANTI-PD THERAPY ELIGIBILITY AND COMPOSITIONS FOR PERFORMING SAME

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	UNDISCOUNTED	\$1290	\$0.00	\$0.00	\$1290	12/24/2025

EXAMINER	ART UNIT	CLASS-SUBCLASS
XIAO, YAN	1642	435-007230

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363).

☐ Change of correspondence address (or Change of Correspondence Address form PTO/AIA/122 or PTO/SB/122) attached.

☐ "Fee Address" indication (or "Fee Address" Indication form PTO/AIA/47 or PTO/SB/47; Rev 03-02 or more recent) attached. **Use of a Customer Number is required.**

2. For printing on the patent front page, list

(1) The names of up to 3 registered patent attorneys or agents OR, alternatively,
(2) The name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed.

1 _____
2 _____
3 _____

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. If an assignee is identified below, the document must have been previously recorded, or filed for recordation, as set forth in 37 CFR 3.11 and 37 CFR 3.81(a). Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE

(B) RESIDENCE: (CITY and STATE OR COUNTRY)

Please check the appropriate assignee category or categories (will not be printed on the patent) : ☐ Individual ☐ Corporation or other private group entity ☐ Government

4a. Fees submitted: ☐ Issue Fee ☐ Publication Fee (if required)

4b. Method of Payment: (Please first reapply any previously paid fee shown above)

☐ Electronic Payment via the USPTO patent electronic filing system ☐ Enclosed check ☐ Non-electronic payment by credit card (Attach form PTO-2038)

☐ The Director is hereby authorized to charge the required fee(s), any deficiency, or credit any overpayment to Deposit Account No. _____

5. Change in Entity Status (from status indicated above)

☐ Applicant certifying micro entity status. See 37 CFR 1.29

☐ Applicant asserting small entity status. See 37 CFR 1.27

☐ Applicant changing to regular undiscounted fee status.

NOTE: Absent a valid certification of Micro Entity Status (see forms PTO/SB/15A and 15B), issue fee payment in the micro entity amount will not be accepted at the risk of application abandonment.

NOTE: If the application was previously under micro entity status, checking this box will be taken to be a notification of loss of entitlement to micro entity status.

NOTE: Checking this box will be taken to be a notification of loss of entitlement to small or micro entity status, as applicable.

NOTE: This form must be signed in accordance with 37 CFR 1.31 and 1.33. See 37 CFR 1.4 for signature requirements and certifications.

Authorized Signature _____

Date _____

Typed or printed name _____

Registration No. _____



UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
18/098,198	01/18/2023	Karina Kulangara	20160057-04/6363.133884C2	9732
22878	7590	09/24/2025	EXAMINER	
Agilent Technologies, Inc. Global IP Operations 5301 Stevens Creek Blvd, 1A-PB Santa Clara, CA 95051			XIAO, YAN	
			ART UNIT	PAPER NUMBER
			1642	

DATE MAILED: 09/24/2025

Determination of Patent Term Adjustment under 35 U.S.C. 154 (b) (Applications filed on or after May 29, 2000)

The Office has discontinued providing a Patent Term Adjustment (PTA) calculation with the Notice of Allowance.

Section 1(h)(2) of the AIA Technical Corrections Act amended 35 U.S.C. 154(b)(3)(B)(i) to eliminate the requirement that the Office provide a patent term adjustment determination with the notice of allowance. See Revisions to Patent Term Adjustment, 78 Fed. Reg. 19416, 19417 (Apr. 1, 2013). Therefore, the Office is no longer providing an initial patent term adjustment determination with the notice of allowance. The Office will continue to provide a patent term adjustment determination with the Issue Notification Letter that is mailed to applicant approximately three weeks prior to the issue date of the patent, and will include the patent term adjustment on the patent. Any request for reconsideration of the patent term adjustment determination (or reinstatement of patent term adjustment) should follow the process outlined in 37 CFR 1.705.

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (571)-272-7702. Questions relating to issue and publication fee payments should be directed to the Customer Service Center of the Office of Patent Publication at 1-(888)-786-0101 or (571)-272-4200.

OMB Clearance and PRA Burden Statement for PTOL-85 Part B

The Paperwork Reduction Act (PRA) of 1995 requires Federal agencies to obtain Office of Management and Budget approval before requesting most types of information from the public. When OMB approves an agency request to collect information from the public, OMB (i) provides a valid OMB Control Number and expiration date for the agency to display on the instrument that will be used to collect the information and (ii) requires the agency to inform the public about the OMB Control Number's legal significance in accordance with 5 CFR 1320.5(b).

The information collected by PTOL-85 Part B is required by 37 CFR 1.311. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 30 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, Virginia 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, Virginia 22313-1450. Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. The United States Patent and Trademark Office (USPTO) collects the information in this record under authority of 35 U.S.C. 2. The USPTO's system of records is used to manage all applicant and owner information including name, citizenship, residence, post office address, and other information with respect to inventors and their legal representatives pertaining to the applicant's/owner's activities in connection with the invention for which a patent is sought or has been granted. The applicable Privacy Act System of Records Notice for the information collected in this form is COMMERCE/PAT-TM-7 Patent Application Files, available in the Federal Register at 78 FR 19243 (March 29, 2013).

