

ANTIBODIES AND METHODS FOR PTK7 DETECTION**TECHNICAL FIELD****CROSS REFERENCE TO RELATED APPLICATIONS**

[0001] This application claims the benefit of US provisional application No. US63/603,097, filed on November 27, 2023, the contents of which is incorporated by reference herein in its entirety.

SEQUENCE LISTING

[0002] The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on November 12, 2023, is named 2023-11-12-Sequence listing-20896-0005PR00.xml and is 33,079 bytes in size.

[0003] The present disclosure generally relates to antibodies of PTK7 and related methods, and specifically to PTK7 antibodies and IHC methods of detecting PTK7 and PTK7 positive cells and tissues.

BACKGROUND

[0004] Immunohistochemistry (IHC) is a widely used ancillary testing method in diagnostic and surgical pathology. The main original purpose of IHC is for cell classification and determination of organ of origin. The use of IHC has further expanded to investigate predictive and prognostic biomarkers in many malignancies including but not limited to those of the breast, gastrointestinal tract, liver, lung, ovary, hematolymphoid, central nervous systems, etc. IHC utilizes antibodies to target certain antigens in specific tissues and cells to facilitate the identification of the cells of interest.

[0005] Selection of a suitable target antigen and proper construction of antibodies that binds to target antigen determine the specificity and sensitivity of IHC. Protein tyrosine kinase 7 (PTK7), also known as colon carcinoma kinase 4 (CCK4), is found to be highly expressed in many types of cancer cells, and it has limited expression on normal tissues in humans. Therefore, it is an attractive biomarker for the detection of cancer cells and further for cancer diagnosis. However, the pace for constructing effective PTK7 antibodies has been slow. There is an urgent need for PTK7 antibodies that can be used for PTK7 positive cell detection and as the key component of a reliable IHC kit. Embodiments of the present disclosure address this and related needs.

SUMMARY

[0006] Provided here are protein tyrosine kinase 7 (PTK7) binding agents (e.g., antibodies)

and methods of using the same. A PTK7 binding agent may comprise: a heavy chain variable (VH) region and a light chain variable (VL) region, the VH region comprising complementarity determining regions HCDR1, HCDR2 and HCDR3 disposed in heavy chain variable region framework regions and the VL region comprising LCDR1, LCDR2 and LCDR3 disposed in light chain variable region framework regions, the VH and VL CDRs having amino acids sequences selected from the sets of amino acid sequences set forth in the group consisting of: SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, LVS, and SEQ ID NO: 7, respectively; SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, QMS, and SEQ ID NO: 14, respectively; and SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, RVS, and SEQ ID NO: 21, respectively.

[0007] In some embodiments, the VH and VL regions have amino acid sequences that are selected from the pairs of amino acid sequences set forth in the group consisting of: SEQ ID NO: 1 and SEQ ID NO: 2, respectively; SEQ ID NO: 8 and SEQ ID NO: 9, respectively; and SEQ ID NO: 15 and SEQ ID NO: 16, respectively.

[0008] In some embodiments, the VH and VL regions have amino acid sequences that are selected from the pairs of amino acid sequences set forth in the group consisting of: SEQ ID NO: 1 and SEQ ID NO: 2, respectively; SEQ ID NO: 8 and SEQ ID NO: 9, respectively; and SEQ ID NO: 15 and SEQ ID NO: 16, respectively, wherein the heavy and light chain framework regions are optionally modified with from 1 to 8 amino acid substitutions, deletions or insertions in the framework regions.

[0009] In some embodiments, the framework regions of the binding agent are human framework regions.

[0010] In some embodiments, the framework regions of the binding agent are mouse framework regions.

[0011] In some embodiments, the binding agent is an antibody. In some embodiments, the binding agent is a monoclonal antibody.

[0012] In some embodiments, the binding agent further comprises a heavy chain constant region and/or a light chain constant region.

[0013] In some embodiments, the heavy chain constant region is of the IgG isotype. In certain embodiments, the heavy chain constant region is a human IgG1 constant region or a human IgG4 constant region. In certain embodiments, the heavy chain constant region is a mouse IgG1 constant region, a mouse IgG2a constant region, a mouse IgG2c constant region, or a mouse IgG3 constant region. In certain embodiments, the heavy chain constant region has the amino acid sequence set forth in SEQ ID NO: 22, 24, or 26.

[0014] In some embodiments, the light chain constant region has the amino acid sequence set forth in SEQ ID NO: 23, 25, or 27.

[0015] In certain embodiments, the binding agent is a monoclonal antibody comprising a VH region having the amino acid sequence set forth in SEQ ID NO: 1, a VL region having the amino acid sequence set forth in SEQ ID NO: 2, a heavy chain constant region having the amino acid sequence set forth in SEQ ID NO: 22 and a light chain constant region having the amino acid sequence set forth in SEQ ID NO: 23.

[0016] In certain embodiments, the binding agent is a monoclonal antibody comprising a VH region having the amino acid sequence set forth in SEQ ID NO: 8, a VL region having the amino acid sequence set forth in SEQ ID NO: 9, a heavy chain constant region having the amino acid sequence set forth in SEQ ID NO: 24 and a light chain constant region having the amino acid sequence set forth in SEQ ID NO: 25.

[0017] In certain embodiments, the binding agent is a monoclonal antibody comprising a VH region having the amino acid sequence set forth in SEQ ID NO: 15, a VL region having the amino acid sequence set forth in SEQ ID NO: 16, a heavy chain constant region having the amino acid sequence set forth in SEQ ID NO: 26 and a light chain constant region having the amino acid sequence set forth in SEQ ID NO: 27.

[0018] Also provided by the current disclosure is a method of detecting a PTK7 expressing cell in a sample, comprising: contacting the sample with a detecting agent comprising the binding agent of the current disclosure, and detecting formation of a binding complex between the detecting agent and PTK7, wherein the detection of the binding complex is indicative of the presence of a PTK7 expressing cell in the sample.

[0019] In some embodiments, the detecting agent further comprises a detectable marker that labels the binding agent. In certain embodiments, the detectable marker includes biotin or an enzyme marker. In certain embodiments, the enzyme marker includes horseradish peroxidase (HRP) or alkaline phosphatase (AP).

[0020] In some embodiments, the detecting the formation of the binding complex between the detecting agent and PTK7 comprises: adding a partner agent of the detectable marker; and detecting a reaction of the detectable marker with the partner agent as an indication of the formation of the binding complex.

[0021] In some embodiments, the detectable marker includes a fluorescent tag. In certain embodiments, the detecting the formation of the binding complex between the detecting agent and PTK7 comprises: detecting a signal from the fluorescent tag as an indication of the formation of the binding complex.

[0022] In some embodiments, the detecting the formation of the binding complex between the detecting agent and PTK7 comprises: contacting the sample with a secondary antibody that is labeled and configured to bind to the binding complex; and detecting a signal from the secondary antibody as an indication of the formation of the binding complex.

[0023] In some embodiments, the secondary antibody is labeled with a detectable marker. In certain embodiments, the detectable marker includes biotin or an enzyme marker. In certain embodiments, the detecting the formation of the binding complex between the detecting agent and PTK7 comprises: adding a partner agent of the detectable marker; and detecting a reaction of the detectable marker with the partner agent as an indication of the formation of the binding complex. In certain embodiments, the detectable marker includes a fluorescent tag. In certain embodiments, the detecting the formation of the binding complex between the detecting agent and PTK7 comprises: detecting a signal from the fluorescent tag as an indication of the formation of the binding complex. In certain embodiments, the fluorescent tag includes fluorescein (FITC), phycoerythrin (PE), preferable R-PE, or allophycocyanin (APC).

[0024] In some embodiments, the sample is from a tissue of a subject. In certain embodiments, the tissue is selected from the group consisting of tonsil, appendix, breast, ovary, colon, prostate, skin, lung, uterus, cervix, kidney, pancreas, bladder, brain, thyroid, ear, nose, throat, and esophagus. In certain embodiments, the tissue is selected from the group consisting of breast, ovary, colon, prostate, skin, lung, uterus, esophagus, and bladder. In certain embodiments, the tissue is breast. In certain embodiments, the tissue is ovary.

[0025] In some embodiments, the sample is from a subject and the presence of PTK7 expressing cell indicates that the subject is suffering from a disease.

[0026] In some embodiments, the sample is from a subject and the method further comprises: quantifying binding PTK expressing cells in the sample to obtain a quantification result, wherein the quantification result over a pre-determined threshold indicates that the subject is suffering from a disease.

[0027] In some embodiments, the disease is cancer.

[0028] In certain embodiments, the disease is selected from the group consisting of breast cancer, ovarian cancer, colon cancer, prostate cancer, melanoma, lung cancer, esophageal cancer, gastric cancer, endometrial cancer, head and neck cancer, and bladder cancer. In certain embodiments, the disease is breast cancer. In certain embodiments, the disease is ovarian cancer. In certain embodiments, the disease is lung squamous cells carcinoma or lung adenocarcinoma.

[0029] In some embodiments, provided by the current disclosure is a method of determining whether a subject is suffering from a disease, comprising: obtaining a sample from the subject; contacting the sample with a detecting agent comprising the binding agent of the current disclosure, determining whether the subject is suffering from the disease by detecting and/or quantifying formation of binding complexes between the detecting agent and PTK7, wherein the presence of the binding complexes or a level of the binding complexes over a pre-determined threshold indicates that the subject is suffering from the disease. In some embodiments, the

disease is cancer.

[0030] In some embodiments, provided by the current disclosure is a method of treating a disease in patient who has been determined to have the disease by the detection methods described herein. In one embodiment, the detection method comprises obtaining a sample from a subject; contacting the sample with a detecting agent comprising the binding agent of the current disclosure; determining whether the subject is suffering from the disease by detecting and/or quantifying formation of binding complexes between the detecting agent and PTK7, wherein the presence of the binding complexes or a level of the binding complexes over a pre-determined threshold indicates that the subject is suffering from the disease. In some embodiments, the binding agent comprises VH and VL regions comprising amino acid sequences set forth in: SEQ ID NO: 1 and SEQ ID NO: 2, respectively; SEQ ID NO: 8 and SEQ ID NO:9, respectively; or SEQ ID NO: 15 and SEQ ID NO: 16, respectively.

[0031] In some embodiments, the disease is selected from the group consisting of breast cancer, ovarian cancer, colon cancer, prostate cancer, melanoma, and lung cancer.

[0032] In certain embodiments, the treatment includes administering chemotherapy to the subject.

[0033] In certain embodiments, the treatment includes administering hormone therapy to the subject.

[0034] In certain embodiments, the treatment includes administering radiation therapy to the subject.

[0035] In certain embodiments, the treatment includes administering immunotherapy to the subject.

[0036] In certain embodiments, the treatment includes administering stem cell therapy to the subject.

[0037] In certain embodiments, the treatment includes administering targeted therapy to the subject.

[0038] In certain embodiments, the treatment includes performing surgery on the subject.

[0039] In certain embodiments, the disease is breast cancer and the treatment includes lumpectomy or mastectomy.

[0040] In certain embodiments, disease is ovarian cancer and the treatment includes hysterectomy or salpingo-oophorectomy.

[0041] Also provided is an anti-cancer agent for use in a method of treating cancer in a patient, the method comprising: contacting a sample from the patient with a detecting agent comprising the binding agent of the current disclosure; determining whether the subject is suffering from the disease by detecting and/or quantifying formation of binding complexes between the detecting agent and PTK7, wherein the presence of the binding complexes or a level of the binding

complexes over a pre-determined threshold indicates that the subject is suffering from the disease; and if the subject is determined to be above said pre-determined threshold and if so treating the disease with the anti-cancer agent. Possible anti-cancer agents include any mentioned herein, for instance a chemotherapy or immunotherapy agent.

[0042] Further provided is an anti-cancer agent for use is the manufacture of a medicament for treating cancer by a method comprising : contacting a sample from the patient with a detecting agent comprising the binding agent of the current disclosure; determining whether the subject is suffering from the disease by detecting and/or quantifying formation of binding complexes between the detecting agent and PTK7, wherein the presence of the binding complexes or a level of the binding complexes over a pre-determined threshold indicates that the subject is suffering from the disease; and if the subject is determined to be above said pre-determined threshold treating the disease with the anti-cancer agent.

[0043] In some embodiments, provided by the current disclosure is a method of monitoring progression of a disease in a subject, comprising the steps of: obtaining a sample from the subject; contacting the sample with a detecting agent comprising the binding agent of the current disclosure; assessing formation of binding complexes between the detecting agent and PTK7 to obtaining a first assessment result; repeating steps (a), (b), and (c) using a sample from the subject at a subsequent point in time to obtaining a second assessment result; and comparing the second assessment result to the first assessment result to determine the progression of the disease in the subject. In certain embodiments, the disease is cancer.

[0044] In some embodiments, provided by the current disclosure is a kit for detecting PTK7 expressing cells in a sample, the kit comprising a detecting agent that comprises the binding agent of the current disclosure. In some embodiments, the kit further includes a second antibody that is labeled and configured to bind to a binding complex that is formed by the detecting agent and PTK7. In some embodiments, the kit further comprises instructions for performing a method of the present invention.

[0045] These and other aspects of the present disclosure may be more fully understood by reference to the following detailed description, non-limiting examples of specific embodiments and the appended drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

[0046] The present disclosure is further described in terms of exemplary embodiments. These exemplary embodiments are described in detail with reference to the drawings. It should be noted that the drawings are not to scale. These embodiments are non-limiting exemplary embodiments, in which like reference numerals represent similar structures throughout the several views of the drawings, and wherein:

[0047] FIG. 1A is a graph illustrating PTK7 antibodies hybridoma screening results of tumor tissue samples 26, 28, 42, 50, 61, 161, 170, 175 by IHC assay, with positive control at the upper left corner of the graph.

[0048] FIG. 1B is a graph illustrating PTK7 antibodies hybridoma screening results of tumor tissue samples 204, 206, 218, 222, 226, 227, 231, 233 by IHC assay, with positive control at the upper left corner of the graph.

[0049] FIG. 1C is a graph illustrating PTK7 antibodies hybridoma screening results of tumor tissue samples 309, 312, 316, 331, 333, 363 by IHC assay, with positive control at the upper left corner of the graph.

[0050] FIG. 1D is a graph illustrating PTK7 antibodies hybridoma screening results of tumor tissue samples 204, 206, 218, 222, 226, 227, 231, 233 by IHC assay, with positive controls at the upper left and lower right corner of the graph.

[0051] FIG. 2A is a graph illustrating PTK7 antibody subclone M6-9H5 (sample 26) IHC staining results in breast cancer.

[0052] FIG. 2B is a graph illustrating PTK7 antibody subclone M6-9H5 (sample 26) IHC staining results in ovarian cancer.

[0053] FIG. 3A is a graph illustrating PTK7 antibody subclone M6-2C6 (sample 222) IHC staining results in breast cancer.

[0054] FIG. 3B is a graph illustrating PTK7 antibody subclone M6-2C6 (sample 222) IHC staining results in ovarian cancer.

[0055] FIG. 4A is a graph illustrating PTK7 antibody subclone M6-14H10 (sample 235) IHC staining results in breast cancer.

[0056] FIG. 4B is a graph illustrating PTK7 antibody subclone M6-14H10 (sample 235) IHC staining results in ovarian cancer.

[0057] FIG. 5A is a graph illustrating PTK7 antibody subclone M10-5F7 (sample 312) IHC staining results in breast cancer.

[0058] FIG. 5B is a graph illustrating PTK7 antibody subclone M10-5F7 (sample 312) IHC staining results in ovarian cancer.

[0059] FIG. 6A is a graph illustrating PTK7 antibody subclone M1-11E5 (sample 58) IHC staining results in breast cancer.

[0060] FIG. 6B is a graph illustrating PTK7 antibody subclone M1-11E5 (sample 58) IHC staining results in ovarian cancer.

[0061] FIG. 7A is a graph illustrating PTK7 antibody subclone M6-17F7 (sample 134) IHC staining results in breast cancer.

[0062] FIG. 7B is a graph illustrating PTK7 antibody subclone M6-17F7 (sample 134) IHC staining results in ovarian cancer.

- [0063] FIG. 8A is a graph illustrating PTK7 antibody subclone M6-8G5 (sample 236) IHC staining results in breast cancer.
- [0064] FIG. 8B is a graph illustrating PTK7 antibody subclone M6-8G5 (sample 236) IHC staining results in ovarian cancer.
- [0065] FIG. 9A is a graph illustrating PTK7 antibody subclone M6-11E2 (sample 259) IHC staining results in breast cancer.
- [0066] FIG. 9B is a graph illustrating PTK7 antibody subclone M6-11E2 (sample 259) IHC staining results in ovarian cancer.
- [0067] FIG. 10 is a graph illustrating PTK7 antibody subclone M6-11E2 (sample 259) IHC staining results at different magnifications: membrane positive in both ovarian cancer and breast cancer, tumor stroma positive.
- [0068] FIG. 11 is a graph illustrating PTK7 antibody subclone M6-8G5 (sample 236) IHC staining results at different magnifications: membrane positive in both ovarian cancer and breast cancer, tumor stroma positive.
- [0069] FIG. 12 is a graph illustrating PTK7 antibody subclone M10-5F7 (sample 312) IHC staining results at different magnifications: membrane positive in ovarian cancer, membrane negative in breast cancer, tumor stroma positive.
- [0070] FIG. 13 is a graph illustrating cell staining technology (CST) IHC staining results at different magnifications: membrane positive in both ovarian cancer and breast cancer, tumor stroma positive.
- [0071] FIG. 14A is a graph illustrating PTK7 antibody subclone M6-11E2 (sample 259) IHC titration results in tonsil.
- [0072] FIG. 14B is a graph illustrating PTK7 antibody subclone M6-11E2 (sample 259) IHC titration results in ovarian cancer-1.
- [0073] FIG. 14C is a graph illustrating PTK7 antibody subclone M6-11E2 (sample 259) IHC titration results in ovarian cancer-2.
- [0074] FIG. 14D is a graph illustrating PTK7 antibody subclone M6-11E2 (sample 259) IHC titration results in breast cancer.
- [0075] FIG. 15A is a graph illustrating PTK7 antibody subclone M6-8G5 (sample 236) IHC titration results in tonsil.
- [0076] FIG. 15B is a graph illustrating PTK7 antibody subclone M6-8G5 (sample 236) IHC titration results in ovarian cancer-1.
- [0077] FIG. 15C is a graph illustrating PTK7 antibody subclone M6-8G5 (sample 236) IHC titration results in ovarian cancer-2.
- [0078] FIG. 15D is a graph illustrating PTK7 antibody subclone M6-8G5 (sample 236) IHC titration results in breast cancer.

[0079] FIG. 16A is a graph illustrating PTK7 antibody subclone M10-5F7 (sample 312) IHC titration results in tonsil.

[0080] FIG. 16B is a graph illustrating PTK7 antibody subclone M10-5F7 (sample 312) IHC titration results in ovarian cancer-1.

[0081] FIG. 16C is a graph illustrating PTK7 antibody subclone M10-5F7 (sample 312) IHC titration results in ovarian cancer-2.

[0082] FIG. 16D is a graph illustrating PTK7 antibody subclone M10-5F7 (sample 312) IHC titration results in breast cancer.

[0083] FIG. 17A is a graph illustrating PTK7 antibody subclone M6-11E2 (sample 259) IHC tissue microarray results in various types of normal and cancer tissues, by manual staining.

[0084] FIG. 17B is a graph illustrating PTK7 antibody subclone M6-11E2 (sample 259) IHC tissue microarray results in various types of normal and cancer tissues, by Leica autostainer.

[0085] FIG. 18A is a graph illustrating PTK7 antibody subclone M6-8G5 (sample 236) IHC tissue microarray results in various types of normal and cancer tissues, by manual staining.

[0086] FIG. 18B is a graph illustrating PTK7 antibody subclone M6-8G5 (sample 236) IHC tissue microarray results in various types of normal and cancer tissues, by Leica autostainer.

[0087] FIG. 19A is a graph illustrating PTK7 antibody subclone M10-5F7 (sample 312) IHC tissue microarray results in various types of normal and cancer tissues, by manual staining.

[0088] FIG. 19B is a graph illustrating PTK7 antibody subclone M10-5F7 (sample 312) IHC tissue microarray results in various types of normal and cancer tissues, by Leica autostainer.

[0089] FIG. 20 is a graph illustrating different levels of PTK7 antigen expression in cell lines PA-1, MDA-MB-453, MCF-7 and Raji (negative control), by IHC staining using subclone M6-11E2 (sample 259) as primary antibody. Going clockwise from the lower left hand panel, the results shown are for the MCF-7, PA-1, MDA-MB-453, and Raji cell lines.

[0090] FIG. 21 is a graph illustrating different levels of PTK7 antigen expression in cell lines PA-1, MDA-MB-453, MCF-7 and Raji (negative control), by IHC staining using subclone M6-8G5 (sample 236) as primary antibody. Going clockwise from the lower left hand panel, the results shown are for the MCF-7, PA-1, MDA-MB-453, and Raji cell lines.

[0091] FIG. 22 is a graph illustrating different levels of PTK7 antigen expression in cell lines PA-1, MDA-MB-453, MCF-7 and Raji (negative control), by IHC staining using subclone M10-5F7 (sample 312) as primary antibody. Going clockwise from the lower left hand panel, the results shown are for the MCF-7, PA-1, MDA-MB-453, and Raji cell lines.

[0092] Additional features will be set forth in part in the description which follows, and in part will become apparent to those skilled in the art upon examination of the following and the accompanying drawings or may be learned by production or operation of the examples. The features of the present disclosure may be realized and attained by practice or use of various

aspects of the methodologies, instrumentalities, and combinations set forth in the detailed examples discussed below.

DETAILED DESCRIPTION

[0093] The following description is presented to enable any person skilled in the art to make and use the present disclosure and is provided in the context of a particular application and its requirements. Various modifications to the disclosed embodiments will be readily apparent to those skilled in the art, and the general principles defined herein may be applied to other embodiments and applications without departing from the spirit and scope of the present disclosure. Thus, the present disclosure is not limited to the embodiments shown but is to be accorded the widest scope consistent with the claims.

[0094] The terminology used herein is to describe particular example embodiments only and is not intended to be limiting. As used herein, the singular forms “a,” “an,” and “the” may be intended to include the plural forms as well, unless the context clearly indicates otherwise. It will be further understood that the terms “comprises,” “comprising,” “includes,” and/or “including” when used in this specification, specify the presence of stated features, integers, steps, operations, elements, and/or components, but do not preclude the presence or addition of one or more other features, integers, steps, operations, elements, components, and/or groups thereof.

[0095] These and other features, and characteristics of the present disclosure, as well as the methods of operation and functions of the related elements of structure and the combination of parts and economies of manufacture, may become more apparent upon consideration of the following description with reference to the accompanying drawing(s), all of which form a part of this specification. It is to be expressly understood, however, that the drawing(s) is for the purpose of illustration and description only and are not intended to limit the scope of the present disclosure. It is understood that the drawings are not to scale

[0096] Where a method of treatment is set out herein, also provided is the product referred to for use in the method of treatment set out. Further provided is use of the product referred in the manufacture of a medicament for treating the condition referred to. Also provided is a pharmaceutical composition comprising the product for use in the method.

[0097] All references mentioned herein are incorporated by reference in their entirety. The priority application for the present application is also incorporated by reference in its entirety.

DEFINITIONS

[0098] For convenience, certain terms in the specification, examples and claims are defined here. Unless stated otherwise, or implicit from context, the following terms and phrases have the

meanings provided below. The definitions are provided to aid in describing particular embodiments, and are not intended to limit the claimed disclosure, because the scope of the disclosure is limited only by the claims. Unless otherwise defined, 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 disclosure belongs.

[0099] As used herein and unless otherwise indicated, the terms "a" and "an" are taken to mean "one", "at least one" or "one or more". Unless otherwise required by context, singular terms used herein shall include pluralities and plural terms shall include the singular.

[0100] Unless the context clearly requires otherwise, throughout the description and the claims, the words "comprise", "comprising", and the like are to be construed in an inclusive sense as opposed to an exclusive or exhaustive sense; that is to say, in the sense of "including, but not limited to". Where something is set out herein using "comprises" or "comprising" language or the like also provided is an embodiment "consisting of" what is set out.

[0101] The terms "decreased," "reduce," "reduced", "reduction", "decrease," and "inhibit" are all used herein generally to mean a decrease by a statistically significant amount relative to a reference.

[0102] The terms "increased", "increase" or "enhance" or "activate" are all used herein to generally mean an increase by a statically significant amount relative to a reference.

[0103] As used herein, the terms "protein" and "polypeptide" are used interchangeably herein to designate a series of amino acid residues each connected to each other by peptide bonds between the alpha-amino and carboxyl groups of adjacent residues. The terms "protein" and "polypeptide" also refer to a polymer of amino acids, including modified amino acids (e.g., phosphorylated, glycosylated, glycosylated, etc.) and amino acid analogs, regardless of its size or function. "Protein" and "polypeptide" are often used in reference to relatively large polypeptides, whereas the term "peptide" is often used in reference to small polypeptides, but usage of these terms in the art overlaps. The terms "protein" and "polypeptide" are used interchangeably herein when referring to an encoded gene product and fragments thereof. Thus, exemplary polypeptides or proteins include gene products, naturally occurring proteins, homologs, orthologs, paralogs, fragments and other equivalents, variants, fragments, and analogs of the foregoing.

[0104] PTK7 (protein tyrosine kinase-like 7) is a member of the receptor protein tyrosine kinase family and is also known as colon carcinoma kinase 4 (CCK4). In humans, this protein is encoded by the *PTK7* gene. PTK7 is overexpressed on the surface of many types of cancer cell, including but not limited to solid tumors. Such solid tumors include but are not limited to breast cancer (BC), lung cancer (LC), esophageal cancer (EsC), gastric cancer (GC), bladder cancer (BLC), endometrial cancer (EC), ovarian cancer (OVC), head and neck cancer (HNC) as

well as hematological malignancies. Human PTK7 polypeptides include, but are not limited to, those having the amino acid sequences set forth in UniProt identifiers Q13308, and those having the amino acid sequences set forth in Genbank such as but not limited to GenBank accession numbers NP_002812.2, NP_690619.1, NP_690620.1, NP_690621.1, NP_001257327.1, XP_011513067.1, XP_011513068.1, XP_047275113.1, XP_054212039.1, XP_054212040.1, and XP_054212041.1, which are incorporated by reference herein. Although lacking detectable catalytic tyrosine kinase activity, PTK7 overexpression is intimately involved in Wnt signaling and promotes cancer cell stemness, survival and tumor progression (See e.g., *Atasaven et al.*, 2013; *Cui et al.*, 2021; *Gärtner*, 2014; *Chen et al.*, 2014; *Jiang et al.*, 2020; *Shin et al.*, 2013; *Liu et al.*, 2017; *Lin et al.*, 2012; *Özçelik et al.*, 2020; *Xiang et al.*, 2022; *Wang et al.*, 2014; *Prebet et al.*, 2010; *Jiang et al.*, 2012; *Damelin et al.*, 2017). PTK7 expression in normal tissues is generally low, although some levels of protein expression have been reported in the digestive tract, such as urinary bladder, kidney, mammary gland, lung, ovary, uterus, and dendritic cells (*Damelin et al.*, 2017). PTK7 is thus a promising target for novel anti-cancer therapy.

[0105] As used herein, an "epitope" refers to the amino acids conventionally bound by an immunoglobulin VH/VL pair, such as the antibodies, antigen binding portions thereof and other binding agents described herein. An epitope can be formed on a polypeptide from contiguous amino acids or noncontiguous amino acids juxtaposed by tertiary folding of a protein. Epitopes formed from contiguous amino acids are typically retained on exposure to denaturing solvents, whereas epitopes formed by tertiary folding are typically lost on treatment with denaturing solvents. An epitope typically includes at least 3, and more usually, at least 5, about 9, or about 8-10 amino acids in a unique spatial conformation. An epitope defines the minimum binding site for an antibody, antigen binding portions thereof and other binding agent, and thus represents the target of specificity of an antibody, antigen binding portion thereof or other immunoglobulin-based binding agent. In the case of a single domain antibody, an epitope represents the unit of structure bound by a variable domain in isolation. As well as the specific binding agents, and in particular antibodies, set out herein, also provided are binding agents, and in particular antibodies, binding the same epitope as the specific antibodies provided. Also provided are binding agents, and in particular antibodies, that cross-block the specific binding agents, and in particular antibodies, set out herein.

[0106] As used herein, "binding agent" is a general term that refers to a molecule that binds to a target, such as human PTK7 protein, with a KD of 10^5 M (10000 nM) or less, e.g., 10^6 M, 10^{-7} M, 10^{-8} M, 10^{-9} M, 10^{-10} M, 10^{-11} M, 10^{-12} M, or less. The binding agent may be an antibody, such as but not be limited to: Fab, Fab', F(ab')₂, scFab, Fv, scFv, VH, VHH, VL, VLRs, and the like, diabodies, monoclonal antibodies (mAbs), polyclonal antibodies (pAbs), mAbdAbs, phage

display-derived binders, affibodies, heteroconjugate antibodies, bispecific antibodies, and evibodies. In one embodiment, the binding agent is an antibody. In one embodiment, the binding agent is an antibody comprising two heavy and two light chains. In one embodiment, the binding agent is a monoclonal antibody. Specific binding can be influenced by, for example, the affinity and avidity of the antibody, antigen binding portion or other binding agent and the concentration of target polypeptide. The person of ordinary skill in the art can determine appropriate conditions under which the antibodies, antigen binding portions and other binding agents described herein selectively bind to PTK7 using any suitable methods, such as titration of an antibody or other binding agent in a suitable cell binding assay. A binding agent specifically bound to PTK7 is not displaced by a non-similar competitor. In certain embodiments, a PTK7 antibody or antigen-binding portion thereof or other binding agent is said to specifically bind to PTK7 when it preferentially recognizes its target antigen, PTK7, in a complex mixture of proteins and/or macromolecules.

[0107] Reference herein to antibodies specifically encompasses antibody-fragments that retain the ability to PTK-7. Hence, wherever an antibody is reference to herein an antigen-binding fragment thereof is also provided. Examples of antibody fragments include Fab, Fab', F(ab')₂, scFab, Fv, scFv, VH, VHH, VL, and VLR antibodies.

[0108] Variant binding agents are disclosed herein, for instance binding agents with a defined number of sequence changes. The variant antibody will be still able to bind PTK7 protein. Hence, in one embodiment, the variant antibody will be still able to bind PTK7 protein. In one embodiment, the variant antibody will still give the same, or similar, results in IHC. For example, a variant antibody will still be useful in performing one of the IHC methods described herein. In one embodiment, a variant will retain at least one other of the specific functions mentioned herein in addition to being able to bind PTK7.

[0109] As used herein, "a detectable marker" refers to a molecule that is attached to or used to label a binding agent or a secondary antibody and can be used to create a detectable signal, which can be detected (e.g., visualized) by color-generation or fluorescence or evaluated by other means. Such detectable marker may include but are not limited to enzyme substrates (e.g., biotin), enzymes (e.g., horseradish peroxidase (HRP), alkaline phosphatase, glucose oxidase, and β -galactosidase, etc.), and fluorescent tags (e.g., fluorescent dye such as but not limited to fluorescein (FITC), phycoerythrin (PE; e.g., R-PE), or allophycocyanin (APC)). A detectable signal can be generated directly by the detectable marker such as fluorescent protein; a detectable signal can also be a chromogenic product of a chemical reaction between the detectable marker and its partner agent.

[0110] As used herein, "a detecting agent" refers to a molecule that includes a binding agent to PTK7. In certain embodiments, the detecting agent is such a binding agent. In certain

embodiments, the detecting agent further includes one or more auxiliary moieties (e.g., detectable makers) that are attached to (e.g., covalently bound to) the binding agent.

[0111] As used herein, “a partner agent” refers to a molecule that can bind to a detectable marker, wherein the partner agent and the detectable marker react to produce a detectable signal. Examples of partner agents include but are not limited to avidin, streptavidin, NeutrAvidin, enzyme substrates including but not limited to 3,3',5,5'-Tetramethylbenzidine (TMB), 3,3'-diaminobenzidine (DAB), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS), 3-amino-9-ethylcarbazole (AEC), o-phenylenediamine dihydrochloride (OPD), homovanillic acid, AmplexRed, Aminoethyl carbazole (AEC), nitro blue tetrazolium chloride (NBT), 5-bromo-4-chloro-3-indolyl phosphate (BCIP), and 5-bromo-4-chloro-3-indolyl- β -D-galactopyranoside (BCIG or X-Gal).

[0112] The binding agents and antibodies described herein are particularly useful in IHC. They may though used for any type of antibody assay or other purpose. In one embodiment, a binding agent of the invention may be used in flow cytometry. In another embodiment, a binding agent of the present invention may be used in an ELISA.

ANTIBODIES AND BINDING AGENTS

[0113] Provided herein are PTK7 binding antibodies (also referred to as PTK7 antibodies) and antigen binding portions thereof and other binding agents that specifically bind to human PTK7. In some embodiments, the PTK7 antibodies, antigen binding portions, and/or other binding agents specifically bind to PTK7+ cells in a subject and can be used to identify/detect/quantify such PTK7+ cells. In some embodiments, the PTK7 antibodies, antigen binding portions, and/or other binding agents specifically bind to PTK7+ cancer cells in a subject and can be used to identify/detect/quantify such PTK+ cancer cells. In some embodiments, the PTK7 antibodies, antigen binding portions, and/or other binding agents specifically bind to PTK7+ cells associated with a disease or condition in a subject, such as a cancer or an autoimmune disease. In some embodiments, the PTK7 antibodies, antigen binding portions, and/or other binding agents can be used to identify/detect/diagnose such disease or condition in a subject, such as a human or an animal.

[0114] In some embodiments, the PTK7 antibodies or antigen binding portions thereof comprise a heavy chain variable (VH) region and a light chain variable (VL) region, the VH and VL regions having the amino acid sequences set forth in the pairs of amino acid sequences selected from SEQ ID NO:1 and SEQ ID NO:2, respectively; SEQ ID NO:8 and SEQ ID NO:9, respectively; and SEQ ID NO:15 and SEQ ID NO:16, respectively. In some embodiments, the PTK7 antibodies or antigen binding portions thereof comprise a heavy chain variable (VH) region and a light chain variable (VL) region, the VH and VL regions having the amino acid

sequences set forth in SEQ ID NO:1 and SEQ ID NO:2, respectively. In some embodiments, the PTK7 antibodies or antigen binding portions thereof comprise a heavy chain variable (VH) region and a light chain variable (VL) region, the VH and VL regions having the amino acid sequences set forth in SEQ ID NO:8 and SEQ ID NO:9, respectively. In some embodiments, the PTK7 antibodies or antigen binding portions thereof comprise a heavy chain variable (VH) region and a light chain variable (VL) region, the VH and VL regions having the amino acid sequences set forth in SEQ ID NO:15 and SEQ ID NO:16, respectively.

[0115] In some embodiments, the PTK7 antibodies or antigen binding portions thereof comprise a heavy chain variable (VH) region and a light chain variable (VL) region, the VH and VL regions having amino acid sequences set forth in the pairs of amino acid sequences selected from SEQ ID NO:1 and SEQ ID NO:2, respectively; SEQ ID NO:8 and SEQ ID NO:9, respectively; and SEQ ID NO:15 and SEQ ID NO:16, respectively; wherein the heavy and light chain variable framework regions are optionally modified with from 1 to 8, 1 to 6, 1 to 4 or 1 to 2 conservative amino acid substitutions in the framework regions, wherein the CDRs of the heavy or light chain variable regions are not modified. In some embodiments, the PTK7 antibodies or antigen binding portions thereof comprise a heavy chain variable (VH) region and a light chain variable (VL) region, the VH and VL regions having the amino acid sequences set forth in the pairs of amino acid sequences selected from SEQ ID NO:1 and SEQ ID NO:2, respectively; SEQ ID NO:8 and SEQ ID NO:9, respectively; and SEQ ID NO:15 and SEQ ID NO:16, respectively; wherein the heavy and light chain variable framework regions are optionally modified with from 1 to 8, 1 to 6, 1 to 4 or 1 to 2 amino acid substitutions, deletions or insertions in the framework regions, wherein the CDRs of the heavy or light chain variable regions are not modified. The phrase "wherein the CDRs of the heavy or light chain variable regions are not modified" refers to the VH and VL CDRs that do not have amino acid substitutions, deletions or insertions.

[0116] In some embodiments, the PTK7 antibodies or antigen binding portions thereof comprise a heavy chain variable (VH) region which has a similarity of at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99% with the amino acid sequence set forth in SEQ ID NO:1, SEQ ID NO:8, or SEQ ID NO:15. In some embodiments, the PTK7 antibodies or antigen binding portions thereof comprise a light chain variable (VL) region which has a similarity of at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99% with the amino acid sequence set forth in SEQ ID NO:2, SEQ ID NO:9, or SEQ ID NO:16. In some embodiments, the PTK7 antibodies or antigen binding portions thereof comprise a heavy chain variable (VH) region and a light chain variable (VL) region, the VH and VL regions having amino acid sequences, which have a similarity of at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99% with the pairs of amino acid sequences selected from

SEQ ID NO:1 and SEQ ID NO:2, respectively; SEQ ID NO:8 and SEQ ID NO:9, respectively; and SEQ ID NO:15 and SEQ ID NO:16, respectively. The levels of similarities result from amino acid substitutions, deletions or insertions to the VH and VL sequences set forth in SEQ ID NO:1 and SEQ ID NO:2, respectively; SEQ ID NO:8 and SEQ ID NO:9, respectively; and SEQ ID NO:15 and SEQ ID NO:16, respectively. The variant antibody will be still able to bind PTK7.

[0117] In some embodiments, the PTK7 antibodies or antigen binding portions thereof comprise a heavy chain variable (VH) region and a light chain variable (VL) region, the VH and VL regions having the amino acid sequences set forth in SEQ ID NO:1 and SEQ ID NO:2, respectively; wherein the heavy and light chain variable framework regions are optionally modified with from 1 to 8, 1 to 6, 1 to 4 or 1 to 2 conservative amino acid substitutions in the framework regions, wherein the CDRs of the heavy or light chain variable regions are not modified. In some embodiments, the PTK7 antibodies or antigen binding portions thereof comprise a heavy chain variable (VH) region and a light chain variable (VL) region, the VH and VL regions having the amino acid sequences set forth in SEQ ID NO:1 and SEQ ID NO:2, respectively; wherein the heavy and light chain variable framework regions are optionally modified with from 1 to 8, 1 to 6, 1 to 4 or 1 to 2 amino acid substitutions, deletions or insertions in the framework regions, wherein the CDRs of the heavy or light chain variable regions are not modified. The variant antibody or antigen binding portion there will be still able to bind PTK7.

[0118] In some embodiments, the PTK7 antibodies or antigen binding portions thereof comprise a heavy chain variable (VH) region sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99% with the sequence set forth in SEQ ID NO:1. In some embodiments, the PTK7 antibodies or antigen binding portions thereof comprise a light chain variable (VL) region that is at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99% with the sequence set forth in SEQ ID NO:2. In some embodiments, the PTK7 antibodies or antigen binding portions thereof comprise a heavy chain variable (VH) region and a light chain variable (VL) region, the VH and VL regions having the amino acid sequences that are at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99% with the sequences set forth in SEQ ID NO:1 and SEQ ID NO:2, respectively. The variant antibody or antigen binding portion there will be still able to bind PTK7.

[0119] In some embodiments, the PTK7 antibodies or antigen binding portions thereof comprise a heavy chain variable (VH) region and a light chain variable (VL) region, the VH and VL regions having the amino acid sequences set forth in SEQ ID NO:8 and SEQ ID NO:9, respectively; wherein the heavy and light chain variable framework regions are optionally modified with from 1 to 8, 1 to 6, 1 to 4 or 1 to 2 conservative amino acid substitutions in the framework regions, wherein the CDRs of the heavy or light chain variable regions are not modified. In some embodiments, the PTK7 antibodies or antigen binding portions thereof

comprise a heavy chain variable region (VH) and a light chain variable region (VL), the VH and VL regions having the amino acid sequences set forth in SEQ ID NO:8 and SEQ ID NO:9, respectively; wherein the heavy and light chain variable framework regions are optionally modified with from 1 to 8, 1 to 6, 1 to 4 or 1 to 2 amino acid substitutions, deletions or insertions in the framework regions, wherein the CDRs of the heavy or light chain variable regions are not modified. The variant antibody or antigen binding portion there will be still able to bind PTK7.

[0120] In some embodiments, the PTK7 antibodies or antigen binding portions thereof comprise a heavy chain variable (VH) region sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99% with the sequence set forth in SEQ ID NO:8. In some embodiments, the PTK7 antibodies or antigen binding portions thereof comprise a light chain variable (VL) region that is at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99% with the sequence set forth in SEQ ID NO:9. In some embodiments, the PTK7 antibodies or antigen binding portions thereof comprise a heavy chain variable (VH) region and a light chain variable (VL) region, the VH and VL regions having the amino acid sequences that are at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99% with the sequences set forth in SEQ ID NO:8 and SEQ ID NO:9, respectively. The variant antibody or antigen binding portion there will be still able to bind PTK7.

[0121] In some embodiments, the PTK7 antibodies or antigen binding portions thereof comprise a heavy chain variable (VH) region and a light chain variable (VL) region, the VH and VL regions having the amino acid sequences set forth in SEQ ID NO:15 and SEQ ID NO:16, respectively; wherein the heavy and light chain variable framework regions are optionally modified with from 1 to 8, 1 to 6, 1 to 4 or 1 to 2 conservative amino acid substitutions in the framework regions, wherein the CDRs of the heavy or light chain variable regions are not modified. In some embodiments, the PTK7 antibodies or antigen binding portions thereof comprise a heavy chain variable (VH) region and a light chain variable (VL) region, the VH and VL regions having the amino acid sequences set forth in SEQ ID NO:15 and SEQ ID NO:16, respectively; wherein the heavy and light chain variable framework regions are optionally modified with from 1 to 8, 1 to 6, 1 to 4 or 1 to 2 amino acid substitutions, deletions or insertions in the framework regions, wherein the CDRs of the heavy or light chain variable regions are not modified. The variant antibody or antigen binding portion there will be still able to bind PTK7.

[0122] In some embodiments, the PTK7 antibodies or antigen binding portions thereof comprise a heavy chain variable (VH) region sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99% with the sequence set forth in SEQ ID NO:15. In some embodiments, the PTK7 antibodies or antigen binding portions thereof comprise a light chain variable (VL) region that is at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99% with the sequence set forth in SEQ ID NO:16. In some

embodiments, the PTK7 antibodies or antigen binding portions thereof comprise a heavy chain variable (VH) region and a light chain variable (VL) region, the VH and VL regions having the amino acid sequences that are at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99% with the sequences set forth in SEQ ID NO:15 and SEQ ID NO:16, respectively. The variant antibody or antigen binding portion there will be still able to bind PTK7.

[0123] In some embodiments, provided herein is a binding agent comprising a heavy chain variable (VH) region and a light chain variable (VL) region, the VH and VL regions having amino acid sequences set forth in the pairs of amino acid sequences selected from SEQ ID NO:1 and SEQ ID NO:2, respectively; SEQ ID NO:8 and SEQ ID NO:9, respectively; and SEQ ID NO:15 and SEQ ID NO:16, respectively; wherein the binding agent specifically binds to human PTK7. In some embodiments, the binding agent of the current disclosure specifically binds to human PTK7 with a higher or a similar binding affinity (lower K_d) than that of antibody cofetuzumab. In some embodiments, provided herein is a binding agent comprising a heavy chain variable region (VH) and a light chain variable (VL) region, the VH and VL regions having the amino acid sequences set forth in the pairs of amino acid sequences selected from SEQ ID NO:1 and SEQ ID NO:2, respectively; SEQ ID NO:8 and SEQ ID NO:9, respectively; and SEQ ID NO:15 and SEQ ID NO:16, respectively; wherein the heavy and light chain variable framework regions are optionally modified with from 1 to 8, 1 to 6, 1 to 4 or 1 to 2 conservative amino acid substitutions in the framework regions and wherein the CDRs of the heavy or light chain variable regions are not modified. In some embodiments, provided herein is a binding agent comprising a heavy chain variable (VH) region and a light chain variable (VL) region, the VH and VL regions having amino acid sequences set forth in the pairs of amino acid sequences selected from SEQ ID NO:1 and SEQ ID NO:2, respectively; SEQ ID NO:8 and SEQ ID NO:9, respectively; and SEQ ID NO:15 and SEQ ID NO:16, respectively; wherein the heavy and light chain variable framework regions are optionally modified with from 1 to 8, 1 to 6, 1 to 4 or 1 to 2 amino acid substitutions, deletions or insertions in the framework regions and wherein the CDRs of the heavy or light chain variable regions are not modified. As described herein, a binding agent includes a PTK7 antibody or antigen binding portion(s) thereof and can optionally include other peptides or polypeptides covalently attached to the PTK7 antibody or antigen binding portion thereof. In any of these embodiments, the binding agent specifically binds to human PTK7.

[0124] In some embodiments, provided herein is a binding agent comprising a heavy chain variable (VH) region and a light chain variable (VL) region, the VH and VL regions having the amino acid sequences set forth in SEQ ID NO: 1 and SEQ ID NO: 2, respectively; wherein the binding agent specifically binds to human PTK7. In some embodiments, provided herein is a binding agent comprising a heavy chain variable (VH) region and a light chain variable (VL) region, the VH and VL regions having the amino acid sequences set forth in SEQ ID NO: 1 and

SEQ ID NO: 2, respectively; wherein the heavy and light chain variable framework regions are optionally modified with from 1 to 8, 1 to 6, 1 to 4 or 1 to 2 conservative amino acid substitutions in the framework regions and wherein the CDRs of the heavy or light chain variable regions are not modified. In some embodiments, provided herein is a binding agent comprising a heavy chain variable (VH) region and a light chain variable (VL) region, the VH and VL regions having the amino acid sequences set forth in SEQ ID NO:1 and SEQ ID NO:2, respectively; wherein the heavy and light chain variable framework regions are optionally modified with from 1 to 8, 1 to 6, 1 to 4 or 1 to 2 amino acid substitutions, deletions or insertions in the framework regions and wherein the CDRs of the heavy or light chain variable regions are not modified.

[0125] In some embodiments, provided herein is a binding agent comprising a heavy chain variable (VH) region and a light chain variable (VL) region, the VH and VL regions having the amino acid sequences set forth in SEQ ID NO: 8 and SEQ ID NO: 9, respectively; wherein the binding agent specifically binds to human PTK7. In some embodiments, provided herein is a binding agent comprising a heavy chain variable (VH) region and a light chain variable (VL) region, the VH and VL regions having the amino acid sequences set forth in SEQ ID NO: 8 and SEQ ID NO:9, respectively; wherein the heavy and light chain variable framework regions are optionally modified with from 1 to 8, 1 to 6, 1 to 4 or 1 to 2 conservative amino acid substitutions in the framework regions and wherein the CDRs of the heavy or light chain variable regions are not modified. In some embodiments, provided herein is a binding agent comprising a heavy chain variable (VH) region and a light chain variable (VL) region, the VH and VL regions having the amino acid sequences set forth in SEQ ID NO:8 and SEQ ID NO: 9, respectively; wherein the heavy and light chain variable framework regions are optionally modified with from 1 to 8, 1 to 6, 1 to 4 or 1 to 2 amino acid substitutions, deletions or insertions in the framework regions and wherein the CDRs of the heavy or light chain variable regions are not modified. The variant antibody or antigen binding portion there will be still able to bind PTK7.

[0126] In some embodiments, provided herein is a binding agent comprising a heavy chain variable (VH) region and a light chain variable (VL) region, the VH and VL regions having the amino acid sequences set forth in SEQ ID NO: 15 and SEQ ID NO: 16, respectively; wherein the binding agent specifically binds to human PTK7. In some embodiments, provided herein is a binding agent comprising a heavy chain variable (VH) region and a light chain variable (VL) region, the VH and VL regions having the amino acid sequences set forth in SEQ ID NO: 15 and SEQ ID NO: 16, respectively; wherein the heavy and light chain variable framework regions are optionally modified with from 1 to 8, 1 to 6, 1 to 4 or 1 to 2 conservative amino acid substitutions in the framework regions and wherein the CDRs of the heavy or light chain variable regions are not modified. In some embodiments, provided herein is a binding agent comprising a heavy chain variable (VH) region and a light chain variable (VL) region, the VH and VL regions having

the amino acid sequences set forth in SEQ ID NO: 15 and SEQ ID NO: 16, respectively; wherein the heavy and light chain variable framework regions are optionally modified with from 1 to 8, 1 to 6, 1 to 4 or 1 to 2 amino acid substitutions, deletions or insertions in the framework regions and wherein the CDRs of the heavy or light chain variable regions are not modified. The variant antibody or antigen binding portion there will be still able to bind PTK7.

[0127] In some embodiments, provided is an antibody or antigen binding portion comprising a heavy chain variable (VH) region and a light chain variable (VL) region, the VH region comprising complementarity determining regions HCDR1, HCDR2 and HCDR3 disposed in heavy chain variable region framework regions and the VL region comprising LCDR1, LCDR2 and LCDR3 disposed in light chain variable region framework regions, the VH and VL CDRs having the amino acids sequences set forth in the sets of amino acid sequences selected from (i) SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, LVS, and SEQ ID NO: 7, respectively; (ii) SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, QMS, and SEQ ID NO: 14, respectively; and (iii) SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, RVS, and SEQ ID NO: 21, respectively. In certain embodiments, the CDR sequences set forth herein are according to the IMGT unique numbering scheme *Lefranc, M. P. The Immunologist*, 7, 132-136 (1999). In some embodiments, each VH and VL region comprises a humanized framework region. In some embodiments, each VH and VL region comprises a human framework region. In some embodiments, each VH and VL region comprises a mouse framework region.

[0128] In some embodiments, provided is an antibody or antigen binding portion comprising a heavy chain variable (VH) region and a light chain variable (VL) region, the VH region comprising complementarity determining regions HCDR1, HCDR2 and HCDR3 disposed in heavy chain variable region framework regions and the VL region comprising LCDR1, LCDR2 and LCDR3 disposed in light chain variable region framework regions, the VH and VL CDRs having the amino acids sequences set forth in SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, LVS, and SEQ ID NO: 7, respectively. In certain embodiments, the CDR sequences set forth herein are according to the IMGT unique numbering scheme. *Lefranc, M. P. The Immunologist*, 7, 132-136 (1999). In some embodiments, each VH and VL region comprises a humanized framework region. In some embodiments, each VH and VL region comprises a human framework region. In some embodiments, each VH and VL region comprises a mouse framework region.

[0129] In some embodiments, provided is an antibody or antigen binding portion comprising a heavy chain variable (VH) region and a light chain variable (VL) region, the VH region comprising complementarity determining regions HCDR1, HCDR2 and HCDR3 disposed in heavy chain variable region framework regions and the VL region comprising LCDR1, LCDR2

and LCDR3 disposed in light chain variable region framework regions, the VH and VL CDRs having the amino acids sequences set forth in SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, QMS, and SEQ ID NO: 14, respectively. In certain embodiments, the CDR sequences set forth herein are according to the IMGT unique numbering scheme *Lefranc, M. P. The Immunologist*, 7, 132-136 (1999). In some embodiments, each VH and VL region comprises a humanized framework region. In some embodiments, each VH and VL region comprises a human framework region. In some embodiments, each VH and VL region comprises a mouse framework region. In some embodiments, each VH and VL region comprises a mouse framework region.