<https://www.govinfo.gov/content/pkg/FR-2013-03-29/pdf/2013-07341.pdf>

Routine uses of the information in this record may include disclosure to:

- 1) law enforcement, in the event that the system of records indicates a violation or potential violation of law;
- 2) a federal, state, local, or international agency, in response to its request;
- 3) a contractor of the USPTO having need for the information in order to perform a contract;
- 4) the Department of Justice for determination of whether the Freedom of Information Act (FOIA) requires disclosure of the record;
- 5) a Member of Congress submitting a request involving an individual to whom the record pertains, when the individual has requested the Member's assistance with respect to the subject matter of the record;
- 6) a court, magistrate, or administrative tribunal, in the course of presenting evidence, including disclosures to opposing counsel in the course of settlement negotiations;
- 7) the Administrator, General Services Administration (GSA), or their designee, during an inspection of records conducted by GSA under authority of 44 U.S.C. 2904 and 2906, in accordance with the GSA regulations and any other relevant (i.e., GSA or Commerce) directive, where such disclosure shall not be used to make determinations about individuals;
- 8) another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c));
- 9) the Office of Personnel Management (OPM) for personnel research purposes; and
- 10) the Office of Management and Budget (OMB) for legislative coordination and clearance.

If you do not furnish the information requested on this form, the USPTO may not be able to process and/or examine your submission, which may result in termination of proceedings, abandonment of the application, and/or expiration of the patent.

Notice of Allowability	Application No. 18/098,198	Applicant(s) Kulangara et al.	
	Examiner YAN XIAO	Art Unit 1642	AIA (First Inventor to File) Status Yes

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address--

All claims being allowable, PROSECUTION ON THE MERITS IS (OR REMAINS) CLOSED in this application. If not included herewith (or previously mailed), a Notice of Allowance (PTOL-85) or other appropriate communication will be mailed in due course. **THIS NOTICE OF ALLOWABILITY IS NOT A GRANT OF PATENT RIGHTS.** This application is subject to withdrawal from issue at the initiative of the Office or upon petition by the applicant. See 37 CFR 1.313 and MPEP 1308.

1. ☒ This communication is responsive to 08/26/2025.
☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

2. ☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

3. ☒ The allowed claim(s) is/are 1-17, 19 and 21-23. As a result of the allowed claim(s), you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to PPHfeedback@uspto.gov.

4. ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
Certified copies:
a) ☐ All b) ☐ Some* c) ☐ None of the:
1. ☐ Certified copies of the priority documents have been received.
2. ☐ Certified copies of the priority documents have been received in Application No. _____.
3. ☐ Copies of the certified copies of the priority documents have been received in this national stage application from the International Bureau (PCT Rule 17.2(a)).
* Certified copies not received: _____.

Applicant has THREE MONTHS FROM THE "MAILING DATE" of this communication to file a reply complying with the requirements noted below. Failure to timely comply will result in ABANDONMENT of this application.
THIS THREE-MONTH PERIOD IS NOT EXTENDABLE.

5. ☐ CORRECTED DRAWINGS (as "replacement sheets") must be submitted.
☐ including changes required by the attached Examiner's Amendment / Comment or in the Office action of Paper No./Mail Date _____.
Identifying indicia such as the application number (see 37 CFR 1.84(c)) should be written on the drawings in the front (not the back) of each sheet. Replacement sheet(s) should be labeled as such in the header according to 37 CFR 1.121(d).

6. ☐ DEPOSIT OF and/or INFORMATION about the deposit of BIOLOGICAL MATERIAL must be submitted. Note the attached Examiner's comment regarding REQUIREMENT FOR THE DEPOSIT OF BIOLOGICAL MATERIAL.

Attachment(s)

1. ☐ Notice of References Cited (PTO-892)	5. ☒ Examiner's Amendment/Comment
2. ☐ Information Disclosure Statements (PTO/SB/08), Paper No./Mail Date _____.	6. ☐ Examiner's Statement of Reasons for Allowance
3. ☐ Examiner's Comment Regarding Requirement for Deposit of Biological Material _____.	7. ☐ Other _____.
4. ☐ Interview Summary (PTO-413), Paper No./Mail Date _____.	

/YAN XIAO/ Primary Examiner, Art Unit 1642	
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Notice of Pre-AIA or AIA Status

1. The present application, filed on or after March 16, 2013, is being examined under the first inventor to file provisions of the AIA.

EXAMINER'S COMMENT

2. The amendment/argument filed 08/26/2025, has been entered.
3. Any comments considered necessary by applicant must be submitted no later than the payment of the Issue Fee and, to avoid processing delays, should preferably accompany the Issue Fee. Such submissions should be clearly labeled "Comments on Statement of Reasons for Allowance."

Conclusion

4. Claims 1-17, 19 and 21-23 have been allowed.
5. Claims 1-17, 19 and 21-23 have been renumbered as claims 1-21, respectively.
6. Any inquiry concerning this communication or earlier communications from the examiner should be directed to YAN XIAO whose telephone number is (571)270-3578. The examiner can normally be reached M-F 8-5 EST.

Examiner interviews are available via telephone, in-person, and video conferencing using a USPTO supplied web-based collaboration tool. To schedule an interview, applicant is encouraged to use the USPTO Automated Interview Request (AIR) at <http://www.uspto.gov/interviewpractice>.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Samira Jean-Louis can be reached on 571-270-3503. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of published or unpublished applications may be obtained from Patent Center. Unpublished application information in Patent Center is available to registered users. To file and manage patent submissions in Patent Center,

Application/Control Number: 18/098,198
Art Unit: 1642

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visit: <https://patentcenter.uspto.gov>. Visit <https://www.uspto.gov/patents/apply/patent-center> for more information about Patent Center and <https://www.uspto.gov/patents/docx> for information about filing in DOCX format. For additional questions, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/YAN XIAO/
Primary Examiner, Art Unit 1642