[0130] In some embodiments, provided is an antibody or antigen binding portion comprising a heavy chain variable (VH) region and a light chain variable (VL) region, the VH region comprising complementarity determining regions HCDR1, HCDR2 and HCDR3 disposed in heavy chain variable region framework regions and the VL region comprising LCDR1, LCDR2 and LCDR3 disposed in light chain variable region framework regions, the VH and VL CDRs having the amino acids sequences set forth in SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, RVS, and SEQ ID NO: 21, respectively. In certain embodiments, the CDR sequences set forth herein are according to the IMGT unique numbering scheme. *Lefranc, M. P. The Immunologist*, 7, 132-136 (1999). In some embodiments, each VH and VL region comprises a humanized framework region. In some embodiments, each VH and VL region comprises a human framework region. In some embodiments, each VH and VL region comprises a mouse framework region.

[0131] In some embodiments, provided is a binding agent comprising a heavy chain variable (VH) region and a light chain variable (VL) region, the VH region comprising complementarity determining regions HCDR1, HCDR2 and HCDR3 disposed in heavy chain variable region framework regions and the VL region comprising LCDR1, LCDR2 and LCDR3 disposed in light chain variable region framework regions, the VH and VL CDRs having the amino acids sequences set forth in SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, LVS, and SEQ ID NO: 7, respectively. In certain embodiments, the CDR sequences set forth herein are according to the IMGT unique numbering scheme. *Lefranc, M. P. The Immunologist*, 7, 132-136 (1999). In some embodiments, each VH and VL region comprises a humanized framework region. In some embodiments, each VH and VL region comprises a human framework region. In some embodiments, each VH and VL region comprises a mouse framework region.

[0132] In some embodiments, provided is a binding agent comprising a heavy chain variable (VH) region and a light chain variable (VL) region, the VH region comprising complementarity determining regions HCDR1, HCDR2 and HCDR3 disposed in heavy chain variable region framework regions and the VL region comprising LCDR1, LCDR2 and LCDR3 disposed in light

chain variable region framework regions, the VH and VL CDRs having the amino acids sequences set forth in SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, QMS, and SEQ ID NO: 14, respectively. In certain embodiments, the CDR sequences set forth herein are according to the IMGT unique numbering scheme. *Lefranc, M. P.* The Immunologist, 7, 132-136 (1999). In some embodiments, each VH and VL region comprises a humanized framework region. In some embodiments, each VH and VL region comprises a human framework region. In some embodiments, each VH and VL region comprises a mouse framework region.

[0133] In some embodiments, provided is a binding agent comprising a heavy chain variable (VH) region and a light chain variable (VL) region, the VH region comprising complementarity determining regions HCDR1, HCDR2 and HCDR3 disposed in heavy chain variable region framework regions and the VL region comprising LCDR1, LCDR2 and LCDR3 disposed in light chain variable region framework regions, the VH and VL CDRs having the amino acids sequences set forth in SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, RVS, and SEQ ID NO: 21, respectively. In certain embodiments, the CDR sequences set forth herein are according to the IMGT unique numbering scheme *Lefranc, M. P.* The Immunologist, 7, 132-136 (1999). In some embodiments, each VH and VL region comprises a humanized framework region. In some embodiments, each VH and VL region comprises a human framework region. In some embodiments, each VH and VL region comprises a mouse framework region.

[0134] In some embodiments, the molecules, compositions, and methods described herein relate to identification/detection/quantification of PTK7+ cells in a subject (e.g., identification/detection/quantification of PTK7+ cells in a cancer or tumor, or PTK7+ cells associated with an autoimmune disease or disorder) by a PTK7 antibody, antigen binding portion thereof, and/or other binding agent *in vitro* or *in vivo*. In some embodiments, the compositions and methods described herein relate to the treatment of PTK7+ cancer in a subject by administering a PTK7 antibody, antigen binding portion thereof, other binding agent or conjugate thereof. Thus, the present invention also provides a pharmaceutical composition comprising a binding agent set out herein and a pharmaceutically acceptable carrier or excipient.

[0135] As used herein, the term "antibody" refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, i.e., molecules that contain an antigen binding site(s) that specifically binds to an antigen, e.g., human PTK7. The term generally refers to antibodies comprised of two immunoglobulin heavy chain variable regions and two immunoglobulin light chain variable regions including full length antibodies (having heavy and light chain constant regions).

[0136] Each heavy chain is composed of a variable region (abbreviated as VH) and a constant region. The heavy chain constant region may include three domains CH1, CH2 and CH3 and optionally a fourth domain, CH4. Each light chain is composed of a variable region (abbreviated as VL) and a constant region. The light chain constant region is a CL domain. The VH and VL regions may be further divided into hypervariable regions referred to as complementarity-determining regions (CDRs) and interspersed with conserved regions referred to as framework regions (FR). Each VH and VL region thus consists of three CDRs and four FRs that are arranged from the N terminus to the C terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, and FR4. This structure is well known to those skilled in the art.

[0137] As used herein, an "antigen-binding portion" of a PTK7 antibody refers to the portions of a PTK7 antibody as described herein having the VH and VL sequences of the PTK7 antibody or the CDRs of a PTK7 antibody and that specifically binds to PTK7. Examples of antigen binding portions include a Fab, a Fab', a F(ab')₂, a Fv, a scFv, a disulfide linked Fv, a single domain antibody (also referred to as a VHH, VNAR, sdAb, or nanobody) or a diabody (see, e.g., Huston et al., Proc. Natl. Acad. Sci. U.S.A., 85, 5879-5883 (1988) and Bird et al., Science 242, 423-426 (1988), which are incorporated herein by reference). As used herein, the terms Fab, F(ab')₂ and Fv refer to the following: (i) a Fab fragment, i.e. a monovalent fragment composed of the VL, VH, CL and CH1 domains; (ii) an F(ab')₂ fragment, i.e. a bivalent fragment comprising two Fab fragments linked to one another in the hinge region via a disulfide bridge; and (iii) an Fv fragment composed of the VL and VH domains, in each case of a PTK7 antibody. Although the two domains of the Fv fragment, namely VL and VH, are encoded by separate coding regions, they may further be linked to one another using a synthetic linker, e.g., a poly-G4S amino acid sequence (G4S)_n disclosed as SEQ ID NO: 58, wherein n = 1 to 5), making it possible to prepare them as a single protein chain in which the VL and VH regions combine in order to form monovalent molecules (known as single chain Fv or scFv). The term "antigen-binding portion" of an antibody is also intended to include such single chain antibodies. Other forms of single chain antibodies such as "diabodies" are likewise included here. Diabodies are bivalent, bispecific antibodies in which VH and VL domains are expressed on a single polypeptide chain, but using a linker connecting the VH and VL domains that is too short for the two domains to be able to combine on the same chain, thereby forcing the VH and VL domains to pair with complementary domains of a different chain (VL and VH, respectively), and to form two antigen-binding sites (see, for example, Holliger, R, et al. (1993) Proc. Natl. Acad. Sci. USA 90:6444-6448; Poljak, R. J, et al. (1994) Structure 2:1121-1123).

[0138] A single-domain antibody is an antibody portion consisting of a single monomeric variable antibody domain. Single domain antibodies can be derived from the variable domain of the antibody heavy chain from camelids (e.g., nanobodies or VHH portions). Furthermore, the

term single-domain antibody includes an autonomous human heavy chain variable domain (aVH) or VNAR portions derived from sharks (see, e.g., Hasler et al., Mol. Immunol. 75:28-37, 2016).

[0139] Techniques for producing single domain antibodies (e.g., DABs or VHH) are known in the art, as disclosed for example in Cossins et al. (2006, Prot Express Purif 51:253-259) and Li et al. (Immunol. Lett. 188:89-95, 2017). Single domain antibodies may be obtained, for example, from camels, alpacas or llamas by standard immunization techniques. (See, e.g., Muyldermans et al., TIBS 26:230-235, 2001; Yau et al., J Immunol Methods 281:161-75, 2003; and Maass et al., J Immunol Methods 324:13-25, 2007.) A VHH may have potent antigen-binding capacity and can interact with novel epitopes that are inaccessible to conventional VH-VL pairs (see, e.g., Muyldermans et al., 2001). Alpaca serum IgG contains about 50% camelid heavy chain only IgG antibodies (HCABs) (see, e.g., Maass et al., 2007). Alpacas may be immunized with antigens and VHHs can be isolated that bind to and neutralize a target antigen (see, e.g., Maass et al., 2007). PCR primers that amplify alpaca VHH coding sequences have been identified and may be used to construct alpaca VHH phage display libraries, which can be used for antibody fragment isolation by standard biopanning techniques well known in the art (see, e.g., Maass et al., 2007).

[0140] In some embodiments, the PTK7 antibodies or antigen binding portions thereof are part of a bispecific or multispecific binding agent. Bispecific and multi-specific antibodies include the following: an scFv1-ScFv2, an ScFv12-Fc-scFv22, an IgG-scFv, a DVD-Ig, a triomab/quadroma, a two-in-one IgG, a scFv2-Fc, a TandAb, and an scFv-HSA-scFv. In some embodiments, an IgG-scFv is an IgG(H)-scFv, scFv-(H)IgG, IgG(L)-scFv, svFc-(L)IgG, 2scFv-IgG or IgG-2scFv. See, e.g., Brinkmann and Kontermann, MAbs 9(2):182-212 (2017); Wang et al., Antibodies, 2019, 8, 43; Dong et al., 2011, MAbs 3:273-88; Natsume et al., J. Biochem. 140(3):359-368, 2006; Cheal et al., Mol. Cancer Ther. 13(7):1803-1812, 2014; and Bates and Power, Antibodies, 2019, 8, 28. Hence, the present invention further provides a bispecific antibody comprising at least one pair of VH and VL regions as set out herein. The present invention also provides a bispecific antibody comprising at least one antigen-binding site comprising a set of six CDRs as set out herein, i.e comprising a set of HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 sequences as set out herein.

Modification of VH and VL Regions

[0141] As to the VH and VL amino acid sequences, one of skill will recognize that individual substitutions, deletions or additions (insertions) to a nucleic acid encoding the VH or VL, or amino acids in a polypeptide that alter a single amino acid or a small percentage of amino acids in the encoded sequence is a "conservatively modified variant", where the alteration results in

the substitution of an amino acid with a chemically similar amino acid (a conservative amino acid substitution) and the altered polypeptide retains the ability to specifically bind to PTK7.

[0142] In some embodiments, a conservatively modified variant of a PTK7 antibody or antigen binding portion thereof can have an alteration(s) in the framework regions (i.e., other than in the CDRs), e.g. a conservatively modified variant of a PTK7 antibody has the amino acid sequences of the VH and VL CDRs (set forth in sets of amino acid sequences (i) SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, LVS, and SEQ ID NO: 7, respectively; (ii) SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, QMS, and SEQ ID NO: 14, respectively; and (iii) SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, RVS, and SEQ ID NO: 21, respectively and has at least one conservative amino acid substitution in a framework region (FR). In some embodiments, the VH and VL amino acid sequences collectively have no more than 8 or 6 or 4 or 2 or 1 conservative amino acid substitutions in the FR, as compared to the amino acid sequences of the unmodified VH and VL regions. In some embodiments, the VH and VL amino acid sequences have 8 to 1, 6 to 1, 4 to 1 or 2 to 1 conservative amino acid substitutions in the FR, as compared to the amino acid sequences of the unmodified VH and VL regions. In further aspects of any of these embodiments, a conservatively modified variant of the PTK7 antibody, antigen binding portion thereof or other binding agent exhibits specific binding to PTK7. A variant antibody or antigen-binding portion thereof will retain the ability to bind PTK7.

[0143] For conservative amino acid substitutions, a given amino acid can be replaced by a residue having similar physiochemical characteristics, e.g., substituting one aliphatic residue for another (such as Ile, Val, Leu, or Ala for one another), or substitution of one polar residue for another (such as between Lys and Arg; Glu and Asp; or Gln and Asn). Other such conservative amino acid substitutions, e.g., substitutions of entire regions having similar hydrophobicity characteristics, are well known. Polypeptides comprising conservative amino acid substitutions can be tested in any one of the assays described herein to confirm that a desired activity, e.g. antigen-binding activity and specificity of a native or reference polypeptide is retained, i.e., to PTK7.

[0144] In some embodiments, provided herein is a PTK7 antibody or antigen binding portion thereof or other binding agent comprising a heavy chain variable (VH) region and a light chain variable (VL) region, the VH and VL regions having amino acid sequences set forth in SEQ ID NO:1 and SEQ ID NO:2, respectively; wherein the heavy and light chain variable framework regions are optionally modified with from 1 to 8, 1 to 6, 1 to 4 or 1 to 2 conservative amino acid substitutions in the framework regions and wherein the CDRs of the heavy or light chain variable regions are not modified. In some embodiments, provided herein is a PTK7 antibody or antigen binding portion thereof or other binding agent comprising a heavy chain variable (VH)

region and a light chain variable (VL) region, the VH and VL regions having amino acid sequences set forth in SEQ ID NO:1 and SEQ ID NO:2, respectively; wherein the heavy and light chain variable framework regions are optionally modified with from 1 to 8, 1 to 6, 1 to 4 or 1 to 2 amino acid substitutions, deletions or insertions in the framework regions and wherein the CDRs of the heavy or light chain variable regions are not modified. A variant antibody or antigen-binding portion thereof will retain the ability to bind PTK7.

[0145] In some embodiments, provided herein is a PTK7 antibody or antigen binding portion thereof or other binding agent comprising a heavy chain variable (VH) region and a light chain variable (VL) region, the VH and VL regions having amino acid sequences set forth in SEQ ID NO:8 and SEQ ID NO:9, respectively; wherein the heavy and light chain variable framework regions are optionally modified with from 1 to 8, 1 to 6, 1 to 4 or 1 to 2 conservative amino acid substitutions in the framework regions and wherein the CDRs of the heavy or light chain variable regions are not modified. In some embodiments, provided herein is a PTK7 antibody or antigen binding portion thereof or other binding agent comprising a heavy chain variable (VH) region and a light chain variable (VL) region, the VH and VL regions having amino acid sequences set forth in SEQ ID NO:8 and SEQ ID NO:9, respectively; wherein the heavy and light chain variable framework regions are optionally modified with from 1 to 8, 1 to 6, 1 to 4 or 1 to 2 amino acid substitutions, deletions or insertions in the framework regions and wherein the CDRs of the heavy or light chain variable regions are not modified. A variant antibody or antigen-binding portion thereof will retain the ability to bind PTK7.

[0146] In some embodiments, provided herein is a PTK7 antibody or antigen binding portion thereof or other binding agent comprising a heavy chain variable (VH) region and a light chain variable (VL) region, the VH and VL regions having amino acid sequences set forth in SEQ ID NO:15 and SEQ ID NO:16, respectively; wherein the heavy and light chain variable framework regions are optionally modified with from 1 to 8, 1 to 6, 1 to 4 or 1 to 2 conservative amino acid substitutions in the framework regions and wherein the CDRs of the heavy or light chain variable regions are not modified. In some embodiments, provided herein is a PTK7 antibody or antigen binding portion thereof or other binding agent comprising a heavy chain variable (VH) region and a light chain variable (VL) region, the VH and VL regions having amino acid sequences set forth in SEQ ID NO:15 and SEQ ID NO:16, respectively; wherein the heavy and light chain variable framework regions are optionally modified with from 1 to 8, 1 to 6, 1 to 4 or 1 to 2 amino acid substitutions, deletions or insertions in the framework regions and wherein the CDRs of the heavy or light chain variable regions are not modified. A variant antibody or antigen-binding portion thereof will retain the ability to bind PTK7.

[0147] In any of these embodiments, the functional activity of the PTK7 binding antibody or antigen binding portion thereof or other binding agent includes specifically binding to PTK7.

Additional functional activities include identification/detection/quantification of PTK7+ cells (e.g., cancer cells or autoimmune cells). In the case where dose dependency does exist, it needs not be identical to that of the reference antibody or antigen-binding portion thereof, but rather substantially similar to or better than the dose-dependence in a given activity as compared to the reference antibody or antigen-binding portion thereof as described herein (i.e., the candidate polypeptide will exhibit greater activity relative to the reference antibody). Hence, in one embodiment, a variant binding agent, and in particular antibody or antigen-binding fragment thereof, may retain a particular function mentioned herein.

[0148] For conservative substitutions, amino acids can be grouped according to similarities in the properties of their side chains (in A. L. Lehninger, in *Biochemistry*, second ed., pp. 73-75, Worth Publishers, New York (1975)): (1) non-polar: Ala (A), Val (V), Leu (L), Ile (I), Pro (P), Phe (F), Trp (W), Met (M); (2) uncharged polar: Gly (G), Ser (S), Thr (T), Cys (C), Tyr (Y), Asn (N), Gln (Q); (3) acidic: Asp (D), Glu (E); and (4) basic: Lys (K), Arg (R), His (H).

[0149] Alternatively, for conservative substitutions naturally occurring residues can be divided into groups based on common side-chain properties: (1) hydrophobic: Norleucine, Met, Ala, Val, Leu, Ile; (2) neutral hydrophilic: Cys, Ser, Thr, Asn, Gln; (3) acidic: Asp, Glu; (4) basic: His, Lys, Arg; (5) residues that influence chain orientation: Gly, Pro; and (6) aromatic: Trp, Tyr, Phe. Non-conservative substitutions will entail exchanging a member of one of these classes or another class.

[0150] Particular conservative substitutions include, for example; Ala to Gly or to Ser; Arg to Lys; Asn to Gln or to His; Asp to Glu; Cys to Ser; Gln to Asn; Glu to Asp; Gly to Ala or to Pro; His to Asn or to Gln; Ile to Leu or to Val; Leu to Ile or to Val; Lys to Arg, to Gln or to Glu; Met to Leu, to Tyr or to Ile; Phe to Met, to Leu or to Tyr; Ser to Thr; Thr to Ser; Trp to Tyr; Tyr to Trp; and/or Phe to Val, to Ile or to Leu.

[0151] In some embodiments, a conservatively modified variant of a PTK7 antibody or antigen binding portion thereof preferably is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or more, identical to the reference VH or VL sequence, wherein the VH and VL CDRs are not modified. The degree of homology (percent identity) between the reference and modified sequence can be determined, for example, by comparing the two sequences using freely available computer programs commonly employed for this purpose on the world wide web (e.g., BLASTp or BLASTn with default settings).

[0152] In some embodiments, the VH and VL amino acid sequences collectively have no more than 8 or 6 or 4 or 2 or 1 conservative amino acid substitutions in the framework regions, as compared to the amino acid sequences of the unmodified VH and VL regions. In some embodiments, the VH and VL amino acid sequences collectively have 8 to 1, or 6 to 1, or 4 to 1,

or 2 to 1 conservative amino acid substitutions in the framework regions, as compared to the amino acid sequences of the unmodified VH and VL regions. In some embodiments, the VH and VL amino acid sequences collectively have no more than 8 or 6 or 4 or 2 or 1 amino acid substitutions, deletions or insertions in the framework regions, as compared to the amino acid sequences of the unmodified VH and VL regions. In some embodiments, the VH and VL amino acid sequences have 8 to 1, 6 to 1, 4 to 1, or 2 to 1 conservative amino acid substitutions in the framework regions, as compared to the amino acid sequences of the unmodified VH and VL regions. In some embodiments, the VH and VL amino acid sequences collectively have no more than 8 or 6 or 4 or 2 or 1 amino acid substitutions, deletions or insertions, as compared to the amino acid sequences of the unmodified VH and VL regions.

[0153] In some embodiments, a binding agent, in particular antibody or antigen-binding fragment thereof, may have a small number of amino acid substitutions, deletions or insertions in the CDR sequences set out provided that the ability to bind PTK7 is retained. In one embodiment, also provided is a binding agent as set out herein with a total of no more than six amino acid sequence changes in the specific CDRs set out herein for the binding agent. In one embodiment, there are no more than a total of four amino acid sequence changes. In one embodiment, there are no more than a total of two amino acid sequence changes. In one embodiment, there is no more than a single amino acid sequence change. In any such embodiments it may be that the amino acid sequence changes are conservative.

[0154] Modification of a native (or reference) amino acid sequence can be accomplished by any of a number of techniques known to one of skill in the art. Mutations can be introduced, for example, at particular loci by synthesizing oligonucleotides containing the desired mutant sequence, flanked by restriction sites enabling ligation to fragments of the native sequence. Following ligation, the resulting reconstructed sequence encodes a variant having the desired amino acid insertion, substitution, or deletion. Alternatively, oligonucleotide-directed site-specific mutagenesis procedures can be employed to provide an altered nucleotide sequence having particular codons altered according to the substitution, deletion, or insertion desired. Techniques for making such alterations are very well established and include, for example, those disclosed by Walder et al. (Gene 42:133, 1986); Bauer et al. (Gene 37:73, 1985); Craik (BioTechniques, January 1985, 12-19); Smith et al. (Genetic Engineering: Principles and Methods, Plenum Press, 1981); and U.S. Pat. Nos. 4,518,584 and 4,737,462, which are herein incorporated by reference in their entireties.

Constant Regions

[0155] In some embodiments, a PTK7 antibody or antigen-binding portion thereof or other binding agent has fully human constant regions. In some embodiments, a PTK7 antibody or

antigen-binding portion thereof or other binding agent has humanized constant regions. In some embodiments, a PTK7 antibody or antigen-binding portion thereof or other binding agent has non-human constant regions. In some embodiments, a PTK7 antibody or antigen-binding portion thereof or other binding agent has mouse constant regions. An immunoglobulin constant region refers to a heavy or light chain constant region. Human heavy chain and light chain constant region amino acid sequences are known in the art. A constant region can be of any suitable type, which can be selected from the classes of immunoglobulins, IgA, IgD, IgE, IgG, and IgM. Several immunoglobulin classes can be further divided into isotypes, e.g., IgG1, IgG2 (e.g., IgG2a, IgG2b, IgG2c), IgG3, IgG4, or IgA1, and IgA2. The heavy-chain constant regions (Fc) that correspond to the different classes of immunoglobulins can be α , δ , ϵ , γ , and μ , respectively. The light chains can be one of either kappa (or κ) and lambda (or λ).

[0156] In some embodiments, a constant region can have an IgG1 isotype. In some embodiments, a constant region can have an IgG2 isotype. In some embodiments, a constant region can have an IgG1 isotype. In some embodiments, a constant region can have an IgG2a isotype. In some embodiments, a constant region can have an IgG1 isotype. In some embodiments, a constant region can have an IgG2b isotype. In some embodiments, a constant region can have an IgG1 isotype. In some embodiments, a constant region can have an IgG2c isotype. In some embodiments, a constant region can have an IgG3 isotype. In some embodiments, a constant region can have an IgG4 isotype. In some embodiments, an Fc domain can have a hybrid isotype comprising constant regions from two or more isotypes. In some embodiments, an immunoglobulin constant region can be an IgG1 or IgG4 constant region.

[0157] In some embodiments, a PTK7 antibody heavy chain has a constant region having the amino acid sequence set forth in SEQ ID NO. 22, SEQ ID NO: 24, or SEQ ID NO: 26. In some embodiments, a PTK7 antibody light chain has a constant region having the amino acid sequence set forth in SEQ ID NO. 23, SEQ ID NO: 25, or SEQ ID NO: 27.

[0158] In some embodiments, a PTK7 antibody comprises a heavy chain having a constant region with the amino acid sequence set forth in SEQ ID NO. 22 and a light chain having a constant region with the amino acid sequence set forth in SEQ ID NO: 23.

[0159] In some embodiments, a PTK7 antibody comprises a heavy chain having a constant region with the amino acid sequence set forth in SEQ ID NO. 24 and a light chain having a constant region with the amino acid sequence set forth in SEQ ID NO: 25.

[0160] In some embodiments, a PTK7 antibody comprises a heavy chain having a constant region with the amino acid sequence set forth in SEQ ID NO. 26 and a light chain having a constant region with the amino acid sequence set forth in SEQ ID NO: 27.

[0161] In some embodiments, a PTK7 antibody comprises a heavy chain with the amino acid sequence set forth in SEQ ID NO. 28 and a light chain with the amino acid sequence set forth in SEQ ID NO: 29.

[0162] In some embodiments, a PTK7 antibody comprises a heavy chain with the amino acid sequence set forth in SEQ ID NO. 30 and a light chain with the amino acid sequence set forth in SEQ ID NO: 31.

[0163] In some embodiments, a PTK7 antibody comprises a heavy chain with the amino acid sequence set forth in SEQ ID NO. 32 and a light chain with the amino acid sequence set forth in SEQ ID NO: 33.

[0164] Furthermore, a PTK7 antibody or an antigen-binding portion thereof or other binding agent may be part of a larger binding agent formed by covalent or noncovalent association of the antibody or antigen binding portion with one or more other proteins or peptides. Relevant to such binding agents are the use, for example, of the streptavidin core region in order to prepare a tetrameric scFv molecule (Kipriyanov, S. M., et al. (1995), Human Antibodies and Hybridomas 6:93-101) and the use of a cysteine residue, a marker peptide and a C-terminal polyhistidiny peptide, e.g. hexahistidiny tag (`hexahistidiny tag` disclosed as SEQ ID NO: 59) in order to produce bivalent and biotinylated scFv molecules (Kipriyanov, S. M., et al. (1994) Mol. Immunol. 31:10471058).

[0165] In some embodiments, where the binding agent is a bispecific antibody, the constant regions of the heavy chains may have "knobs-into-holes" modifications to facilitate heterodimer formation and hence bispecific antibody production.

Fc Domain Modifications to Alter Effector Function

[0166] In some embodiments, an Fc region or Fc domain of a PTK7 antibody or antigen binding portion thereof or other binding agent has substantially no binding to at least one Fc receptor selected from FcγRI (CD64), FcγRIIA (CD32a), FcγRIIB (CD32b), FcγRIIA (CD16a), and FcγRIIB (CD16b). In some embodiments, an Fc region or domain exhibits substantially no binding to any of the Fc receptors selected from FcγRI (CD64), FcγRIIA (CD32a), FcγRIIB (CD32b), FcγRIIA (CD16a), and FcγRIIB (CD16b). As used herein, "substantially no binding" refers to weak to no binding to a selected Fcγ receptor or receptors. In some embodiments, "substantially no binding" refers to a reduction in binding affinity (i.e., increase in K_d) to a Fc γ receptor of at least 1000-fold. In some embodiments, an Fc domain or region is an Fc null. As used herein, an "Fc null" refers to an Fc region or Fc domain that exhibits weak to no binding to any of the Fcγ receptors. In some embodiments, an Fc null domain or region exhibits a reduction in binding affinity (i.e., increase in K_d) to Fc γ receptors of at least 1000-fold.

[0167] In some embodiments, an Fc domain has reduced or substantially no effector function activity. As used herein, “effector function activity” refers to antibody dependent cellular cytotoxicity (ADCC), antibody dependent cellular phagocytosis (ADCP) and/or complement dependent cytotoxicity (CDC). In some embodiments, an Fc domain exhibits reduced ADCC, ADCP or CDC activity, as compared to a wildtype Fc domain. In some embodiments, an Fc domain exhibits a reduction in ADCC, ADCP and CDC, as compared to a wildtype Fc domain. In some embodiments, an Fc domain exhibits substantially no effector function (i.e., the ability to stimulate or effect ADCC, ADCP or CDC). As used herein, “substantially no effector function” refers to a reduction in effector function activity of at least 1000-fold, as compared to a wildtype or reference Fc domain.

[0168] In some embodiments, an Fc domain has reduced or no ADCC activity. As used herein reduced or no ADCC activity refers to a decrease in ADCC activity of an Fc domain by a factor of at least 10, at least 20, at least 30, at least 50, at least 100 or at least 500.

[0169] In some embodiments, an Fc domain has reduced or no CDC activity. As used herein reduced or no CDC activity refers to a decrease in CDC activity of an Fc domain by of a factor of at least 10, at least 20, at least 30, at least 50, at least 100 or at least 500.

[0170] In vitro and/or in vivo cytotoxicity assays can be conducted to confirm the reduction/depletion of ADCC and/or CDC activity. For example, Fc receptor (FcR) binding assays can be conducted to ensure that the antibody lacks Fcγ receptor binding (hence likely lacking ADCC activity). The primary cells for mediating ADCC, NK cells, express FcγRIII only, whereas monocytes express FcγRI, FcγRII and FcγRIII. FcR expression on hematopoietic cells is summarized in Table 3 on page 464 of Ravetch and Kinet, *Annu. Rev. Immunol.* 9:457-492 (1991). Non-limiting examples of in vitro assays to assess ADCC activity of a molecule of interest are described in U.S. Pat. No. 5,500,362 (see, e.g. Hellstrom, I. et al. *Proc. Nat'l Acad. Sci. USA* 83:7059-7063 (1986)) and Hellstrom, I et al., *Proc. Nat'l Acad. Sci. USA* 82:1499-1502 (1985); U.S. Pat. No. 5,821,337 (see Bruggemann, M. et al., *J. Exp. Med.* 166:1351-1361 (1987)). Alternatively, non-radioactive assay methods may be employed (see, for example, ACTITM non-radioactive cytotoxicity assay for flow cytometry (CellTechnology, Inc. Mountain View, Calif.; and CytoTox 96TM non-radioactive cytotoxicity assay (Promega, Madison, Wis.)). Useful effector cells for such assays include peripheral blood mononuclear cells (PBMC) and Natural Killer (NK) cells. Alternatively, or additionally, ADCC activity of the molecule of interest may be assessed in vivo, e.g., in an animal model such as that disclosed in Clynes et al., *Proc. Nat'l Acad. Sci. USA* 95:652-656 (1998).

[0171] C1q binding assays may also be carried out to confirm that an antibody or Fc domain or region is unable to bind C1q and hence lacks CDC activity or has reduced CDC activity. See, e.g., C1q and C3c binding ELISA in WO 2006/029879 and WO 2005/100402. To assess

complement activation, a CDC assay may be performed (see, for example, Gazzano-Santoro et al., J. Immunol. Methods 202:163 (1996); Cragg, M. S. et al., Blood 101:1045-1052 (2003); and Cragg, M. S. and M. J. Glennie, Blood 103:2738-2743 (2004)).

[0172] In some embodiments, an Fc domain has reduced or no ADCP activity. As used herein reduced or no ADCP activity refers to a decrease in ADCP activity of an Fc domain by a factor of at least 10, at least 20, at least 30, at least 50, at least 100 or at least 500.

[0173] ADCP binding assays may also be carried out to confirm that an antibody or Fc domain or region lacks ADCP activity or has reduced ADCP activity. See, e.g., US20190079077 and US20190048078 and the references disclosed therein.

[0174] A PTK7 antibody or antigen binding portion thereof or other binding agent with reduced effector function activity includes those with substitution of one or more of Fc region residues, such as, for example, 238, 265, 269, 270, 297, 327 and 329, according to the EU number of Kabat (see, e.g., U.S. Pat. No. 6,737,056). Such Fc mutants include Fc mutants with substitutions at two or more of amino acid positions 265, 269, 270, 297 and 327, including the so-called "DANA" Fc mutant with substitution of residues 265 and 297 to alanine, according to the EU numbering of Kabat (see U.S. Pat. No. 7,332,581). Certain antibody variants with diminished binding to FcRs are also known. (See, e.g., U.S. Pat. No. 6,737,056; WO 2004/056312, and Shields et al., J. Biol. Chem. 9(2): 6591-6604 (2001).) A PTK7 antibody or antigen binding portion thereof or other binding agent with diminished binding to FcRs can be prepared containing such amino acid modifications.

[0175] In some embodiments, a PTK7 antibody or antigen binding portion thereof or other binding agent comprises an Fc domain or region with one or more amino acid substitutions which diminish Fcγ₁ binding, e.g., substitutions at positions 234 and 235 of the Fc region (EU numbering of residues). In some embodiments, the substitutions are L234A and L235A (LALA), according to the EU numbering of Kabat. In some embodiments, the Fc domain comprises D265A and/or P329G in an Fc region derived from a human IgG1 Fc region, according to the EU numbering of Kabat. In some embodiments, the substitutions are L234A, L235A and P329G (LALA-PG), according to the EU numbering of Kabat, in an Fc region derived from a human IgG1 Fc region. (See, e.g., WO 2012/130831). In some embodiments, the substitutions are L234A, L235A and D265A (LALA-DA) in an Fc region derived from a human IgG1 Fc region, according to the EU numbering of Kabat.

[0176] In some embodiments, alterations are made in the Fc region that result in altered (i.e., either diminished) C1q binding and/or Complement Dependent Cytotoxicity (CDC), e.g., as described in U.S. Pat. No. 6,194,551, WO 99/51642, and Idusogie et al. J. Immunol. 164: 4178-4184 (2000).

Methods of Making Antibodies, Antigen Binding Portions and Other Binding Agents

[0177] In various embodiments, PTK7 antibodies, antigen binding portions thereof and other binding agents can be produced in human, murine or other animal-derived cells lines.

Recombinant DNA expression can be used to produce PTK7 antibodies, antigen binding portions thereof and other binding agents. This allows the production of PTK7 antibodies as well as a spectrum of PTK7 antigen binding portions and other binding agents (including fusion proteins) in a host species of choice. The production of PTK7 antibodies, antigen binding portions thereof and other binding agents in bacteria, yeast, transgenic animals and chicken eggs are also alternatives for cell-based production systems. The main advantages of transgenic animals are potential high yields from renewable sources.

[0178] In some embodiments, a PTK7 VH polypeptide having the amino acid sequence set forth in SEQ ID NOs:1, 8, or 15 is encoded by a nucleic acid. In some embodiments, a PTK7 VL polypeptide having the amino acid sequence set forth in SEQ ID NOs: 2, 9, or 16 is encoded by a nucleic acid. In some embodiments, a nucleic acid encodes a PTK7 VH polypeptide having the amino acid sequence set forth in SEQ ID NOs: 1, 8, or 15. In some embodiments, a nucleic acid encodes a PTK7 VL polypeptide having the amino acid sequence set forth in SEQ ID NOs: 2, 9, or 16. In some embodiments, the nucleic acid encodes a PTK7 VH polypeptide having the amino acid sequence set forth in SEQ ID NO:1. In some embodiments, the nucleic acid encodes a PTK7 VH polypeptide having the amino acid sequence set forth in SEQ ID NO:8. In some embodiments, the nucleic acid encodes a PTK7 VH polypeptide having the amino acid sequence set forth in SEQ ID NO:15. In some embodiments, the nucleic acid encodes a PTK7 VH polypeptide having the amino acid sequence set forth in SEQ ID NO:2. In some embodiments, the nucleic acid encodes a PTK7 VH polypeptide having the amino acid sequence set forth in SEQ ID NO:9. In some embodiments, the nucleic acid encodes a PTK7 VH polypeptide having the amino acid sequence set forth in SEQ ID NO:16.

[0179] In some embodiments, the nucleic acid encodes VH and VL polypeptides having the amino acid sequences set forth in SEQ ID NOs:1 and 2. In some embodiments, the nucleic acid encodes VH and VL polypeptides having the amino acid sequences set forth in SEQ ID NOs:8 and 9. In some embodiments, the nucleic acid encodes VH and VL polypeptides having the amino acid sequences set forth in SEQ ID NOs:15 and 16.

[0180] As used herein, the term "nucleic acid" or "nucleic acid sequence" or "polynucleotide sequence" or "nucleotide" refers to a polymeric molecule incorporating units of ribonucleic acid, deoxyribonucleic acid or an analog thereof. The nucleic acid can be either single-stranded or double-stranded. A single-stranded nucleic acid can be one strand nucleic acid of a denatured double-stranded DNA. In some embodiments, the nucleic acid can be a cDNA, e.g., a nucleic acid lacking introns. In one embodiment, a single nucleic acid encoding both the light and heavy

chains of an antibody is provided. In a further embodiment, a pair of nucleic acids is provided with one encoding the light chain of the antibody and the other the heavy chain.

[0181] Nucleic acid molecules encoding the amino acid sequence of a PTK7 antibody, antigen binding portion thereof as well as other binding agents can be prepared by a variety of methods known in the art. These methods include, but are not limited to, preparation of synthetic nucleotide sequences encoding of a PTK7 antibody, antigen binding portion or other binding agent(s). In addition, oligonucleotide-mediated (or site-directed) mutagenesis, PCR-mediated mutagenesis, and cassette mutagenesis can be used to prepare nucleotide sequences encoding a PTK7 antibody or antigen binding portion as well as other binding agents. A nucleic acid sequence encoding at least a PTK7 antibody, antigen binding portion thereof, binding agent, or a polypeptide thereof, as described herein, can be recombined with vector DNA in accordance with conventional techniques, such as, for example, blunt-ended or staggered-ended termini for ligation, restriction enzyme digestion to provide appropriate termini, filling in of cohesive ends as appropriate, alkaline phosphatase treatment to avoid undesirable joining, and ligation with appropriate ligases or other techniques known in the art. Techniques for such manipulations are disclosed, e.g., by Maniatis et al., *Molecular Cloning, Lab. Manual* (Cold Spring Harbor Lab. Press, NY, 1982 and 1989), and Ausubel et al., *Current Protocols in Molecular Biology* (John Wiley & Sons), 1987-1993, and can be used to construct nucleic acid sequences and vectors that encode a PTK7 antibody or antigen binding portion thereof or a VH or VL polypeptide thereof or other binding agent.

[0182] A nucleic acid molecule, such as DNA, is said to be "capable of expressing" a polypeptide if it contains nucleotide sequences that contain transcriptional and translational regulatory information and such sequences are "operably linked" to nucleotide sequences that encode the polypeptide. An operable linkage is a linkage in which the regulatory DNA sequences and the DNA sequence sought to be expressed (e.g., a PTK7 antibody or antigen binding portion thereof or other binding agent) are connected in such a way as to permit gene expression of a polypeptide(s) or antigen binding portions in recoverable amounts. The precise nature of the regulatory regions needed for gene expression may vary from organism to organism, as is well known in the analogous art. See, e.g., Sambrook et al., 1989; Ausubel et al., 1987-1993.

[0183] Accordingly, the expression of a PTK7 antibody or antigen-binding portion thereof as described herein can occur in either prokaryotic or eukaryotic cells. Suitable hosts include bacterial or eukaryotic hosts, including yeast, insects, fungi, bird and mammalian cells either in vivo or in situ, or host cells of mammalian, insect, bird or yeast origin. The mammalian cell or tissue can be of human, primate, hamster, rabbit, rodent, cow, pig, sheep, horse, goat, dog or cat origin, but any other mammalian cell may be used. Further, by use of, for example, the yeast

ubiquitin hydrolase system, in vivo synthesis of ubiquitin-transmembrane polypeptide fusion proteins can be accomplished. The fusion proteins so produced can be processed in vivo or purified and processed in vitro, allowing synthesis of a PTK7 antibody or antigen binding portion thereof or other binding agent as described herein with a specified amino terminus sequence. Moreover, problems associated with retention of initiation codon-derived methionine residues in direct yeast (or bacterial) expression maybe avoided. (See, e.g., Sabin et al., 7 Bio/Technol. 705 (1989); Miller et al., 7 Bio/Technol. 698 (1989).) Any of a series of yeast gene expression systems incorporating promoter and termination elements from the actively expressed genes coding for glycolytic enzymes produced in large quantities when yeast are grown in medium rich in glucose can be utilized to obtain recombinant PTK7 antibodies or antigen-binding portions thereof or other binding agents. Known glycolytic genes can also provide very efficient transcriptional control signals. For example, the promoter and terminator signals of the phosphoglycerate kinase gene can be utilized.

[0184] Production of PTK7 antibodies or antigen-binding portions thereof or other binding agents in insects can be achieved, for example, by infecting an insect host with a baculovirus engineered to express a polypeptide by methods known to those of ordinary skill in the art. See Ausubel et al., 1987-1993.

[0185] In some embodiments, the introduced nucleic acid sequence(s) (encoding a PTK7 antibody or antigen binding portion thereof or other binding agent or a polypeptide thereof) is incorporated into a plasmid or viral vector capable of autonomous replication in a recipient host cell. Any of a wide variety of vectors can be employed for this purpose and are known and available to those of ordinary skill in the art. See, e.g., Ausubel et al., 1987-1993. Factors of importance in selecting a particular plasmid or viral vector include: the ease with which recipient cells that contain the vector may be recognized and selected from those recipient cells which do not contain the vector; the number of copies of the vector which are desired in a particular host; and whether it is desirable to be able to "shuttle" the vector between host cells of different species.

[0186] Exemplary prokaryotic vectors known in the art include plasmids such as those capable of replication in *E. coli*. Other gene expression elements useful for the expression of DNA encoding PTK7 antibodies or antigen-binding portions thereof or other binding agents include, but are not limited to (a) viral transcription promoters and their enhancer elements, such as the SV40 early promoter. (Okayama et al., 3 Mol. Cell. Biol. 280 (1983)), Rous sarcoma virus LTR (Gorman et al., 79 PNAS 6777 (1982)), and Moloney murine leukemia virus LTR (Grosschedl et al., 41 Cell 885 (1985)); (b) splice regions and polyadenylation sites such as those derived from the SV40 late region (Okayama et al., 1983), and (c) polyadenylation sites such as in SV40 (Okayama et al., 1983). Immunoglobulin-encoding DNA genes can be expressed as described

by Liu et al., *infra*, and Weidle et al., 51 *Gene* 21 (1987), using as expression elements the SV40 early promoter and its enhancer, the mouse immunoglobulin H chain promoter enhancers, SV40 late region mRNA splicing, rabbit S-globin intervening sequence, immunoglobulin and rabbit S-globin polyadenylation sites, and SV40 polyadenylation elements.

[0187] For immunoglobulin encoding nucleotide sequences, the transcriptional promoter can be, for example, human cytomegalovirus, the promoter enhancers can be cytomegalovirus and mouse/human immunoglobulin.

[0188] In some embodiments, for expression of DNA coding regions in rodent cells, the transcriptional promoter can be a viral LTR sequence, the transcriptional promoter enhancers can be either or both the mouse immunoglobulin heavy chain enhancer and the viral LTR enhancer, and the polyadenylation and transcription termination regions. In other embodiments, DNA sequences encoding other proteins are combined with the above-recited expression elements to achieve expression of the proteins in mammalian cells.

[0189] Each coding region or gene fusion is assembled in, or inserted into, an expression vector. Recipient cells capable of expressing the PTK7 variable region(s) or antigen binding portions thereof or other binding agents are then transfected singly with nucleotides encoding a PTK7 antibody or an antibody polypeptide or antigen-binding portion thereof or other binding agent, or are co-transfected with a polynucleotide(s) encoding VH and VL chain coding regions or other binding agents. The transfected recipient cells are cultured under conditions that permit expression of the incorporated coding regions and the expressed antibody chains or intact antibodies or antigen binding portions or other binding agents are recovered from the culture.

[0190] In some embodiments, the nucleic acids containing the coding regions encoding a PTK7 antibody or antigen-binding portion thereof or other binding agent are assembled in separate expression vectors that are then used to co-transfect a recipient host cell. Each vector can contain one or more selectable genes. For example, in some embodiments, two selectable genes are used, a first selectable gene designed for selection in a bacterial system and a second selectable gene designed for selection in a eukaryotic system, wherein each vector has a set of coding regions. This strategy results in vectors which first direct the production, and permit amplification, of the nucleotide sequences in a bacterial system. The DNA vectors so produced and amplified in a bacterial host are subsequently used to co-transfect a eukaryotic cell, and allow selection of a co-transfected cell carrying the desired transfected nucleic acids (e.g., containing PTK7 antibody heavy and light chains). Non-limiting examples of selectable genes for use in a bacterial system are the gene that confers resistance to ampicillin and the gene that confers resistance to chloramphenicol. Selectable genes for use in eukaryotic transfectants include the xanthine guanine phosphoribosyl transferase gene (designated gpt)

and the phosphotransferase gene from Tn5 (designated neo). Alternatively the fused nucleotide sequences encoding VH and VL chains can be assembled on the same expression vector.

[0191] For transfection of the expression vectors and production of the PTK7 antibodies or antigen binding portions thereof or other binding agents, the recipient cell line can be a Chinese Hamster ovary cell line (e.g., DG44) or a myeloma cell. Myeloma cells can synthesize, assemble and secrete immunoglobulins encoded by transfected immunoglobulin genes and possess the mechanism for glycosylation of the immunoglobulin. For example, in some embodiments, the recipient cell is the recombinant Ig-producing myeloma cell SP2/0. SP2/0 cells only produce immunoglobulins encoded by the transfected genes. Myeloma cells can be grown in culture or in the peritoneal cavity of a mouse, where secreted immunoglobulin can be obtained from ascites fluid.

[0192] An expression vector encoding a PTK7 antibody or antigen-binding portion thereof or other binding agent can be introduced into an appropriate host cell by any of a variety of suitable means, including such biochemical means as transformation, transfection, protoplast fusion, calcium phosphate-precipitation, and application with polycations such as diethylaminoethyl (DEAE) dextran, and such mechanical means as electroporation, direct microinjection and microprojectile bombardment. Johnston et al., 240 Science 1538 (1988), as known to one of ordinary skill in the art.

[0193] Yeast provides certain advantages over bacteria for the production of immunoglobulin heavy and light chains. Yeast gene expression systems can be routinely evaluated for the levels of production, secretion and the stability of antibodies, and assembled PTK7 antibodies and antigen binding portions thereof and other binding agents.

[0194] Bacterial strains (e.g., E. coli K12 strains) can also be utilized as hosts for the production of the antibody molecules or antigen binding portions thereof or other binding agents as described herein.

[0195] Host mammalian cells can be grown in vitro or in vivo. Mammalian cells provide post-translational modifications to immunoglobulin molecules including leader peptide removal, folding and assembly of VH and VL chains, glycosylation of the antibody molecules, and secretion of functional antibody and/or antigen binding portions thereof or other binding agents.

[0196] Mammalian cells which can be useful as hosts for the production of antibody proteins, in addition to the cells of lymphoid origin described above, include cells of fibroblast origin, such as Vero or CHO-K1 cells. Exemplary eukaryotic cells that can be used to express immunoglobulin polypeptides include, but are not limited to, COS cells, including COS 7 cells; 293 cells, including 293-6E cells; CHO cells, including CHO--S and DG44 cells; PERC6TM cells (Crucell); and NSO cells. In some embodiments, a particular eukaryotic host cell is selected based on its ability to make desired post-translational modifications to the heavy chains and/or

light chains. For example, in some embodiments, CHO cells produce polypeptides that have a higher level of sialylation than the same polypeptide produced in 293 cells.

[0197] In some embodiments, one or more PTK7 antibodies or antigen-binding portions thereof or other binding agents can be produced in vivo in an animal that has been engineered or transfected with one or more nucleic acid molecules encoding the polypeptides, according to any suitable method.

[0198] In some embodiments, an antibody or antigen-binding portion thereof is produced in a cell-free system. Non-limiting exemplary cell-free systems are described, e.g., in Sitaraman et al., *Methods Mol. Biol.* 498: 229-44 (2009); Spirin, *Trends Biotechnol.* 22: 538-45 (2004); and Endo et al., *Biotechnol. Adv.* 21: 695-713 (2003).

[0197] Many vector systems are available for the expression of the VH and VL chains in mammalian cells (see Glover, 1985). Various approaches can be followed to obtain intact antibodies. Additionally, plants have emerged as a convenient, safe and economical alternative expression system for recombinant antibody production, which are based on large scale culture of microbes or animal cells.

[0198] For intact antibodies, the variable regions (VH and VL regions) of the PTK7 antibodies are typically linked to at least a portion of an immunoglobulin constant region (Fc) or domain, typically that of a human immunoglobulin. Human constant region DNA sequences can be isolated in accordance with well-known procedures from a variety of human cells, such as immortalized B-cells (WO 87/02671). A PTK7 binding antibody can contain both light chain and heavy chain constant regions. The heavy chain constant region can include CH1, hinge, CH2, CH3, and, optionally, CH4 regions. In some embodiments, the CH2 domain can be deleted or omitted.

[0199] Techniques described for the production of single chain antibodies (see, e.g. U.S. Pat. No. 4,946,778; Bird, *Science* 242:423-42 (1988); Huston et al., *Proc. Natl. Acad. Sci. USA* 85:5879-5883 (1988); and Ward et al., *Nature* 334:544-54 (1989)) can be adapted to produce single chain antibodies that specifically bind to PTK7. Single chain antibodies are formed by linking the heavy and light chain variable regions of the Fv region via an amino acid bridge, resulting in a single chain polypeptide. Techniques for the assembly of functional Fv portions in *E. coli* can also be used (see, e.g. Skerra et al., *Science* 242:1038-1041 (1988); which is incorporated by reference herein in its entirety).

[0200] In some embodiments, an antigen binding portion or other binding agent comprises one or more scFvs. In some embodiments, an antigen binding portion or other binding agent is a single-domain antibody is an antibody portion consisting of a single monomeric variable antibody domain. Techniques for producing single domain antibodies (DABs or VHH) are known

in the art, as disclosed for example in Cossins et al. (2006, *Prot Express Purif* 51:253-259) and Li et al. (*Immunol. Lett.* 188:89-95, 2017).

[0201] Techniques for making multispecific antibodies include, but are not limited to, recombinant co-expression of two immunoglobulin heavy chain-light chain pairs having different specificities (see, e.g., Milstein and Cuello, *Nature* 305: 537 (1983)), WO 93/08829, and Traunecker et al., *EMBO J.* 10: 3655 (1991)), and "knob-in-hole" engineering (see, e.g., U.S. Pat. No. 5,731,168; Carter (2001), *J Immunol Methods* 248, 7-15).

[0202] Engineered antibodies with three or more functional antigen binding sites, including "Octopus antibodies," also can be binding agents (see, e.g. US 2006/0025576A1).

[0203] The binding agents (e.g., antibodies or antigen binding portions) herein also include a "Dual Acting FAb" or "DAF" comprising an antigen binding site that binds to two different antigens (see, e.g., US 2008/0069820 and Bostrom et al., 2009, *Science* 323:1610-14).

"Crossmab" antibodies are also included herein (see e.g. WO 2009/080251, WO 2009/080252, WO2009/080253, WO2009/080254, and WO2013/026833).

[0204] In some embodiments, the binding agents comprise different antigen-binding sites, fused to one or the other of the two subunits of the Fc domain; thus, the two subunits of the Fc domain may be comprised in two non-identical polypeptide chains. Recombinant co-expression of these polypeptides and subsequent dimerization leads to several possible combinations of the two polypeptides. To improve the yield and purity of the bispecific molecules in recombinant production, it will thus be advantageous to introduce in the Fc domain of the binding agent a modification promoting the association of the desired polypeptides.

[0205] Generally, this method involves replacement of one or more amino acid residues at the interface of the two Fc domains by charged amino acid residues so that homodimer formation becomes electrostatically unfavorable but heterodimerization electrostatically favorable.

[0206] In some embodiments, a binding agent is a "bispecific T cell engager" or BiTE (see, e.g., WO2004/106381, WO2005/061547, WO2007/042261, and WO2008/119567). This approach utilizes two antibody variable domains arranged on a single polypeptide. For example, a single polypeptide chain can include two single chain Fv (scFv) portions, each having a variable heavy chain (VH) and a variable light chain (VL) domain separated by a polypeptide linker of a length sufficient to allow intramolecular association between the two domains. This single polypeptide further includes a polypeptide spacer sequence between the two scFvs. Each scFv recognizes a different epitope, and these epitopes may be specific for different proteins, such that both proteins are bound by the BiTE.

[0207] In some embodiments, a binding agent is multispecific, such as an IgG-scFv. IgG-scFv formats include IgG(H)-scFv, scFv-(H)IgG, IgG(L)-scFv, svFc-(L)IgG, 2scFv-IgG and IgG-2scFv. These and other bispecific antibody formats and methods of making them have been

described in for example, Brinkmann and Kontermann, MAbs 9(2):182-212 (2017); Wang et al., Antibodies, 2019, 8, 43; Dong et al., 2011, MAbs 3:273-88; Natsume et al., J. Biochem. 140(3):359-368, 2006; Cheal et al., Mol. Cancer Ther. 13(7):1803-1812, 2014; and Bates and Power, Antibodies, 2019, 8, 28.

[0208] IgG-like dual-variable domain antibodies (DVD-Ig) have been described by Wu et al., 2007, Nat Biotechnol 25:1290-97; Hasler et al., Mol. Immunol. 75:28-37, 2016 and in WO 08/024188 and WO 07/024715. Triomabs have been described by Chelius et al., MAbs 2(3):309-319, 2010. 2-in-1-IgGs have been described by Kontermann et al., Drug Discovery Today 20(7):838-847, 2015. Tandem antibody or TandAb have been described by Kontermann et al., id. ScFv-HSA-scFv antibodies have also been described by Kontermann et al. (id.).

[0209] Intact (e.g., whole) antibodies, their dimers, individual light and heavy chains, or antigen binding portions thereof and other binding agents can be recovered and purified by known techniques, e.g., immunoabsorption or immunoaffinity chromatography, chromatographic methods such as HPLC (high performance liquid chromatography), ammonium sulfate precipitation, gel electrophoresis, or any combination of these. See generally, Scopes, Protein Purification (Springer-Verlag, N.Y., 1982). Substantially pure PTK7 binding antibodies or antigen binding portions thereof or other binding agents of at least about 90% to 95% homogeneity are advantageous, as are those with 98% to 99% or more homogeneity, particularly for pharmaceutical uses. Once purified, partially or to homogeneity as desired, an intact PTK7 antibody or antigen binding portions thereof or other binding agent can then be used therapeutically or in developing and performing assay procedures, immunofluorescent staining, and the like. See generally, Vols. I & II Immunol. Meth. (Lefkovits & Pernis, eds., Acad. Press, NY, 1979 and 1981).

CONJUGATES

[0210] A binding agent, in particular antibody, of the present invention may be provided in the form of a conjugate. In one embodiment, a binding agent, in particular antibody, of the present invention may be provided in the form of the binding agent, in particular antibody, conjugated to a label. The label may be, for example, any of those mentioned herein. In one embodiment, a binding agent, in particular antibody, of the present invention may be provided in the form of the binding agent, in particular antibody, conjugated to a drug. The drug may be, for example, a chemotherapeutic agent. In one embodiment, a binding agent, in particular antibody, of the present invention may be provided in the form of the binding agent, in particular antibody, conjugated to a moiety allowing easier purification of the conjugate. The moiety may be, for example, biotin.

IHC METHODS

[0211] The present invention provides for the use of a binding agent of the invention in detecting and/or quantifying PTK7 protein. In one particular embodiment, the invention provides for the use of a binding agent of the invention for immunohistochemistry (IHC).

[0212] Also provided by the current disclosure are immunohistochemistry (IHC) methods of detecting a PTK7 expressing cell in a sample employing a binding agent of the present invention. In some embodiments, the method comprises: contacting a sample with a detecting agent comprising any one of the antibodies, antigen binding portions thereof, and other binding agents of the current disclosure, and detecting formation of a binding complex between the detecting agent and PTK7. In certain embodiments, the detection of the binding complex is indicative of the presence of a PTK7 expressing cell in the sample.

[0213] In accordance with the present disclosure, the term "sample" from a subject may be a sample of tissue or any bodily fluid of said subject that may comprise PTK7. Exemplary bodily fluids include for example blood, saliva, nasal mucus or lymph fluid. In certain embodiments, the sample is a blood sample, such as selected from the group comprising whole blood, serum, plasma, capillary blood, arterial blood, venous blood or any mixture thereof. Exemplary tissue samples include but are limited to tissues of tonsil, appendix, breast, ovary, colon, prostate, skin, lung, uterus, cervix, kidney, pancreas, bladder, brain, thyroid, ear, nose, throat, esophagus, and liver. In one embodiment the tissue is ovarian tissue. In another embodiment, the tissue is breast cancer tissue. In one embodiment the tissue is tissue from a biopsy for breast cancer. The skilled person is aware of various means and methods that may be applied to obtain a sample from a subject suitable for the purposes of the herein disclosed methods, products and uses and in the required quantities. In certain embodiments, the sample may be obtained from the subject by a physician, whereas in other cases, the sample may be obtained by the subject itself. In certain embodiments, the sample may originate from a single subject, i.e., a single individual. In certain embodiments, the sample may comprise tissues or bodily fluids from more than one subject. In some embodiments, the sample may be tissue sections. In embodiments where a tissue sample is utilized, the method is therefore an *in vitro* method. The binding agents of the invention may though also be used for *in vivo* imaging.

[0214] In accordance with the present disclosure, the "subject", or interchangeably referred to herein as "patient", refers to a mammal, preferably a human, but may alternatively also refer to a different mammal, such as a non-human primate or other mammalian animal or even a non-mammalian animal, any of which produces PTK7 expressing cells. In certain embodiments, the subject is a human patient. In certain embodiments, the subject is a human cancer patient.

[0215] In some embodiments, the detecting agent further comprises a detectable marker that

labels the binding agent. The definitions and examples of the detectable marker and detecting agent can be found elsewhere in the current disclosure.

[0216] As used herein, the terms “label” or “labeled”, with regard to the binding agent, are intended to embrace such embodiments wherein the binding is labelled by coupling, such as by physical coupling or covalent binding, a detectable substance, such as a radioactive agent or another molecule providing a detectable signal, such as, without intended to being limiting, a fluorescent tag (i.e., a fluorophore, such as a small organic chemical fluorophore or fluorescent protein), a binding molecule (e.g., biotin, which can bind to avidin or streptavidin with high affinity), or an enzymatically active label (“enzyme marker”), i.e. an enzyme, such as horseradish peroxidase (HRP) or alkaline phosphatase (AP), whose presence can be assessed and optionally be quantified based on its reactivity with, and/or conversion of, a substrate substance. Various suitable detectable markers are known in the art and some of which are also described herein.

[0217] In some embodiments, the detectable marker includes biotin, which can bind to avidin or streptavidin with high affinity. Such duals (biotin-avidin, biotin-streptavidin) have been used rather extensively in labeling and target molecule (e.g., an antigen) detection.

[0218] In some embodiments, the detectable marker includes an enzyme marker. In certain embodiments, the enzyme marker includes horseradish peroxidase (HRP) or alkaline phosphatase (AP). Such markers catalyze certain reactions that may generate detectable signals that can indicate the presence of formation of the complex between the binding agent and PTK7. Common substrates of HRP include but are not limited to 3,3',5,5'-Tetramethylbenzidine (TMB), 3,3'-diaminobenzidine (DAB), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS), 3-amino-9-ethylcarbazole (AEC), o-phenylenediamine dihydrochloride (OPD), homovanillic acid, AmplexRed, and luminol.

[0219] In some embodiments, the detecting the formation of the binding complex between the detecting agent and PTK7 comprises: adding a partner agent of the detectable marker; and detecting a reaction of the detectable marker with the partner agent as an indication of the formation of the binding complex.

[0220] The definition and examples of the partner agent can be found elsewhere in the current disclosure. In essence, the detectable marker and the partner agent react to generate a signal (e.g., coloration of tissue) that can be detected (e.g., by microscopy and imaging techniques). In certain embodiments, the partner agent is the binding partner of biotin (e.g., avidin or streptavidin). In certain embodiments, the partner agent is the substrate of the enzyme marker (e.g., the substrates of HRP or the substrates of AP).

[0221] One example of a pair of detectable marker and partner agent is biotin and DAB. By labeling the binding agent with biotin, contacting the sample with the biotinylated binding agent

(i.e., the detecting agent), and adding DAB, cells that are positive for PTK7 would coloration and can be visualized, imaged, and if necessary, quantified, according to pre-determined protocols.

[0222] In some embodiments, the detectable marker includes a fluorescent tag, which provides a means to produce a detectable signal without the need to add the partner agent. In certain embodiments, the detecting the formation of the binding complex between the detecting agent and PTK7 comprises: detecting a signal from the fluorescent tag as an indication of the formation of the binding complex. In certain embodiments, the fluorescent tag includes fluorescein (FITC), phycoerythrin (PE), preferably R-PE, or allophycocyanin (APC).

[0223] In some embodiments, the detecting the formation of the binding complex between the detecting agent and PTK7 comprises: contacting the sample with a secondary antibody that is labeled and configured to bind to the binding complex; and detecting a signal from the secondary antibody as an indication of the formation of the binding complex.

[0224] In accordance with the present disclosure, "secondary antibody" refers to any kind of "binding moiety", e.g., binding protein, capable of specific binding to an IgA, IgG and/or IgM class antibody or a fragment thereof. Non-limiting examples of binding moieties include antibodies, for example antibodies immunologically or genetically derived from any species, for example human, chicken, camel, llama, lamprey, shark, goat, rodent, cow, dog, rabbit, etc., antibody fragments, domains or parts thereof, for example Fab, Fab', F(ab')₂, scFab, Fv, scFv, VH, VHH, VL, VLRs, and the like, diabodies, monoclonal antibodies (mAbs), and polyclonal antibodies (pAbs).

[0225] In a some embodiments, the secondary antibody is an immunoglobulin (Ig), e.g., IgG raised in a non-human species, wherein the secondary antibody specifically binds immunoglobulins of one or more specific Ig classes or fragments thereof (e.g. a constant domain of a particular Ig class) of another selected species, e.g., mouse or human.

[0226] In some embodiments, secondary antibodies are labeled with a detectable marker so that a detectable signal can be produced directly by the secondary antibody or after the labeled secondary antibody reacts with another molecule. Means and methods for developing, screening and identification of suitable binding molecules of various scaffolds, including, without being limiting, those described herein above, toward desired target structures, such as the IgA and/or IgG class antibodies, are known and employed in the art. Exemplary nowadays routinely-performed methods include, without intended to being limiting, high-throughput (HT) combinatorial library-based display and selection methods, such as phage display, ribosome display, mRNA display, and cell surface display (e.g. yeast display).

[0227] In certain embodiments, the detecting the formation of the binding complex between the detecting agent and PTK7 comprises: adding a partner agent of the detectable marker; and detecting a reaction of the detectable marker with the partner agent as an indication of the

formation of the binding complex.

[0228] In certain embodiments, the detectable marker includes biotin or an enzyme marker. Descriptions for the detectable marker, biotin, the enzyme marker, and partner agent for the secondary antibodies are essentially the same as provided elsewhere for such molecules or portions thereof for the binding agent.

[0229] In certain embodiments, the detectable marker includes a fluorescent tag. In certain embodiments, the detecting the formation of the binding complex between the detecting agent and PTK7 comprises: detecting a signal from the fluorescent tag as an indication of the formation of the binding complex. In certain embodiments, the fluorescent tag includes fluorescein (FITC), phycoerythrin (PE), preferably R-PE, or allophycocyanin (APC).

[0230] In some embodiments, the sample is from a tissue of a subject. In certain embodiments, the tissue is selected from the group consisting of tonsil, appendix, breast, ovary, colon, prostate, skin, lung, uterus, cervix, kidney, pancreas, bladder, brain, thyroid, ear, nose, throat, and esophagus. In certain embodiments, the tissue is selected from the group consisting of breast, ovary, colon, prostate, skin, lung, uterus, esophagus, and bladder. In certain embodiments, the tissue is breast. In certain embodiments, the tissue is ovary. In certain embodiments, the tissue is prostate. As well as the methods set out herein, the present invention also provides a tissue sample stained using a binding agent of the present invention.

[0231] In some embodiments, the sample is from a subject and the presence of PTK7 expressing cell indicates that the subject is suffering from a disease.

[0232] In some embodiments, the sample is from a subject and the method further comprises: quantifying binding PTK expressing cells in the sample to obtain a quantification result, wherein the quantification result over a pre-determined threshold indicates that the subject is suffering from a disease.

[0233] In certain embodiments, the occurrence of the disease is associated with the presence of PTK7 expression. In certain embodiments, the occurrence of the disease is associated with the presence of PTK7 expressing cells. In certain embodiments, the occurrence of the disease is associated with the number of PTK7 expressing cells in a tissue section being over a pre-determined number threshold. In certain embodiments, the occurrence of the disease is associated with the ratio of PTK7 expressing cells in total cells in a tissue section being over a pre-determined ration threshold.

[0234] In some embodiments, the disease is cancer. In certain embodiments, the disease is selected from the group consisting of breast cancer, ovarian cancer, colon cancer, prostate cancer, melanoma, lung cancer, esophageal cancer, gastric cancer, endometrial cancer, head and neck cancer, and bladder cancer.

[0235] In certain embodiments, the disease is breast cancer. In certain embodiments, the

disease is ovarian cancer. In certain embodiments, the disease is lung squamous cells carcinoma or lung adenocarcinoma.

[0236] In certain embodiments, the disease is colon cancer. In certain embodiments, the disease is prostate cancer. In certain embodiments, the disease is melanoma. In certain embodiments, the disease is lung cancer. In certain embodiments, the disease is esophageal cancer. In certain embodiments, the disease is gastric cancer. In certain embodiments, the disease is endometrial cancer. In certain embodiments, the disease is head and neck cancer. In certain embodiments, the disease is bladder cancer.

DISEASE DETECTION AND TREATMENT

[0237] In some embodiments, provided by the current disclosure is a method of determining whether a subject is suffering from a disease, comprising: obtaining a sample from the subject; contacting the sample with a detecting agent comprising the binding agent of the current disclosure, determining whether the subject is suffering from the disease by detecting and/or quantifying formation of binding complexes between the detecting agent and PTK7, wherein the presence of the binding complexes or a level of the binding complexes over a pre-determined threshold indicates that the subject is suffering from the disease. In some embodiments the method is performed on a sample that has already been obtained from the subject and hence the method does not comprise the step of sample collection.

[0238] In certain embodiments, the occurrence of the disease is associated with the presence of PTK7 expression. In certain embodiments, the occurrence of the disease is associated with the presence of PTK7 expressing cells. In certain embodiments, the occurrence of the disease is associated with the number of PTK7 expressing cells in a tissue section being over a pre-determined number threshold. In certain embodiments, the occurrence of the disease is associated with the ratio of PTK7 expressing cells in total cells in a tissue section being over a pre-determined ration threshold.

[0239] In some embodiments, the disease is cancer. In certain embodiments, the disease is selected from the group consisting of breast cancer, ovarian cancer, colon cancer, prostate cancer, melanoma, lung cancer, esophageal cancer, gastric cancer, endometrial cancer, head and neck cancer, and bladder cancer.

[0240] In certain embodiments, the disease is breast cancer. In certain embodiments, the disease is ovarian cancer. In certain embodiments, the disease is lung squamous cells carcinoma or lung adenocarcinoma.

[0241] In certain embodiments, the disease is colon cancer. In certain embodiments, the disease is prostate cancer. In certain embodiments, the disease is melanoma. In certain embodiments, the disease is lung cancer. In certain embodiments, the disease is esophageal

cancer. In certain embodiments, the disease is gastric cancer. In certain embodiments, the disease is endometrial cancer. In certain embodiments, the disease is head and neck cancer. In certain embodiments, the disease is bladder cancer.

[0242] In some embodiments, provided by the current disclosure is a method of treating a disease in patient who has been determined to have the disease by the detection methods described herein. In one embodiment, the detection method comprises obtaining a sample from a subject; contacting the sample with a detecting agent comprising the binding agent of the current disclosure; determining whether the subject is suffering from the disease by detecting and/or quantifying formation of binding complexes between the detecting agent and PTK7, wherein the presence of the binding complexes or a level of the binding complexes over a pre-determined threshold indicates that the subject is suffering from the disease; and treating the disease. In some embodiments, the binding agent comprises VH and VL regions comprising amino acid sequences set forth in: SEQ ID NO: 1 and SEQ ID NO: 2, respectively; SEQ ID NO: 8 and SEQ ID NO: 9, respectively; or SEQ ID NO: 15 and SEQ ID NO: 16, respectively. Also provided is an anti-cancer agent for use in such a method.

[0243] In certain embodiments, the occurrence of the disease is associated with the presence of PTK7 expression. In certain embodiments, the occurrence of the disease is associated with the presence of PTK7 expressing cells. In certain embodiments, the occurrence of the disease is associated with the number of PTK7 expressing cells in a tissue section being over a pre-determined number threshold. In certain embodiments, the occurrence of the disease is associated with the ratio of PTK7 expressing cells in total cells in a tissue section being over a pre-determined ration threshold.

[0244] In some embodiments, the disease is cancer. In certain embodiments, the disease is selected from the group consisting of breast cancer, ovarian cancer, colon cancer, prostate cancer, melanoma, lung cancer, esophageal cancer, gastric cancer, endometrial cancer, head and neck cancer, and bladder cancer.

[0245] In certain embodiments, the disease is breast cancer. In certain embodiments, the disease is ovarian cancer. In certain embodiments, the disease is lung squamous cells carcinoma or lung adenocarcinoma.

[0246] In certain embodiments, the disease is colon cancer. In certain embodiments, the disease is prostate cancer. In certain embodiments, the disease is melanoma. In certain embodiments, the disease is lung cancer. In certain embodiments, the disease is esophageal cancer. In certain embodiments, the disease is gastric cancer. In certain embodiments, the disease is endometrial cancer. In certain embodiments, the disease is head and neck cancer. In certain embodiments, the disease is bladder cancer.

[0247] In certain embodiments, the treatment includes administering chemotherapy to the

subject.

[0248] In certain embodiments, the treatment includes administering hormone therapy to the subject.

[0249] In certain embodiments, the treatment includes administering radiation therapy to the subject.

[0250] In certain embodiments, the treatment includes administering immunotherapy to the subject.

[0251] In certain embodiments, the treatment includes administering stem cell therapy to the subject.

[0252] In certain embodiments, the treatment includes administering targeted therapy to the subject.

[0253] In certain embodiments, the treatment includes performing surgery on the subject.

[0254] In certain embodiments, the disease is breast cancer and the treatment includes lumpectomy or mastectomy.

[0255] In certain embodiments, disease is ovarian cancer and the treatment includes hysterectomy or salpingo-oophorectomy.

[0256] In some embodiments, provided by the current disclosure is a method of monitoring progression of a disease in a subject, comprising the steps of: obtaining a sample from the subject; contacting the sample with a detecting agent comprising the binding agent of the current disclosure; assessing formation of binding complexes between the detecting agent and PTK7 to obtaining a first assessment result; repeating steps (a), (b), and (c) using a sample from the subject at a subsequent point in time to obtaining a second assessment result; and comparing the second assessment result to the first assessment result to determine the progression of the disease in the subject.

[0257] In certain embodiments, the occurrence of the disease is associated with the presence of PTK7 expression. In certain embodiments, the occurrence of the disease is associated with the presence of PTK7 expressing cells. In certain embodiments, the occurrence of the disease is associated with the number of PTK7 expressing cells in a tissue section being over a pre-determined number threshold. In certain embodiments, the occurrence of the disease is associated with the ratio of PTK7 expressing cells in total cells in a tissue section being over a pre-determined ratio threshold.

[0258] In some embodiments, the disease is cancer. In certain embodiments, the disease is selected from the group consisting of breast cancer, ovarian cancer, colon cancer, prostate cancer, melanoma, lung cancer, esophageal cancer, gastric cancer, endometrial cancer, head and neck cancer, and bladder cancer.

[0259] In certain embodiments, the disease is breast cancer. In certain embodiments, the

disease is ovarian cancer. In certain embodiments, the disease is lung squamous cells carcinoma or lung adenocarcinoma.

[0260] In certain embodiments, the disease is colon cancer. In certain embodiments, the disease is prostate cancer. In certain embodiments, the disease is melanoma. In certain embodiments, the disease is lung cancer. In certain embodiments, the disease is esophageal cancer. In certain embodiments, the disease is gastric cancer. In certain embodiments, the disease is endometrial cancer. In certain embodiments, the disease is head and neck cancer. In certain embodiments, the disease is bladder cancer.

KITS

[0261] Also provided by the current disclosure is a kit for detecting PTK7 expressing cells in a sample. In some embodiments, such a kit typically comprises one or more components necessary for performing the IHC methods herein described (i.e., assays). Components may be compounds, reagents, containers and/or equipment.

[0262] In some embodiments, the kit comprises a detecting agent that includes any one of the antibodies, antibody fragments, and other detecting agents (as a whole referred to as "binding agents") of the current disclosure.

[0263] In some embodiments, the binding agent of the kit is labeled with a detectable marker.

[0264] In some embodiments, the kit further includes a second antibody that is labeled and configured to bind to a binding complex that can be formed by the detecting agent and PTK7. In certain embodiments, the secondary antibody is labeled with a detectable marker.

[0265] In some embodiments, the kit further includes a partner agent. The descriptions for the detectable marker and the partner agent are provided elsewhere in the current disclosure.

[0266] In some embodiments, the kit further includes buffers to be used in the assay.

[0267] In some embodiments, the kit further comprises instructions for performing a method of the present invention.

[0268] In some embodiments, the kit comprises all of the reagents mentioned herein in relation to a method of the present invention. In some embodiments, the kit further comprises instructions for performing the method.

USE

[0269] The current disclosure also provides a use of the binding agents in detecting a PTK7 expressing cell in a sample, a use of the binding agent for the manufacture of a kit for detecting a PTK7 expressing cell in a sample and/or a user of determining whether a subject is suffering from a disease.

[0270] In some embodiments, the disease is cancer. In certain embodiments, the disease is

selected from the group consisting of breast cancer, ovarian cancer, colon cancer, prostate cancer, melanoma, lung cancer, esophageal cancer, gastric cancer, endometrial cancer, head and neck cancer, and bladder cancer.

[0271] In certain embodiments, the disease is breast cancer. In certain embodiments, the disease is ovarian cancer. In certain embodiments, the disease is lung squamous cells carcinoma or lung adenocarcinoma.

[0272] In certain embodiments, the disease is colon cancer. In certain embodiments, the disease is prostate cancer. In certain embodiments, the disease is melanoma. In certain embodiments, the disease is lung cancer. In certain embodiments, the disease is esophageal cancer. In certain embodiments, the disease is gastric cancer. In certain embodiments, the disease is endometrial cancer. In certain embodiments, the disease is head and neck cancer. In certain embodiments, the disease is bladder cancer.

[0273] Also provided is use of a binding agent of the present invention in detecting and/or quantifying PTK7.

[0274] Also provided is the use of a binding agent of the present invention to stain a tissue sample. Further provided is the use of a binding agent of the present invention in flow cytometry.

FURTHER EMBODIMENTS

[0275] Any of the binding agents, in particular antibodies, set out herein may be used to detect a cancer expressing PTK7. In one embodiment, a binding agent, and in particular antibody, of the present invention may be chosen for a method or use as set out herein based on the staining pattern it displays, for instance as set out in the Tables of the present application. In one embodiment, it may be chosen because it, for instance, shows stronger binding to the target cancer tissue than other antibodies set out herein. It may be chosen, for instance, because it stains membrane PTK7. It may be chosen, for instance, because it stains intracellular PTK7.

[0276] In one embodiment, an antibody, or antigen-binding fragment therefore comprising the six CDRs of the M10-5F7 antibody may be used to detect ovarian cancer (HCDR1 of SEQ ID NO: 17, HCDR2 of SEQ ID NO: 18, HCDR3 of SEQ ID NO: 19, LCDR1 of SEQ ID NO: 20, LCDR2 of RVS, and LCDR3 of SEQ ID NO: 21). In one embodiment, the antibody, or antigen binding fragment thereof comprises the VH and VL sequences of the M10-5F7 antibody (respectively SEQ ID Nos: 15 and 16). Variant antibodies may also be used.

[0277] In one embodiment, an antibody, or antigen-binding fragment therefore comprising the six CDRs of the M10-5F7 antibody may be used to detect tonsil cancer. In one embodiment, the antibody, or antigen binding fragment thereof comprises the VH and VL sequences of the M10-5F7 antibody. Variant antibodies may also be used.

[0278] In one embodiment, an antibody, or antigen-binding fragment therefore comprising the

six CDRs of the M10-5F7 antibody may be used to detect kidney cancer. In one embodiment, the antibody, or antigen binding fragment thereof comprises the VH and VL sequences of the M10-5F7 antibody. Variant antibodies may also be used.

[0279] In one embodiment, an antibody, or antigen-binding fragment thereof comprising the six CDRs of the M10-5F7 antibody may be used to detect uterine cancer. In one embodiment, the antibody, or antigen binding fragment thereof comprises the VH and VL sequences of the M10-5F7 antibody. Variant antibodies may also be used.

[0280] In one embodiment, an antibody, or antigen-binding fragment thereof comprising the six CDRs of the M6-8G5 antibody (HCDR1 of SEQ ID NO: 10, HCDR2 of SEQ ID NO: 11, HCDR3 of SEQ ID NO: 12, LCDR1 of SEQ ID NO: 13, LCDR2 of QMS, and LCDR3 of SEQ ID NO: 14) may be used to detect ovarian cancer. In one embodiment, the antibody, or antigen binding fragment thereof comprises the VH and VL sequences of the M6-8G5 antibody (respectively SEQ ID Nos: 8 and 9). Variant antibodies may also be used.

[0281] In one embodiment, an antibody, or antigen-binding fragment thereof comprising the six CDRs of the M6-8G5 antibody may be used to detect breast cancer. In one embodiment, the antibody, or antigen binding fragment thereof comprises the VH and VL sequences of the M6-8G5 antibody. Variant antibodies may also be used.

[0282] In one embodiment, an antibody, or antigen-binding fragment thereof comprising the six CDRs of the M6-8G5 antibody may be used to detect pancreatic cancer. In one embodiment, the antibody, or antigen binding fragment thereof comprises the VH and VL sequences of the M6-8G5 antibody. Variant antibodies may also be used.

[0283] In one embodiment, an antibody, or antigen-binding fragment thereof comprising the six CDRs of the M6-8G5 antibody may be used to detect lung cancer. In one embodiment, the antibody, or antigen binding fragment thereof comprises the VH and VL sequences of the M6-8G5 antibody. Variant antibodies may also be used.

[0284] In one embodiment, an antibody, or antigen-binding fragment thereof comprising the six CDRs of the M6-8G5 antibody may be used to detect uterine cancer. In one embodiment, the antibody, or antigen binding fragment thereof comprises the VH and VL sequences of the M6-8G5 antibody. Variant antibodies may also be used.

[0285] In one embodiment, an antibody, or antigen-binding fragment thereof comprising the six CDRs of the M6-8G5 antibody may be used to detect kidney cancer. In one embodiment, the antibody, or antigen binding fragment thereof comprises the VH and VL sequences of the M6-8G5 antibody. Variant antibodies may also be used.

[0286] In one embodiment, an antibody, or antigen-binding fragment thereof comprising the six CDRs of the M6-8G5 antibody may be used to detect pancreatic cancer. In one embodiment, the antibody, or antigen binding fragment thereof comprises the VH and VL sequences of the

M6-8G5 antibody. Variant antibodies may also be used.

[0287] In one embodiment, an antibody, or antigen-binding fragment therefore comprising the six CDRs of the M6-8G5 antibody may be used to detect prostate cancer. In one embodiment, the antibody, or antigen binding fragment thereof comprises the VH and VL sequences of the M6-8G5 antibody. Variant antibodies may also be used.

[0288] In one embodiment, an antibody, or antigen-binding fragment therefore comprising the six CDRs of the M6-11E2 antibody (HCDR1 of SEQ ID NO: 3, HCDR2 of SEQ ID NO: 4, HCDR3 of SEQ ID NO: 5, LCDR1 of SEQ ID NO: 6, LCDR2 of LVS, and LCDR3 of SEQ ID NO: 7) may be used to detect breast cancer. In one embodiment, the antibody, or antigen binding fragment thereof comprises the VH and VL sequences of the M6-11E2 antibody (respectively SEQ ID Nos: 1 and 2). Variant antibodies may also be used.

[0289] In one embodiment, an antibody, or antigen-binding fragment therefore comprising the six CDRs of the M6-11E2 antibody may be used to detect ovarian cancer. In one embodiment, the antibody, or antigen binding fragment thereof comprises the VH and VL sequences of the M6-11E2 antibody. Variant antibodies may also be used.

[0290] In one embodiment, an antibody, or antigen-binding fragment therefore comprising the six CDRs of the M6-11E2 antibody may be used to detect pancreatic cancer. In one embodiment, the antibody, or antigen binding fragment thereof comprises the VH and VL sequences of the M6-11E2 antibody. Variant antibodies may also be used.

[0291] In one embodiment, an antibody, or antigen-binding fragment therefore comprising the six CDRs of the M6-11E2 antibody may be used to detect stomach cancer. In one embodiment, the antibody, or antigen binding fragment thereof comprises the VH and VL sequences of the M6-11E2 antibody. Variant antibodies may also be used.

OTHERS

[0292] The IHC methods and assays of the present disclosure should be understood to encompass not only the specific steps and reagents herein described. At the very least, herein provided are: the detection/quantification of PTK7 or complex and/or the detection/quantification of PTK7 expressing cells/tissues. Such methods can be used for the prognosis and diagnosis of diseases (e.g., cancer) and kit for the same purposes according to the present disclosure. The methods/assays that can employ the binding agents of the current disclosure may include: immunodiffusion techniques, immunoelectrophoretic techniques, light scattering immunoassays, agglutination techniques, labeled immunoassays such as those from the group comprising radiolabeled immunoassays, enzyme immunoassays such as colorimetric assays, chemiluminescence immunoassays and immunofluorescence techniques. The person skilled

in the art is familiar with these methods, which are also described in the state of the art, for example in Zane, H. D. (2001): Immunology—Theoretical & Practical Concepts in Laboratory Medicine, W. B. Saunders Company, in particular in Chapter 14. Preferably the test format is an ELISA and a microtiter plate comprising wells is used as a diagnostically useful carrier.

SEQUENCE CHART

[0293] The Table below provides a summary of the CDR sequences, VL sequences, VH sequences, constant region sequences, entire heavy chain sequences and entire light chain sequences of the antibody clones of the present invention. The present invention provides antibodies comprising the sets of six CDRs set out as well as variant versions. It also provides antibodies comprising one of the pairs of VL and VH sequences from those clones as well as variant sequences of those sequences. It also provides antibodies with a pair of the entire heavy and light chain sequences of one of those antibodies as well as variants thereof.

Sequence	SEQ ID NO.
11E2 VH	1
11E2 VL	2
11E2 HCDR1	3
11E2 HCDR2	4
11E2 HCDR3	5
11E2 LCDR1	6
11E2 LCDR2	LVS
11E2 LCDR3	7
8G5 VH	8
8G5 VL	9
8G5 HCDR1	10
8G5 HCDR2	11
8G5 HCDR3	12
8G5 LCDR1	13
8G5 LCDR2	QMS
8G5 LCDR3	14
5F7 VH	15
5F7 VL	16
5F7 HCDR1	17
5F7 HCDR2	18
5F7 HCDR3	19
5F7 LCDR1	20
5F7 LCDR2	RVS
5F7 LCDR3	21
11E2 CH	22

11E2 CL	23
8G5 CH	24
8G5 CL	25
5F7 CH	26
5F7 CL	27
11E2 Heavy Chain	28
11E2 Light Chain	29
8G5 Heavy Chain	30
8G5 Light Chain	31
5F7 Heavy Chain	32
5F7 Light Chain	33

EXAMPLES

MATERIAL AND METHOD

1. IHC method

[0294] Sample preparation: baked FFPE slides in a dry oven for 1 hour at 60°C to improve sample adhesion to the slide.

[0295] Deparaffinization and rehydration: deparaffinized FFPE tissue by immersing in fresh xylene twice for 10 min each; rehydrated with a series of grade EtOH washes (100%, 95%, 85%, 75%) for 5 min, respectively.

[0296] Washed sections in dH₂O for 3 min, and in PBS twice for 3 min each.

[0297] Epitope retrieval: heated the slides in the pH9.0 EDTA antigen retrieval solution (1X) in a pressure cooker until boiling is initiated, followed with 3 min at high-temperature and high-pressure epitope retrieval. Cooled down to room temperature. Washed sections with PBS twice for 3 min.

[0298] Blocking: removed wash buffer, drew a hydrophobic circle around the tissue with a PAP pen. Blocked sections with peroxidase-blocking reagent for 15 min at room temperature. Washed sections with PBS twice for 3 min each.

[0299] Primary antibody incubation: conducted product-specific protocol for antibody dilution. Removed wash buffer and cover sections with 100-400µL primary antibody diluted in recommended antibody diluent. Incubated for 1 hour at 37°C in a humidified chamber. Washed sections in PBS twice for 3 min each.

[0300] Secondary antibody incubation: removed wash buffer and covered sections with 1-3 drops of HRP conjugated goat anti-rabbit & mouse reagent. Incubated for 30 min at 37°C in a humidified chamber. Wash sections in PBS twice for 3 min each.

[0301] DAB staining: DAB-containing substrate working solution was prepared by mixing thoroughly 1 drop concentrated DAB solution per 1mL DAB substrate buffer. Removed wash buffer and covered tissue section in 1X DAB working solution for 3 min at room temperature.

Washed sections in PBS twice for 3 min each.

[0302] Counterstain: completely submersed sections in hematoxylin and incubated for 1 min. Immersed slides in bluing solution. Rinsed slides in tap water for 3 min.

[0303] Dehydration and clearing: dehydrated with a series of grade EtOH washes (70%, 85%, 95%, 100%) for 3 min, respectively. Immersed in fresh xylene for twice for 10 min each.

[0304] Mounting: mounted sections with mounting medium.

2. FFPE Blocks Preparation on Cell line

[0305] Harvested cells respectively during the logarithmic growth period and counted cell number using Count-star.

[0306] About 5×10^6 cells (1×10^7) were collected after centrifuged at 1000 rpm at 4°C for 24 hour.

[0307] Discarded the supernatant, then added 30 mL 10% NBF into a 50mL tube with cell pellet.

[0308] Prepared cell suspension by reversing the tube several times. Then the tube was placed on bench flatwise and fixed the cells in 10%NBF for 30 min.

[0309] Centrifuged samples at 1000 rpm for 5 min at 4°C, discarded all the fixative carefully without disturbing cell pellet at the tube bottom, and placed samples in the incubator at 50°C for preheating.

[0310] Heated HistoGel in boiling water bath for 3-10 minutes and placed liquid HistoGel in the incubator at 50°C.

[0311] Added one or two drop of HistoGel on deposits of cell, vortexed for seconds to mix thoroughly. Then placed mixture on ice for 5-10 minutes to solidify the gel.

[0312] Placed the cell gel into a long hole embedding box. In a 250 mL beaker, rinsed the cell gel slowly with running water for 1h, then added 50%, 75%, 85%, 95% ethanol to the cell block sequentially, let the cell gel soak for 1h in each ethanol solution. Finally, soaked the cell gel in 100% ethanol twice, for 1h each time.

[0313] Removed the cell blocks from 100% ethanol and soaked them in xylene twice, 1h each time.

[0314] Soaked the cell block in liquid paraffin twice, 1h each time, but can stay overnight.

[0315] Prepared FFPE block according to standard embedding process. Tissues were embedded in paraffin on Paraffin Embedding Station.

Example 1 – Hybridoma campaign for top clones

[0316] The PTK7 ECD protein was prepared in house and used as antigen. Monoclonal antibodies against PTK7 were developed by sequentially immunizing animals with PTK7-his antigen proteins with an adjuvant, and the experimental animals are Balb/c mice.

[0317] The animals were immunized with 100 µg of antigen per animal for the first shot, subsequently 50 µg of antigen per animal was used for immunization for the second and third

shot, and 25 µg for the booster immunization. The immune adjuvant used in the experiments was Freund's Adjuvant (complete and incomplete). All animals were immunized by intraperitoneal injection. The mice with good immunized titer and serum immunohistochemistry (IHC) positive were chosen for booster immunization. After booster immunization, mice were sacrificed and soaked in 75% alcohol. The spleen was dissected out, grounded with a grinding rod, and filtered through a cell strainer to prepare a single cell suspension. The spleen cell suspension was centrifuged at 1,500 rpm for 5 min, and the supernatant was discarded. 5 mL red blood cell lysate was added to lyse red blood cells at room temperature for 5 min and PBS was added to reach 20 mL. After centrifugation at 1,500 rpm for 5 min, the supernatant was discarded. Viable cells were counted after resuspension. The Sp2/0 cells in the culture flask were collected and after centrifuged at 1,500 rpm for 5 min, the supernatant was discarded. Viable cells were counted after resuspension. The spleen cells were mixed with Sp2/0 cells at a ratio of 4:1 and subjected to centrifugation at 1,500 rpm for 5 min, the supernatant was discarded. The cells were resuspended in 20 mL electroporation buffer. After centrifugation at 1,500 rpm for 7 min, the supernatant was discarded, and the step was repeated once. The cells were resuspended with an appropriate amount of electroporation buffer to ensure the cell concentration of about 2×10^7 cells/mL. The cell suspension was added to a 9 mL electroporation tank for fusion. After fusion, the cell suspension was transferred to 20 mL RPMI 1640 complete medium containing 20% FBS and then left at room temperature for 20 min. The fused cells were resuspended with RPMI 1640 medium containing 1xHAT, 1xBIOMYC3, and 20% FBS. The cell suspension was added to several 96-well cell culture plates at 100 µL/well to ensure that the cell volume per well was about 5×10^4 cells/well, and the plates were placed in a 37°C cell incubator. After 7 days, additional 100 µL of RPMI 1640 complete medium containing 20 % FBS, 1xHAT, and 1xBIOMYC-3 was added to each well. After 10 days of fusion, the cell culture supernatants from hybridoma parent clones were collected and used for screening by binding to human PTK7-his protein by ELISA and IHC method. The ELSIA and IHC both positive clones were subsequently chosen for further validation.

Example 2 – IHC method- mice serum validation

[0318] FFPE (Formalin-Fixed Paraffin-Embedded) sections of ovarian cancer were baked in a dry oven for 1 hour at 60°C, and then deparaffinized in fresh xylene for 10min and rehydrated through graded concentrations of ethanol (100%, 95%, 85%, 75%) to distilled water, the sections were washed with PBS twice for 3 min each. Epitope retrieval was performed by heating the sections in the pH 9.0 EDTA antigen retrieval solution (1X) in a pressure cooker until boiling was initiated. The sections were cooled down to room temperature. The sections were washed with distilled water twice for 3 min. Endogenous peroxidase activity was blocked by incubating the

sections in peroxidase-blocking reagent for 15 min at room temperature, then washed with PBS twice for 3 min each. Immunodetection of PTK7 was performed using the primary antibody (mice serum) against PTK7 at dilution of 1:500, 1:2000, respectively. The sections were covered with 100-400 μ L of primary antibody dilutions and incubated for 1 hour at 37°C. The sections were washed in PBS twice for 3 min each. Sections were then incubated with the HRP conjugated goat anti-rabbit & mouse reagent for 30 mins at 37°C and were visualized using DAB-containing substrate working solution as chromogen for 3 min at room temperature, resulting in brown staining. The sections were washed with PBS twice for 3 min each. Finally, the sections were counterstained (with hematoxylin and incubated for 1 min, rinsed in tap water for 3 min), dehydrated (through graded concentrations of ethanol (70%, 85%, 95%, 100%) for 3 min, respectively) and mounted in mounting medium.

Example 3 – IHC method-hybridoma screening (manual)

[0319] The sections of formalin-fixed, paraffine-embedded tumor tissue were baked in a dry oven for 1 hour at 60°C, and then deparaffinized in fresh xylene for 10 min and rehydrated through graded concentrations of ethanol (100%, 95%, 85%, 75%) to distilled water, the sections were washed with PBS twice for 3 mins. Epitope retrieval was performed by heating the slides in the pH9.0 EDTA antigen retrieval solution (1X) in a pressure cooker until boiling was initiated. The sections cooled down to room temperature. The sections were washed with distilled water twice for 3 min. Endogenous peroxidase activity was blocked by incubating the sections in peroxidase-blocking reagent for 15 min at room temperature. Then the sections were washed with PBS twice for 3 min each. Immunodetection of PTK7 was performed using the primary antibody (hybridoma supernatant) against PTK7 and the sections were covered with 100-400 μ L primary antibody dilutions and incubated for 1 hour at 37°C. The sections were washed in PBS twice for 3 min each. The sections were then incubated with the HRP conjugated goat anti-rabbit & mouse reagent for 30 mins at 37°C and were visualized using DAB-containing substrate working solution as chromogen for 3 min at room temperature, resulting in brown staining. The sections were washed with PBS twice for 3 min each. Finally, the sections were counterstained (with hematoxylin and incubated for 1 min, rinsed in tap water for 3 min), dehydrated (through graded concentrations of ethanol (70%, 85%, 95%, 100%) for 3 min, respectively) and mounted in mounting medium. The results are shown in FIGs. 1A-1D.

Example 4 – IHC method-subclone screening (manual)

[0320] The slices of formalin-fixed, paraffine-embedded tumor tissue were baked in a dry oven for 1 hour at 60°C, and then deparaffinized in fresh xylene for 10 min and rehydrated through graded concentrations of ethanol (100%, 95%, 85%, 75%) to distilled water. The sections were

washed with PBS twice for 3 mins. Epitope retrieval was performed by heating the slides in the pH9.0 EDTA antigen retrieval solution (1X) in a pressure cooker until boiling is initiated. The sections cooled down to room temperature. The sections were washed with distilled water twice for 3 min. Endogenous peroxidase activity was blocked by incubating the sections in peroxidase-blocking reagent for 15 min at room temperature, then the sections were washed with PBS twice for 3 min each. Immunodetection of PTK7 was performed using the primary antibody (hybridoma supernatant) against PTK7 and the sections were covered with 100-400 µL primary antibody dilutions, and incubated for 1 hour at 37°C. The sections were washed in PBS twice for 3 min each. The sections were then incubated with the HRP conjugated goat anti-rabbit & mouse reagent for 30 mins at 37°C and were visualized using DAB-containing substrate working solution as chromogen for 3 min at room temperature, resulting in brown staining. The sections were washed with PBS twice for 3 min each. Finally, the sections were counterstained (with hematoxylin and incubated for 1 min, rinsed in tap water for 3 min), dehydrated (through graded concentrations of ethanol (70%, 85%, 95%, 100%) for 3 min, respectively) and mounted in mounting medium. Table 1 shows IHC screening results of top 8 subclones (underlined) in ovarian and breast cancers.

Table 1: Top8 subclone IHC screening results in ovarian cancer and breast cancer

Sample number	Clone number	Ovarian	Breast cancer
26	M1-13A8	Cytoplasm positive and nuclear membrane positive	Cytoplasm positive and nuclear membrane positive
28	M1-15B5	Membrane 2+, stroma 2+	Membrane 2+, stroma 2+
204	M6-9E10	Stoma positive	Membrane 2+, stroma 2+
<u>206</u>	<u>M6-9H5</u>	Membrane 1+, stroma 1+	Membrane 1+, stroma 1+
<u>222</u>	<u>M6-2C6</u>	Membrane 3+, stroma 3+	Membrane 3+, stroma 3+
226	M6-13A4	negative	negative
<u>235</u>	<u>M6-14H10</u>	Membrane 3+, stroma 3+ with strong positive staining in immune cells	Membrane 3+, stroma 3+ with strong positive staining in immune cells
246	M6-15H4	Pan staining	Pan staining
270	M6-18H11	Cytoplasm positive	Cytoplasm positive

<u>312</u>	<u>M10-5F7</u>	Membrane 3+, stroma negative	negative
<u>58</u>	<u>M1-11E5</u>	Cytoplasm positive and nuclear membrane positive	Cytoplasm positive and membrane positive
<u>134</u>	<u>M6-17F7</u>	Membrane 3+, stroma positive	Membrane positive
148	M6-20A4	negative	negative
156	M10-1A6	negative	negative
<u>236</u>	<u>M6-8G5</u>	Membrane 3+, stroma 3+	Membrane 3+, stroma 3+
254	M6-11B1	negative	negative
<u>259</u>	<u>M6-11E2</u>	Membrane 3+, stroma 3+	Membrane 3+, stroma 3+

[0321] Selection of 206-M6-9H5 (M10091-01)(see FIGs. 2A-2B), 222-M6-2C6 (M10091-04) (see FIGs. 3A-3B), 235-M6-14H10 (M10091-05) (see FIGs. 4A-4B), 312-M10-5F7 (M10091-08) (see FIGs. 5A-5B), 58-M1-11E5 (M10091-06) (see FIGs. 6A-6B), 134-M6-17F7 (M10091-03) (see FIGs. 7A-7B), 236-M6-8G5 (M10091-07) (see FIGs. 8A-8B), 259-M6-11E2 (M10091-02) (see FIGs. 9A-9B), based on good and distinctive staining results.

[0322] Top 3 subclone IHC results: Table 2 shows the recommended concentration of top3 subclones for IHC test; table 3 shows the IDs and tissue sources of the tumor sections used in the titration assays of the top 3 subclones; table 4 shows the tissue types used in tissue microarray (TMA) of the top 3 subclones.

[0323] 11E2: Ovarian cancer cell membrane positive, breast cancer cell membrane positive, tumor stroma positive. (see FIG.10)

[0324] 8G5: Ovarian cancer cell membrane positive, breast cancer cell membrane positive, tumor stroma positive. (see FIG.11)

[0325] 5F7: Ovarian cancer cell membranes are positive, breast cancer tumor cells are negative, and tumor stroma is positive. (see FIG.12)

[0326] CST: Ovarian cancer cell membranes are positive, breast cancer cell membranes are positive, and tumor stroma is positive. (see FIG.13)

Table 2: Top 3 subclone recommended IHC concentration

No.	Clone number	Conc.	Volume	Buffer	IHC recommended test concentration

1	M10091-11E2	0.88mg/ml	1.2ml	PBS+0.05% NaN ₃	1 µg/mL
2	M10091-8G5	0.96mg/ml	1.1ml	20mM Tris+1%BSA+0.05% NaN ₃	2 µg/mL
3	M10091-5F7	1mg/ml	1.1ml	20mM Tris+1%BSA+0.05% NaN ₃	2 µg/mL

Table 3: Samples

Sample	Tissue type	Comment
TMA026	Tissue micro array	Table 4
OC2300219/OC2300104	Ovarian cancer tissue	NA
BC2201004	Breast cancer tissue	NA
TO2309705	Tonsil tissue	NA

Table 4: Tissue type

tonsil	colon	appendix	spleen	pancreas	liver
stomach	lung	kidney	cardiac muscle	brain	uterus
breast	colorectal cancer	thyroid gland	prostate cancer	breast cancer	ovarian cancer
squamous cell lung carcinoma	lung adenocarcinoma	hepatocellular carcinoma	renal carcinoma	gastric cancer	malignant melanoma

Example 5 – IHC method-Top clone optimization for TMA (manual)

[0327] The sections of formalin-fixed, paraffine-embedded tumor tissue were baked in a dry oven for 1 hour at 60°C, and then deparaffinized in fresh xylene for 10min and rehydrated through graded concentrations of ethanol (100%, 95%, 85%, 75%) to distilled water. The sections were washed with PBS twice for 3 mins. Epitope retrieval was performed by heating the sections in the pH9.0 EDTA antigen retrieval solution (1X) in a pressure cooker until boiling was initiated. The sectioned cooled down to room temperature and then were washed with distilled water twice for 3 min. Endogenous peroxidase activity was blocked by incubating the sections in peroxidase-blocking reagent for 15 min at room temperature, the sections were washed with PBS twice for 3 min each. Immunodetection of PTK7 was performed using the primary antibody against PTK7

at recommend concentration (1, 2, 4, 8 µg/mL, respectively) and the sections were covered with 100-400 µL primary antibody dilutions and incubated for 1 hour at 37°C. The sections were washed in PBS twice for 3 min each. The sections were then incubated with the HRP conjugated goat anti-rabbit & mouse reagent for 30 mins at 37°C and were visualized using DAB-containing substrate working solution as chromogen for 3 min at room temperature, resulting in brown staining. The sections were washed with PBS twice for 3 min each. Finally, the sections were counterstained (with hematoxylin and incubated for 1 min, rinsed in tap water for 3 min), dehydrated (through graded concentrations of ethanol (70%, 85%, 95%, 100%) for 3 min, respectively) and mounted in mounting medium.

[0328] Subclone 11E2 results are shown in FIGs. 14A-14D. Subclone 8G5 results are shown in FIGs. 15A-15D. Subclone 5F7 results are shown in FIGs. 16A-16D.

Example 6 – IHC method-TMA

[0329] One-millimeter cores from paraffin blocks of tumors were used to generate TMAs. Before staining, microarrays were baked in a dry oven for 1 hour at 60°C, these sections were deparaffinized in fresh xylene for 10min and rehydrated through graded concentrations of ethanol (100%, 95%, 85%, 75%) to distilled water, then washed with PBS twice for 3 min. Epitope retrieval was performed by heating the sections in the pH9.0 EDTA antigen retrieval solution (1X) in a pressure cooker until boiling is initiated. The sections cooled down to room temperature then were washed with distilled water twice for 3 min. Endogenous peroxidase activity was blocked by incubating the sections in peroxidase-blocking reagent for 15 min at room temperature, then sections were washed with PBS twice for 3 min each. Immunodetection of PTK7 was performed using the primary antibody against PTK7 at recommend concentration (1, 2, 2 µg/mL, respectively) and the sections were covered with 100-400 µL of primary antibody dilutions and incubated for 1 hour at 37°C. The sections were washed in PBS twice for 3 min each. The sections were then incubated with the HRP conjugated goat anti-rabbit & mouse reagent for 30 mins at 37°C and were visualized using DAB-containing substrate working solution as chromogen for 3 min at room temperature, resulting in brown staining. The sections were washed with PBS twice for 3 min each. Finally, the sections were counterstained (with hematoxylin and incubate for 1 min, rinsed in tap water for 3 min), dehydrated (through graded concentrations of ethanol (70%, 85%, 95%, 100%) for 3 min, respectively) and mounted in mounting medium. Table 5 shows the TMA-Leica. Table 6 shows the TMA- manual.

Table 5: TMA – IHC by Leica Autostainer

Leica

Tissue	M10091-02 (11E2)	M10091-07 (8G5)	M10091-08 (5F7)	CST PTK7/CCK4 (D2Z1N) Rabbit mAb
tonsil	Overall weak squamous epithelium has cell membrane localization staining and basal cell layer staining	Squamous epithelium has cell membrane localization staining and basal cell layer staining	Moderate to strong cytoplasmic staining	Basal cell layer staining
colon	Glandular cell membrane, stroma staining	Glandular cell membrane, stroma staining	stroma staining	Glandular cell membrane, stroma staining
Appendix	Glandular cell membrane, stroma staining	Glandular cell membrane, stroma staining	Glandular cell membrane, stroma staining, vascular endothelial staining	Glandular cell membrane, stroma staining
Spleen	No staining			limited cell staining
Pancreas	No staining	Strong staining of pancreatic cells	Weak staining of pancreatic cells and staining of vascular endothelium	Strong staining of pancreatic cells and nonspecific staining
Liver	No staining			Granular cytoplasmic staining of hepatocytes
Stomach				Diffuse cytoplasmic staining on the glands at the base of the gastric fovea
Lung	No staining	A small amount of alveolar cell cytoplasmic staining	No staining	A small amount of alveolar cell cytoplasmic staining

Kidney	weak staining of distal tubule and proximal tubule	strong staining of distal tubule and weak staining of proximal tubule	strong staining of distal tubule and weak staining of proximal tubule	strong staining of distal tubule and weak staining of proximal tubule
Myocardium	No staining			background staining
Brain	very limited cell staining	limited cell staining	limited cell staining	limited cell staining
Uterus	gland staining	gland staining	No staining	gland staining
Mammary gland	failed			
Thyroid	slight non-specific staining			
colon cancer	Tumor stroma staining	Tumor stroma staining	tumor cell and stroma staining	strong staining
prostate cancer	Weak staining of cell membrane	Strong staining of cell membrane	Weak staining of cell membrane	cytoplasmic staining
breast cancer	Weak staining of cell membrane	Strong staining of cell membrane	cytoplasmic staining	Cell membrane and cytoplasmic staining
ovarian cancer	Positive staining	Positive staining	Positive staining, no stroma staining	Positive staining
lung squamous cell carcinoma	failed			
lung adenocarcinoma	stroma staining	stroma staining	lung adenocarcinoma cell and stroma staining	lung adenocarcinoma cell and stroma staining
liver cancer	No staining			granular cytoplasmic staining
stomach cancer	only a few atypical cells staining in the tissue			
malignant melanoma	Tumor cell membrane staining			Tumor cell nuclear staining

Table 6: TMA – IHC by manual staining

Manual

Tissue	M10091-02 (11E2)	M10091-07 (8G5)	M10091-08 (5F7)	CST PTK7/CCK4 (D2Z1N) Rabbit mAb
tonsil	Overall weak squamous epithelium has cell membrane localization staining and basal cell layer staining	Squamous epithelium has cell membrane localization staining and basal cell layer staining	Moderate to strong cytoplasmic staining	Basal cell layer staining
colon	Glandular cell membrane, stroma staining	Glandular cell membrane, stroma staining	stroma staining	Glandular cell membrane, stroma staining
Appendix	Glandular cell membrane, stroma staining	Glandular cell membrane, stroma staining	stroma staining, vascular endothelial staining	Glandular cell membrane, stroma staining
Spleen	No staining			limited cell staining
Pancreas	Strong staining of pancreatic cells	Strong staining of pancreatic cells	Weak staining of pancreatic cells and staining of vascular endothelium	Strong staining of pancreatic cells and nonspecific staining
Liver	No staining			Granular cytoplasmic staining of hepatocytes
Stomach	Diffuse cytoplasmic staining on the glands at the base of the gastric fovea	No staining		Diffuse cytoplasmic staining on the glands at the base of the gastric fovea
Lung	A small amount of alveolar cell cytoplasmic staining	A small amount of alveolar cell cytoplasmic staining	No staining	A small amount of alveolar cell cytoplasmic staining

Kidney	Cytoplasmic staining, strong staining in distal tubules, weak staining in proximal tubules	Cytoplasmic staining, strong staining in distal tubules, weak staining in proximal tubules	cytoplasmic staining	Cytoplasmic staining, strong staining in distal tubules, weak staining in proximal tubules
Myocardium	No staining			Background staining
Brain	very limited cell staining	limited cell staining	limited cell staining	limited cell staining
Uterus	gland staining	gland staining	No staining	gland staining
Mammary gland	breast duct staining			
Thyroid	slight non-specific staining			
colon cancer	Tumor stroma staining	Tumor stroma staining	tumor cell staining	strong staining
prostate cancer	Weak to moderate membrane staining in prostate cancer cells and ducts			
breast cancer	Cell membrane staining	Cell membrane staining	No staining	Cell membrane staining
ovarian cancer	Positive staining	Positive staining	Positive staining, no stroma staining	Positive staining
lung squamous cell carcinoma	Squamous tumor cells moderately staining	Squamous tumor cells strong staining	Squamous tumor cells moderately staining	Squamous tumor cells strong staining
lung adenocarcinoma	no staining in adenocarcinoma cells, stroma staining	no staining in adenocarcinoma cells, stroma staining	both adenocarcinoma cells and stroma staining	both adenocarcinoma cells and stroma staining
liver cancer	No staining			granular cytoplasmic staining
stomach cancer	only a few atypical cells staining in the tissue			
malignant melanoma	Tumor cell membrane staining	Tumor cell membrane staining	No staining of tumor cells, staining of vascular endothelium	Tumor cell nuclear staining

[0330] Subclone 11E2 TMA results are shown in FIGs. 17A-17B. Subclone 8G5 TMA results are shown in FIGs. 18A-18B. Subclone 5F7 TMA results are shown in FIGs. 19A-19B.

Example 7 – IHC method- tumor cell blocks (manual)

[0331] The sections of formalin-fixed, paraffine-embedded tumor cells were baked in a dry oven for 1 hour at 60°C, then deparaffinized in fresh xylene for 10min and rehydrated through graded concentrations of ethanol (100%, 95%, 85%, 75%) to distilled water. The sections were washed with PBS twice for 3 min. Epitope retrieval was performed by heating the slides in the pH9.0 EDTA antigen retrieval solution (1X) in a pressure cooker until boiling was initiated. The sections cooled down to room temperature. The sections were washed with distilled water twice for 3 min. Endogenous peroxidase activity was blocked by incubating the sections in peroxidase-blocking reagent for 15 min at room temperature, then the sections washed with PBS twice for 3 min each. Immunodetection of PTK7 was performed using the primary antibody against PTK7 at recommend concentration (1, 2, 2 µg/mL, respectively) and the sections were covered with 100-400 µL primary antibody dilutions, incubation for 1 hour at 37°C. The sections were washed in PBS twice for 3 min each. The sections were then incubated with the HRP conjugated goat anti-rabbit & mouse reagent for 30 mins at 37°C and were visualized using DAB-containing substrate working solution as chromogen for 3 min at room temperature, resulting in brown staining. The sections were washed with PBS twice for 3 min each. Finally, the sections were counterstained (with hematoxylin and incubated for 1 min, rinsed in tap water for 3 min), dehydrated (through graded concentrations of ethanol (70%, 85%, 95%, 100%) for 3 min, respectively) and mounted in mounting medium.

[0332] Top 3 cell line IHC.

[0333] PTK7 antigen expression level: PA-1>MDA-MB-453>MCF-7. Raji is negative control. Subclone 11E2 results are shown in FIG. 20. Subclone 8G5 results are shown in FIG. 21. Subclone 5F7 results are shown in FIG. 22. PA-1 is a cancer cell line originally isolated from an ovarian cancer patient. MDA-MB-453 is a cancer cell line originally from a patient with metastatic breast carcinoma. MCF-7 is a cancer cell line isolated originally from a breast adenocarcinoma patient.

[0334] Having thus described the basic concepts, it may be rather apparent to those skilled in the art after reading this detailed disclosure that the foregoing detailed disclosure is intended to be presented by way of example only and is not limiting. Various alterations, improvements, and modifications may occur and are intended to those skilled in the art, though not expressly stated herein. These alterations, improvements, and modifications are intended to be suggested by this disclosure and are within the spirit and scope of the exemplary embodiments of this disclosure.

[0335] Moreover, certain terminology has been used to describe embodiments of the present disclosure. For example, the terms “one embodiment,” “an embodiment,” and “some embodiments” mean that a particular feature, structure or characteristic described in connection with the embodiment is included in at least one embodiment of the present disclosure. Therefore, it is emphasized and should be appreciated that two or more references to “an embodiment” or “one embodiment” or “an alternative embodiment” in various portions of this specification are not necessarily all referring to the same embodiment. Furthermore, the particular features, structures or characteristics may be combined as suitable in one or more embodiments of the present disclosure.

[0336] Further, it will be appreciated by one skilled in the art, aspects of the present disclosure may be illustrated and described herein in any of a number of patentable classes or context including any new and useful process, machine, manufacture, or composition of matter, or any new and useful improvement thereof.

[0337] Furthermore, the recited order of processing elements or sequences, or the use of numbers, letters, or other designations, therefore, is not intended to limit the claimed processes and methods to any order except as may be specified in the claims. Although the above disclosure discusses through various examples what is currently considered to be a variety of useful embodiments of the disclosure, it is to be understood that such detail is solely for that purpose and that the appended claims are not limited to the disclosed embodiments, but, on the contrary, are intended to cover modifications and equivalent arrangements that are within the spirit and scope of the disclosed embodiments.

[0338] Similarly, it should be appreciated that in the foregoing description of embodiments of the present disclosure, various features are sometimes grouped together in a single embodiment, figure, or description thereof to streamline the disclosure aiding in the understanding of one or more of the various embodiments. This method of disclosure, however, is not to be interpreted as reflecting an intention that the claimed subject matter requires more features than are expressly recited in each claim. Rather, claim subject matter lie in less than all features of a single foregoing disclosed embodiment.

FURTHER EMBODIMENTS – BINDING AGENTS

1. A binding agent of protein tyrosine kinase 7 (PTK7), comprising:
 - a heavy chain variable (VH) region and a light chain variable (VL) region, the VH region comprising complementarity determining regions HCDR1, HCDR2 and HCDR3 disposed in heavy chain variable region framework regions and the VL region comprising LCDR1, LCDR2 and LCDR3 disposed in light chain variable region framework regions, the VH and VL CDRs having amino acids sequences selected from the sets of amino acid sequences set forth in the group consisting of:
 - a. SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, LVS, and SEQ ID NO: 7, respectively;
 - b. SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, QMS, and SEQ ID NO: 14, respectively; and
 - c. SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, RVS, and SEQ ID NO: 21, respectively.
2. The binding agent of 1, wherein the VH and VL regions have amino acid sequences that are selected from the pairs of amino acid sequences set forth in the group consisting of:
 - a. SEQ ID NO: 1 and SEQ ID NO: 2, respectively;
 - b. SEQ ID NO: 8 and SEQ ID NO: 9, respectively; and
 - c. SEQ ID NO: 15 and SEQ ID NO: 16, respectively.
3. The binding agent of 1, wherein the VH and VL regions have amino acid sequences that are selected from the pairs of amino acid sequences set forth in the group consisting of:
 - a. SEQ ID NO: 1 and SEQ ID NO: 2, respectively;
 - b. SEQ ID NO: 8 and SEQ ID NO: 9, respectively; and
 - c. SEQ ID NO: 15 and SEQ ID NO: 16, respectively,

wherein the heavy and light chain framework regions are optionally modified with from 1 to 8 amino acid substitutions, deletions or insertions in the framework regions.
4. The binding agent of any one of claims 1-3, wherein the framework regions are human framework regions.
5. The binding agent of any one of 1-3, wherein the framework regions are mouse framework regions.
6. The binding agent of any one of 1 -5, wherein the binding agent is an antibody.
7. The binding agent of any one of 1-6, wherein one of 1 to 6, wherein the binding agent further comprises a heavy chain constant region.
8. The binding agent of any one of claims 1-6, wherein the binding agent is a monoclonal antibody.

9. The binding agent of 8, wherein the heavy chain constant region is of the IgG isotype.
10. The binding agent of 8, wherein the heavy chain constant region is a human IgG1 constant region or a human IgG4 constant region.
11. The binding agent of 8, wherein the heavy chain constant region is a mouse IgG1 constant region, a mouse IgG2a constant region, a mouse IgG2c constant region, or a mouse IgG3 constant region.
12. The binding agent of 8, wherein the heavy chain constant region has the amino acid sequence set forth in SEQ ID NO: 22, 24, or 26.
13. The binding agent of any one of 1 to 11, wherein the binding agent further comprises a light chain constant region.
14. The binding agent of 13, wherein the light chain constant region has the amino acid sequence set forth in SEQ ID NO: 23, 25, or 27.
15. The binding agent of 1, wherein the binding agent is a monoclonal antibody comprising a VH region having the amino acid sequence set forth in SEQ ID NO: 1, a VL region having the amino acid sequence set forth in SEQ ID NO: 2, a heavy chain constant region having the amino acid sequence set forth in SEQ ID NO: 22 and a light chain constant region having the amino acid sequence set forth in SEQ ID NO: 23.
16. The binding agent of 1, wherein the binding agent is a monoclonal antibody comprising a VH region having the amino acid sequence set forth in SEQ ID NO: 8, a VL region having the amino acid sequence set forth in SEQ ID NO: 9, a heavy chain constant region having the amino acid sequence set forth in SEQ ID NO: 24 and a light chain constant region having the amino acid sequence set forth in SEQ ID NO: 25.
17. The binding agent of 1, wherein the binding agent is a monoclonal antibody comprising a VH region having the amino acid sequence set forth in SEQ ID NO: 15, a VL region having the amino acid sequence set forth in SEQ ID NO: 16, a heavy chain constant region having the amino acid sequence set forth in SEQ ID NO: 26 and a light chain constant region having the amino acid sequence set forth in SEQ ID NO: 27.

FURTHER EMBODIMENTS - IHC METHODS:

A1. A method of detecting a PTK7 expressing cell in a sample, comprising:

contacting the sample with a detecting agent comprising the binding agent of any one of further numbered embodiments 1-17, and

detecting formation of a binding complex between the detecting agent and PTK7, wherein the detection of the binding complex is indicative of the presence of a PTK7 expressing cell in the sample.

A2. The method of A1, wherein the detecting agent further comprises a detectable marker that labels the binding agent.

A2.1. The method of A2, wherein the detectable marker includes biotin or an enzyme marker.

A2.2. The method of A2.1, wherein the enzyme marker includes horseradish peroxidase (HRP) or alkaline phosphatase (AP).

A2.3. The method of any one of A2 - A2.2, wherein the detecting the formation of the binding complex between the detecting agent and PTK7 comprises:

adding a partner agent of the detectable marker; and

detecting a reaction of the detectable marker with the partner agent as an indication of the formation of the binding complex.

A2.4. The method of A2, wherein the detectable marker includes a fluorescent tag.

A2.5. The method of A2.4, wherein the detecting the formation of the binding complex between the detecting agent and PTK7 comprises:

detecting a signal from the fluorescent tag as an indication of the formation of the binding complex.

A3. The method of A1, wherein the detecting the formation of the binding complex between the detecting agent and PTK7 comprises:

contacting the sample with a secondary antibody that is labeled and configured to bind to the binding complex; and

detecting a signal from the secondary antibody as an indication of the formation of the binding complex.

A3.1. The method of A3, wherein the secondary antibody is labeled with a detectable marker.

A3.2. The method of A3.1, wherein the detectable marker includes biotin or an enzyme marker.

A3.3. The method of any one of A3.1-A3.2, wherein the detecting the formation of the binding complex between the detecting agent and PTK7 comprises:

adding a partner agent of the detectable marker; and

detecting a reaction of the detectable marker with the partner agent as an indication of the formation of the binding complex.

A3.4. The method of claim A3.1, wherein the detectable marker includes a fluorescent tag.

A3.5. The method of claim A3.4, wherein the detecting the formation of the binding complex between the detecting agent and PTK7 comprises:

detecting a signal from the fluorescent tag as an indication of the formation of the binding complex.

A3.6. The method of A3.4, wherein the fluorescent tag includes fluorescein (FITC), phycoerythrin (PE), preferable R-PE, or allophycocyanin (APC).

A4. The method of A1, wherein the sample is from a tissue of a subject.

A4.1. The method of A4, wherein the tissue is selected from the group consisting of tonsil, appendix, breast, ovary, colon, prostate, skin, lung, uterus, cervix, kidney, pancreas, bladder, brain, thyroid, ear, nose, throat, and esophagus.

A4.2. The method of any one of A4-A4.1, wherein the tissue is selected from the group consisting of breast, ovary, colon, prostate, skin, lung, uterus, esophagus, and bladder.

A4.3. The method of any one of A4-A4.2, wherein the tissue is breast.

A4.4. The method of any one of A4-A4.2, wherein the tissue is ovary.

A5. The method of A1, wherein the sample is from a subject and the presence of PTK7 expressing cell indicates that the subject is suffering from a disease.

A5.1. The method of A5.1, wherein the sample is from a subject and the method further comprises: quantifying binding PTK expressing cells in the sample to obtain a quantification result, wherein the quantification result over a pre-determined threshold indicates that the subject is suffering from a disease.

A5.2. The method of A5 or A5.1, wherein the disease is cancer.

A5.3. The method of A5 or A5.1, wherein the disease is selected from the group consisting of breast cancer, ovarian cancer, colon cancer, prostate cancer, melanoma, lung cancer, esophageal cancer, gastric cancer, endometrial cancer, head and neck cancer, and bladder cancer.

A5.4. The method of claim A5 or A5.1, wherein the disease is breast cancer.

A5.5. The method of claim A5 or A5.1, wherein the disease is ovarian cancer.

A5.6. The method of claim A5 or A5.1, wherein the disease is lung squamous cells carcinoma or lung adenocarcinoma.

FURTHER EMBODIMENTS - DISEASE DIAGNOSIS, TREATMENT AND MONITORING

B1. A method of determining whether a subject is suffering from a disease, comprising:

obtaining a sample from the subject;

contacting the sample with a detecting agent comprising the binding agent of any one of further numbered embodiments 1-17, and

determining whether the subject is suffering from the disease by detecting and/or quantifying formation of binding complexes between the detecting agent and PTK7, wherein the presence of the binding complexes or a level of the binding complexes over a pre-determined threshold indicates that the subject is suffering from the disease.

B1.1. The method of B1, wherein the disease is cancer.

B2. A method of treating a disease, comprising:

obtaining a sample from a subject;

contacting the sample with a detecting agent comprising the binding agent of any one of

1-17;

determining whether the subject is suffering from the disease by detecting and/or quantifying formation of binding complexes between the detecting agent and PTK7, wherein the presence of the binding complexes or a level of the binding complexes over a pre-determined threshold indicates that the subject is suffering from the disease; and

treating the disease.

B2.1. The method of B2, wherein the disease is cancer.

B2.2. The method of B2.1, wherein the disease is selected from the group consisting of breast cancer, ovarian cancer, colon cancer, prostate cancer, melanoma, lung cancer, esophageal cancer, gastric cancer, endometrial cancer, head and neck cancer, and bladder cancer.

B2.3. The method of any one of B2-B2.2, wherein the treatment includes administering chemotherapy to the subject.

B2.4. The method of any one of B2-B2.2, wherein the treatment includes administering hormone therapy to the subject.

B2.5. The method of any one of B2-B2.2, wherein the treatment includes administering radiation therapy to the subject.

B2.6. The method of any one of B2-B2.2, wherein the treatment includes administering immunotherapy to the subject.

B2.7. The method of any one of B2-B2.2, wherein the treatment includes administering stem cell therapy to the subject.

B2.8. The method of any one of B2-B2.2, wherein the treatment includes administering targeted therapy to the subject.

B2.9. The method of any one of B2-B2.2, wherein the treatment includes performing surgery on the subject.

B2.10. The method of any one of B2-B2.2, wherein the disease is breast cancer and the treatment includes lumpectomy or mastectomy.

B2.11. The method of any one of B2-B2.2, wherein the disease is ovarian cancer and the treatment includes hysterectomy or salpingo-oophorectomy.

B3. A method of monitoring progression of a disease in a subject, comprising the steps of:

- (a) obtaining a sample from the subject;
- (b) contacting the sample with a detecting agent comprising the binding agent of any one of further numbered embodiments 1-17;
- (c) assessing formation of binding complexes between the detecting agent and PTK7 to obtaining a first assessment result;
- (d) repeating steps (a), (b), and (c) using a sample from the subject at a subsequent point in time to obtaining a second assessment result; and

- (e) comparing the second assessment result to the first assessment result to determine the progression of the disease in the subject.

B3.1. The method of B3, wherein the disease is cancer.

FURTHER EMBODIMENTS - KITS

C1. A kit for detecting PTK7 expressing cells in a sample, the kit comprising a detecting agent that comprises the binding agent of any one of further numbered embodiments 1-17.

C2. The kit of claim C1, further comprising a second antibody that is labeled and configured to bind to a binding complex that is formed by the detecting agent and PTK7.

SEQUENCE LISTING

SEQ ID NO: 1

QVTLKESGPGILQPSQTLSTCSFSGFSLNTFGMGVSWIRQPSGNGLEWLAHIYWDDGKDYNP
SLKSRLTISKDTSNNQVFLKITTVDTTDTATYYCAYGFAYWGQGTTLTVSA

SEQ ID NO: 2

DVVMQTPTLTSVTIGQPASISCKSSQSLLSINGKTYLNWLLQRPQGSPKRLIHLVSKLDSGVPD
RFTGSGSGTDFTLKISRVEAEDLGVYYCVQGTTHFPHTFGGGTKLEIK

SEQ ID NO: 3 GFSLNTFGMG

SEQ ID NO: 4 IYWDDGK

SEQ ID NO: 5 AYGFA

SEQ ID NO: 6 QSLLSINGKTY

SEQ ID NO: 7 VQGTTHFPHT

SEQ ID NO: 8

EVMLVESGGGSAKPGGSLKLSCEASGFTFSSYAMSWVRQTPGKRLEWVASISNGGSYTNYPD
SVKGRFTISRDNKNTLYLQMTSLRSEDTAIYYCSNTGTSYYGFYWGQGTTLTVSS

SEQ ID NO: 9

DIVMTQAAFSSPVTLGTSASISCRSSESLYSNGITYLYWYLQKPGQSPQLLIYQMSNLASGVPD
RFSSSGSGTDFTLRISRVEAEDVGVYYCAQNLELPYTFGGGTRLEIK

SEQ ID NO: 10 GFTFSSYA

SEQ ID NO: 11 ISNGGSYT

SEQ ID NO: 12 SNTGTSYYGFY

SEQ ID NO: 13 ELLYSNGITY

SEQ ID NO: 14 AQNLELPYT

SEQ ID NO: 15

EVQLQQSGPVLVKPGASVKISCKASGYFTDYNMHWRQSHGKSLEWIGYIYPYNGGTGYNQ

KFKSKATLTVDNSSNTAYLELRSLTSEDSAVYYCARGGWYFDVWGAGTTVTVSS

SEQ ID NO: 16

DAVMTQTPLSLPVSLGDQASISCRSSQSLENSNGNTYLNWYLQKPGQSPQLLIYRVSNRFGSV
LDRFSGSGSGTDFTLKISRVEAEDLGVYFCLQITHVPFTFGSGTKLEIK

SEQ ID NO: 17 GYTFTDYN

SEQ ID NO: 18 IYPYNGGT

SEQ ID NO: 19 ARGGWYFDV

SEQ ID NO: 20 QSLENSNGNTY

SEQ ID NO: 21 LQITHVPFT

SEQ ID NO: 22

AKTTPPSVYPLAPGSAAQTNSMVTLGCLVKGYFPEPVTVTWNSGSLSSGVHTFPAVLQSDLYTL
SSSVTVPSSTWPSETVTCNVAHPASSTKVDKKIVPRDCGCKPCICTVPEVSSVFIFPPKPKDVLTI
TLTPKVTCTVVDISKDDPEVQFSWFVDDVEVHTAQTQPREEQFNSTFRSVSELPIMHQDWLNG
KEFKCRVNSAAFPAPIEKTISKTKGRPKAPQVYTIPPPKEQMAKDKVSLTCMITDFFPEDITVEW
QWNGQPAENYKNTQPIMDTDGSYFVYSKLVQKSNWEAGNTFTCSVLHEGLHNHHTEKSLSH
SPGK

SEQ ID NO: 23

RADAAPTVSIFPPSSEQLTSGGASVVCFLNNFYPKDINVKWKIDGSERQNGVLNSWTDQDSKD
STYSMSSTLTLTKEDEYERHNSYTCEATHKTSTSPIVKSFNRECE

SEQ ID NO: 24

AKTTPPSVYPLAPGSAAQTNSMVTLGCLVKGYFPEPVTVTWNSGSLSSGVHTFPAVLQSDLYTL
SSSVTVPSSTWPSETVTCNVAHPASSTKVDKKIVPRDCGCKPCICTVPEVSSVFIFPPKPKDVLTI
TLTPKVTCTVVDISKDDPEVQFSWFVDDVEVHTAQTQPREEQFNSTFRSVSELPIMHQDWLNG
KEFKCRVNSAAFPAPIEKTISKTKGRPKAPQVYTIPPPKEQMAKDKVSLTCMITDFFPEDITVEW
QWNGQPAENYKNTQPIMDTDGSYFVYSKLVQKSNWEAGNTFTCSVLHEGLHNHHTEKSLSH
SPGK

SEQ ID NO: 25

RADAAPTVSIFPPSSEQLTSGGASVVCFLNNFYPKDINVKWKIDGSERQNGVLNSWTDQDSKD
STYSMSSTLTLTKEDEYERHNSYTCEATHKTSTSPIVKSFNRECE

SEQ ID NO: 26

AKTTPPSVYPLAPGSAAQTNSMVTLGCLVKGYFPEPVTVTWNSGSLSSGVHTFPAVLQSDLYTL
SSSVTVPSSTWPSETVTCNVAHPASSTKVDKKIVPRDCGCKPCICTVPEVSSVFIFPPKPKDVLTI
TLTPKVTCTVVDISKDDPEVQFSWFVDDVEVHTAQTQPREEQFNSTFRSVSELPIMHQDWLNG
KEFKCRVNSAAFPAPIEKTISKTKGRPKAPQVYTIPPPKEQMAKDKVSLTCMITDFFPEDITVEW
QWNGQPAENYKNTQPIMDTDGSYFVYSKLVQKSNWEAGNTFTCSVLHEGLHNHHTEKSLSH
SPGK

SEQ ID NO: 27

RADAAPTVSIFPPSSEQLTSGGASVVCFLNNFYPKDINVKWKIDGSERQNGVLNSWTDQDSKD
STYSMSSTLTLTCKDEYERHNSYTCEATHKTSTSPIVKSFNRECE

SEQ ID NO: 28

QVTLKESGPGILQPSQTLSTCSFSGFSLNTFGMGVSWIRQPSGNGLEWLAHIYWDDGKDYNP
SLKSRLTISKDTSNNQVFLKITTVDTTDTATYYCAYGFAYWGQGLTVTVSAAKTTPPSVYPLAPG
SAAQTNSMVTLGCLVKGYFPEPVTVTWNSGSLSSGVHTFPAVLQSDLYTLSSSVTVPSSTWPS
ETVTCNVAHPASSTKVDDKIVPRDCGCKPCICTVPEVSSVFIFPPKPKDVLITLTPKVTCTVVDIS
KDDPEVQFSWFVDDVEVHTAQTQPREEQFNSTFRSVSELPIMHQDWLNGKEFKCRVNSAAFP
APIEKTISKTKGRPKAPQVYTIPPPKEQMAKDKVSLTCMITDFFPEDITVEWQWNGQPAENYKNT
QPMDDTGSYFVYSKLVQKSNWEAGNTFTCSVLHEGLNHHTEKSLSHSPGK

SEQ ID NO: 29

DVVMQTPLTSLVTIGQPASISCKSSQSLLSINGKTYLNWLLQRPQGQSPKRLIHLVSKLDGSGVPD
RFTGSGSGTDFTLRISRVEAEDLVVYCVQGTTHFPHTFGGGKLEIKRADAAPTVSIFPPSSEQL
TSGGASVVCFLNNFYPKDINVKWKIDGSERQNGVLNSWTDQDSKSTYSMSSTLTLTCKDEYER
HNSYTCEATHKTSTSPIVKSFNRECE

SEQ ID NO: 30

EVMLVESGGGSAKPGGSLKLSCEASGFTFSSYAMSWVRQTPGKRLEWVASISNGGSYTNYPD
SVKGRFTISRDNANKNTLYLQMTSLRSEDAIYYCSNTGTSYYGFYWGQGTTLTVSSAKTTPPS
VYPLAPGSAAQTNSMVTLGCLVKGYFPEPVTVTWNSGSLSSGVHTFPAVLQSDLYTLSSSVTVPS
SSTWPSETVTCNVAHPASSTKVDDKIVPRDCGCKPCICTVPEVSSVFIFPPKPKDVLITLTPKVT
CVVVDISKDDPEVQFSWFVDDVEVHTAQTQPREEQFNSTFRSVSELPIMHQDWLNGKEFKCR
VNSAAFPAPIEKTISKTKGRPKAPQVYTIPPPKEQMAKDKVSLTCMITDFFPEDITVEWQWNGQP
AENYKNTQPMDDTGSYFVYSKLVQKSNWEAGNTFTCSVLHEGLNHHTEKSLSHSPGK

SEQ ID NO: 31

DIVMTQAAFSSPVTLGTSASISCRSSESLYSNGITYLYWYLQKPGQSPQLLIYQMSNLASGVPD
RFSSSGSGTDFTLRISRVEAEDVGVYCAQNLELPYTFGGGTRLEIKRADAAPTVSIFPPSSEQL
TSGGASVVCFLNNFYPKDINVKWKIDGSERQNGVLNSWTDQDSKSTYSMSSTLTLTCKDEYER
HNSYTCEATHKTSTSPIVKSFNRECE

SEQ ID NO: 32

EVQLQQSGPVLVKPGASVKISCKASGYTFTDYNMHWRQSHGKSLEWIGYIYPYNGGTGYNQ
KFKSKATLTVDNSSNTAYLELRSLTSEDSAVYYCARGGWYFDVWGAGTTVTVSSAKTTPPSVYP
LAPGSAAQTNSMVTLGCLVKGYFPEPVTVTWNSGSLSSGVHTFPAVLQSDLYTLSSSVTVPSST
WPSETVTCNVAHPASSTKVDDKIVPRDCGCKPCICTVPEVSSVFIFPPKPKDVLITLTPKVTCTV
VDISKDDPEVQFSWFVDDVEVHTAQTQPREEQFNSTFRSVSELPIMHQDWLNGKEFKCRVNS
AAFPAPIEKTISKTKGRPKAPQVYTIPPPKEQMAKDKVSLTCMITDFFPEDITVEWQWNGQPAEN

YKNTQPIMDTDGSYFVYSKLVQKSNWEAGNTFTCSVLHEGLHNHHTKSLSHSPGK

SEQ ID NO: 33

DAVMTQTPLSLPVSLGDQASISCRSSQSLENSNGNTYLNWYLQKPGQSPQLLIYRVSNRFSGV
LDRFSGSGSGTDFTLKISRVEAEDLGVYFCLQITHVPFTFGSGTKLEIKRADAAPTVSIFPPSSEQ
LTSGGASVVCFLNNFYPKDINVKWKIDGSERQNGVLNSWTDQDSKDSTYSMSSTLTLTKEYE
RHNSYTCEATHKTSTSPIVKSFNRECE

WHAT IS CLAIMED IS:

1. A binding agent for protein tyrosine kinase 7 (PTK7), the binding agent comprising:
a heavy chain variable (VH) region and a light chain variable (VL) region, the VH region comprising complementarity determining regions HCDR1, HCDR2 and HCDR3 disposed in heavy chain variable region framework regions and the VL region comprising LCDR1, LCDR2 and LCDR3 disposed in light chain variable region framework regions, wherein the VH and VL are selected from one of the following pairs of VH and VL set out in a. to c. below:
 - a. the VH comprising a HCDR1 comprising SEQ ID NO: 3, a HCDR2 comprising SEQ ID NO: 4, and a HCDR3 comprising SEQ ID NO: 5, and the VL comprising a LCDR1 comprising SEQ ID NO: 6, a LCDR2 comprising LVS, and a LCDR3 comprising SEQ ID NO: 7;
 - b. the VH comprising a HCDR1 comprising SEQ ID NO: 10, a HCDR2 comprising SEQ ID NO: 11, and a HCDR3 comprising SEQ ID NO: 12, and the VL comprising a LCDR1 of SEQ ID NO: 13, a LCDR2 comprising QMS, and a LCDR3 comprising SEQ ID NO: 14; and
 - c. the VH comprising a HCDR1 comprising SEQ ID NO: 17, a HCDR2 comprising SEQ ID NO: 18, and a HCDR3 comprising SEQ ID NO: 19, and the VL comprising a LCDR1 of SEQ ID NO: 20, a LCDR2 comprising RVS, and a LCDR3 comprising SEQ ID NO: 21(LCDR3).
2. The binding agent of claim 1, wherein the VH and VL region are selected from one of the following pairs of VH and VL regions:
 - a. a VH comprising SEQ ID NO: 1 and a VL comprising SEQ ID NO: 2;
 - b. a VH comprising SEQ ID NO: 8 and a VL comprising SEQ ID NO: 9; and
 - c. a VH comprising SEQ ID NO: 15 and a VL comprising SEQ ID NO: 16.
3. The binding agent of claim 1, wherein the VH and VL regions have amino acid sequences that one of the following pairs of VH and VL sequences set forth below:
 - a. a VH comprising SEQ ID NO: 1 and a VL comprising SEQ ID NO: 2;
 - b. a VH comprising SEQ ID NO: 8 and a VL comprising SEQ ID NO: 9; and
 - c. a VH comprising SEQ ID NO: 15 and a VL comprising SEQ ID NO: 6,wherein the heavy and light chain framework regions are optionally modified with from 1 to 8 amino acid substitutions, deletions or insertions in the framework regions and the antibody retains the ability to bind PTK7.
4. The binding agent of any one of claims 1-3, wherein the framework regions are human framework regions.
5. The binding agent of any one of claims 1-3, wherein the framework regions are mouse

framework regions.

6. The binding agent of any one of claims 1-5, wherein the binding agent is an antibody.
7. The binding agent of any one of claims 1-6, wherein the binding agent is a monoclonal antibody.
8. The binding agent of any of the preceding claims, wherein the binding agent comprises a heavy chain comprising the VH and a heavy chain constant region.
9. The binding agent of claim 8, wherein the heavy chain constant region is of the IgG isotype.
10. The binding agent of claim 8, wherein the heavy chain constant region is a human IgG1 constant region or a human IgG4 constant region.
11. The binding agent of claim 8, wherein the heavy chain constant region is a mouse IgG1 constant region, a mouse IgG2a constant region, a mouse IgG2c constant region, or a mouse IgG3 constant region.
12. The binding agent of claim 8, wherein the heavy chain constant region has the amino acid sequence set forth in SEQ ID NO: 22, 24, or 26.
13. The binding agent of any of the preceding claims, wherein the binding agent comprises a light chain comprising the VL and a light chain constant region.
14. The binding agent of claim 13, wherein the light chain constant region has the amino acid sequence set forth in SEQ ID NO: 23, 25, or 27.
15. The binding agent of claim 1, wherein the binding agent is an antibody comprising:
 - a. a heavy chain comprising a VH region having the amino acid sequence set forth in SEQ ID NO: 1 and a heavy chain constant region having the amino acid sequence set forth in SEQ ID NO: 22; and
 - b. a light chain comprising a VL region having the amino acid sequence set forth in SEQ ID NO: 2 and a light chain constant region having the amino acid sequence set forth in SEQ ID NO: 23.
16. The binding agent of claim 1, wherein the binding agent is an antibody comprising:
 - a. a heavy chain comprising a VH region having the amino acid sequence set forth in SEQ ID NO: 8 and a heavy chain constant region having the amino acid sequence set forth in SEQ ID NO: 24; and
 - b. a light chain comprising a VL region having the amino acid sequence set forth in SEQ ID NO: 9 and a light chain constant region having the amino acid sequence set forth in SEQ ID NO: 25.
17. The binding agent of claim 1, wherein the binding agent is an antibody comprising:
 - a. a heavy chain comprising a VH region having the amino acid sequence set forth in SEQ ID NO: 15 and a heavy chain constant region having the amino acid sequence set forth in SEQ ID NO: 26; and

- b. a light chain comprising a VL region having the amino acid sequence set forth in SEQ ID NO: 16 and a light chain constant region having the amino acid sequence set forth in SEQ ID NO: 27.
18. The binding agent of claim 1 comprising a heavy chain of SEQ ID NO: 28 and a light chain of SEQ ID NO: 29.
19. The binding agent of claim 1 comprising a heavy chain of SEQ ID NO: 30 and a light chain of SEQ ID NO: 31.
20. The binding agent of claim 1 comprising a heavy chain of SEQ ID NO: 32 and a light chain of SEQ ID NO: 33.
21. A method of detecting a PTK7 expressing cell in a sample, comprising:
 contacting the sample with a detecting agent comprising the binding agent of any one of claims 1-20, and
 detecting formation of a binding complex between the detecting agent and PTK7, wherein the detection of the binding complex is indicative of the presence of a PTK7 expressing cell in the sample.
22. The method of claim 21, wherein the detecting agent further comprises a detectable marker that labels the binding agent.
23. The method of claim 21, wherein the detectable marker includes biotin or an enzyme marker.
24. The method of claim 23, wherein the enzyme marker includes horseradish peroxidase (HRP) or alkaline phosphatase (AP).
25. The method of any one of claims 21-24, wherein the detecting the formation of the binding complex between the detecting agent and PTK7 comprises:
 adding a partner agent of the detectable marker; and
 detecting a reaction of the detectable marker with the partner agent as an indication of the formation of the binding complex.
26. The method of claim 22, wherein the detectable marker includes a fluorescent tag.
27. The method of claim 21, wherein the detecting the formation of the binding complex between the detecting agent and PTK7 comprises:
 detecting a signal from the fluorescent tag as an indication of the formation of the binding complex.
28. The method of claim 21, wherein the detecting the formation of the binding complex between the detecting agent and PTK7 comprises:
 contacting the sample with a secondary antibody that is labeled and configured to bind to the binding complex; and
 detecting a signal from the secondary antibody as an indication of the formation of the binding complex.

29. The method of claim 28, wherein the secondary antibody is labeled with a detectable marker.
30. The method of claim 29, wherein the detectable marker includes biotin or an enzyme marker.
31. The method of any one of claims 25 to 30, wherein the detecting the formation of the binding complex between the detecting agent and PTK7 comprises:
- adding a partner agent of the detectable marker; and
 - detecting a reaction of the detectable marker with the partner agent as an indication of the formation of the binding complex.
32. The method of claim 25, wherein the detectable marker includes a fluorescent tag.
33. The method of claim 32, wherein the detecting the formation of the binding complex between the detecting agent and PTK7 comprises:
- detecting a signal from the fluorescent tag as an indication of the formation of the binding complex.
34. The method of claim 33 wherein the fluorescent tag includes fluorescein (FITC), phycoerythrin (PE), preferable R-PE, or allophycocyanin (APC).
35. The method of claim 21, wherein the sample is from a tissue of a subject.
36. The method of claim 35, wherein the tissue is selected from the group consisting of tonsil, appendix, breast, ovary, colon, prostate, skin, lung, uterus, cervix, kidney, pancreas, bladder, brain, thyroid, ear, nose, throat, and esophagus.
37. The method of claim 35, wherein the tissue is selected from the group consisting of breast, ovary, colon, prostate, skin, lung, uterus, esophagus, and bladder.
38. The method of any one of claims 35 to 37, wherein the tissue is breast.
39. The method of any one of claims 35 to 37, wherein the tissue is ovary.
40. The method of claim 21, wherein the sample is from a subject and the presence of PTK7 expressing cell indicates that the subject is suffering from a disease.
41. The method of claim 37, wherein the sample is from a subject and the method further comprises: quantifying binding PTK expressing cells in the sample to obtain a quantification result, wherein the quantification result over a pre-determined threshold indicates that the subject is suffering from a disease.
42. The method of claim 40 or 41, wherein the disease is cancer.
43. The method of claim 40 or 41, wherein the disease is selected from the group consisting of breast cancer, ovarian cancer, colon cancer, prostate cancer, melanoma, lung cancer, esophageal cancer, gastric cancer, endometrial cancer, head and neck cancer, and bladder cancer.
44. The method of claim 42 or 43, wherein the disease is breast cancer.
45. The method of claim 42 or 43, wherein the disease is ovarian cancer.
46. The method of claim 42 or 43, wherein the disease is lung squamous cells carcinoma or lung adenocarcinoma.

47. The method of any one of claims 21 to 46, wherein the method is an immunohistochemistry method.
48. Use of a binding agent according to any one of claims 1 to 20 to detect PTK7 protein.
49. A method of determining whether a subject is suffering from a disease, comprising:
obtaining a sample from the subject;
contacting the sample with a detecting agent comprising the binding agent of any one of claims 1-20, and
determining whether the subject is suffering from the disease by detecting and/or quantifying formation of binding complexes between the detecting agent and PTK7, wherein the presence of the binding complexes or a level of the binding complexes over a pre-determined threshold indicates that the subject is suffering from the disease.
50. The method of claim 49 wherein the disease is cancer.
51. A method of treating a disease in a subject, wherein the subject has been determined to have the disease by the method of claim 40.
52. The method of claim 51, wherein the binding agent comprises VH and VL regions comprising amino acid sequences set forth in: SEQ ID NO: 1 and SEQ ID NO: 2, respectively; SEQ ID NO: 8 and SEQ ID NO:9, respectively; or SEQ ID NO: 15 and SEQ ID NO: 16, respectively.
53. A method of treating a disease, comprising:
obtaining a sample from a subject;
contacting the sample with a detecting agent comprising the binding agent of any one of claims 1-20;
determining whether the subject is suffering from the disease by detecting and/or quantifying formation of binding complexes between the detecting agent and PTK7, wherein the presence of the binding complexes or a level of the binding complexes over a pre-determined threshold indicates that the subject is suffering from the disease; and
treating the disease.
54. The claims of 53, wherein the disease is cancer.
55. The method of claim 54, wherein the disease is selected from the group consisting of breast cancer, ovarian cancer, colon cancer, prostate cancer, melanoma, lung cancer, esophageal cancer, gastric cancer, endometrial cancer, head and neck cancer, and bladder cancer.
56. The method of any one of claims 53 to 55, wherein the treatment includes administering chemotherapy to the subject.
57. The method of any one of claims 53 to 56, wherein the treatment includes administering hormone therapy to the subject.
58. The method of any one of claims 53 to 56, wherein the treatment includes administering radiation therapy to the subject.

59. The method of any one of claims 53 to 56, wherein the treatment includes administering immunotherapy to the subject.
60. The method of any one of claims 53 to 56, wherein the treatment includes administering stem cell therapy to the subject.
61. The method of any one of claims 53 to 56, wherein the treatment includes administering targeted therapy to the subject.
62. The method of any one of claims 53 to 56, wherein the treatment includes performing surgery on the subject.
63. The method of any one of claims 53 to 56, wherein the disease is breast cancer and the treatment includes lumpectomy or mastectomy.
64. The method of any one of claims 53 to 56, wherein the disease is ovarian cancer and the treatment includes hysterectomy or salpingo-oophorectomy.
65. A method of monitoring progression of a disease in a subject, comprising the steps of:
- (a) contacting a sample from the subject with a detecting agent comprising the binding agent of any one of claims 1-20;
 - (b) assessing formation of binding complexes between the detecting agent and PTK7 to obtaining a first assessment result;
 - (c) repeating steps (a), (b), and (c) using a sample from the subject at a subsequent point in time to obtaining a second assessment result; and
 - (d) comparing the second assessment result to the first assessment result to determine the progression of the disease in the subject.
66. The method of 65, wherein the disease is cancer.
67. A kit for detecting PTK7 expressing cells in a sample, the kit comprising a detecting agent that comprises the binding agent of any one of claims 1-21.
68. The kit of claim 67, further comprising a second antibody that is labeled and configured to bind to a binding complex that is formed by the detecting agent and PTK7.

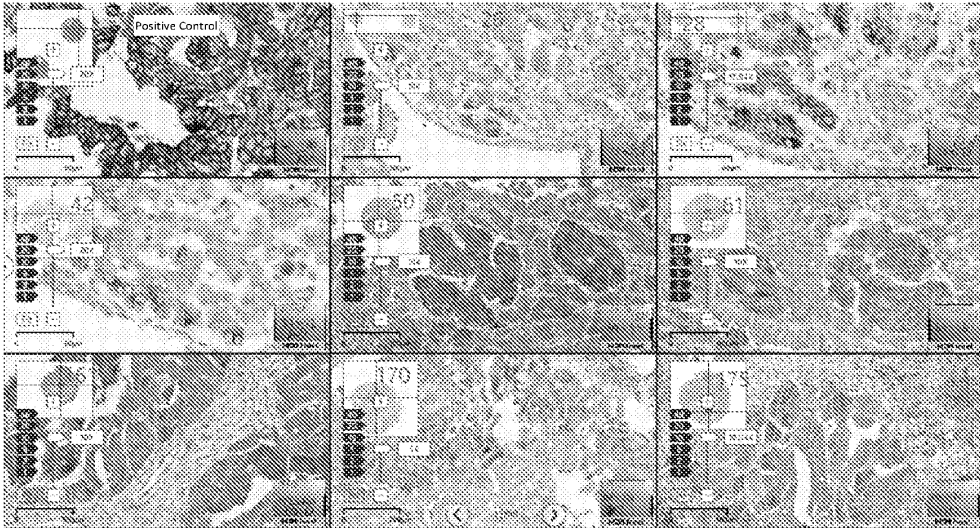


FIG. 1A

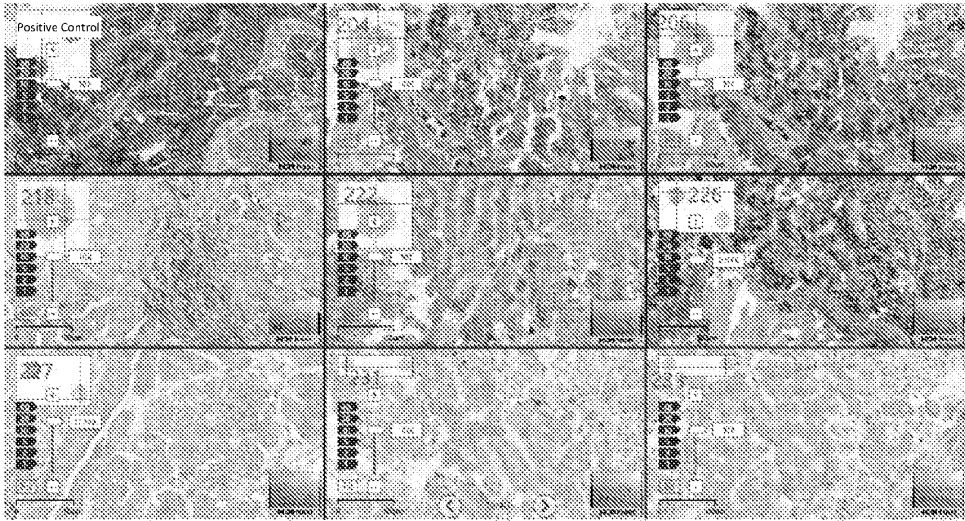


FIG. 1B

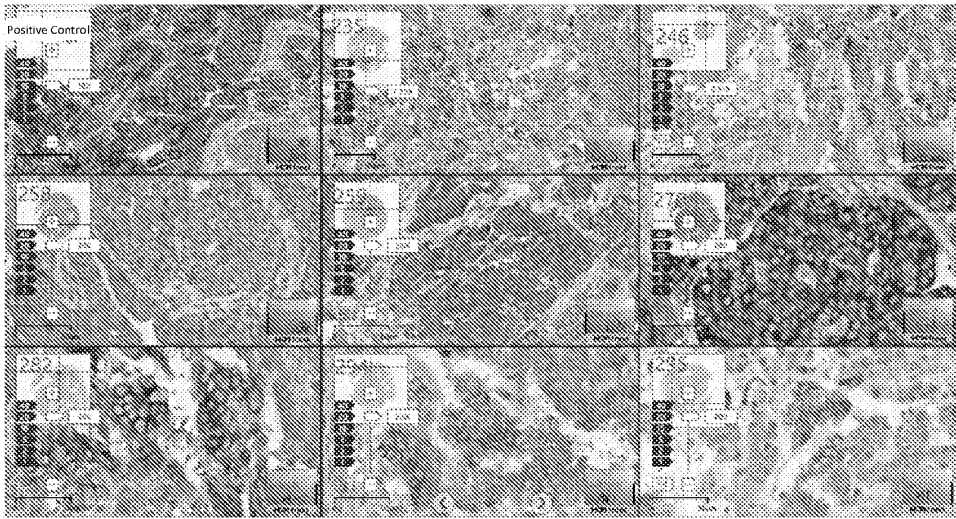


FIG. 1C

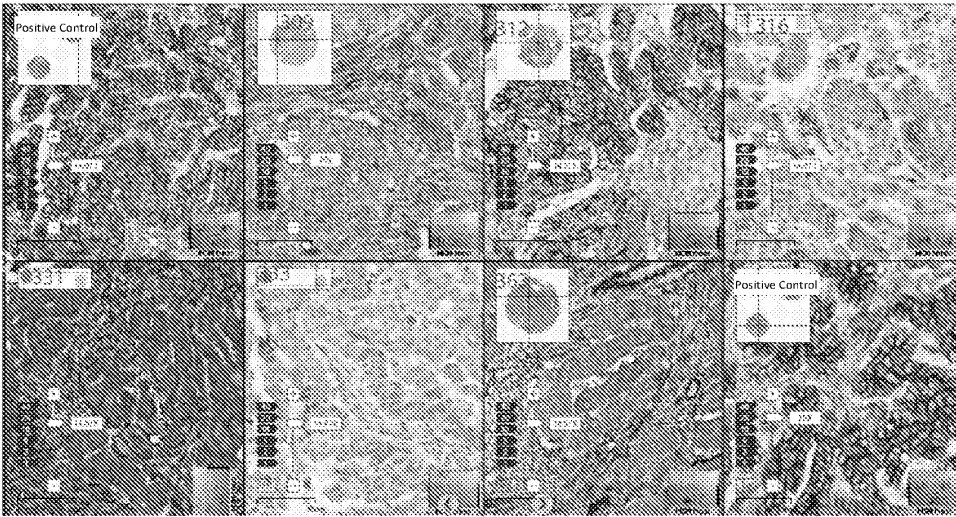


FIG. 1D

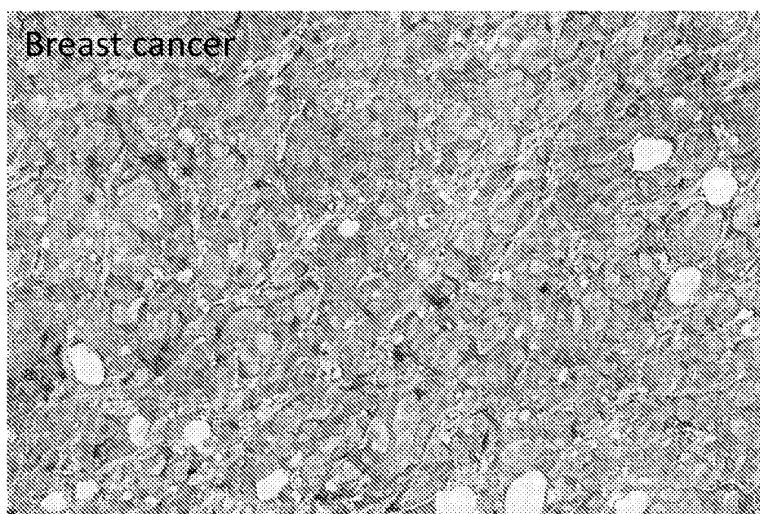


FIG. 2A

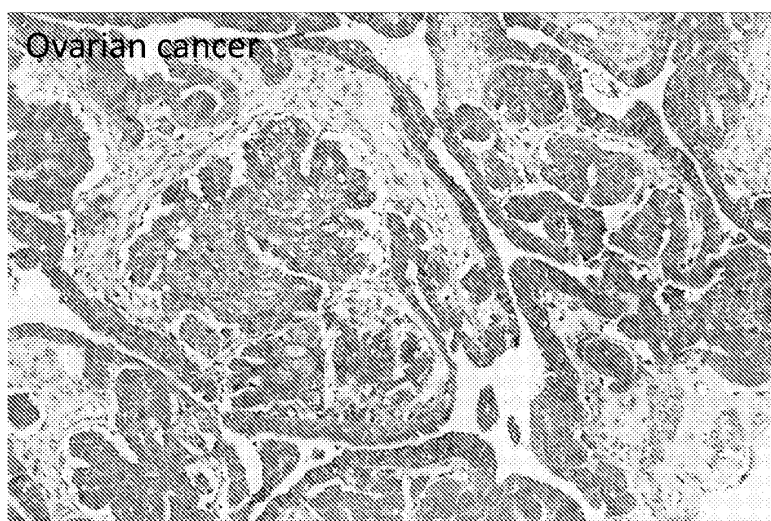


FIG. 2B

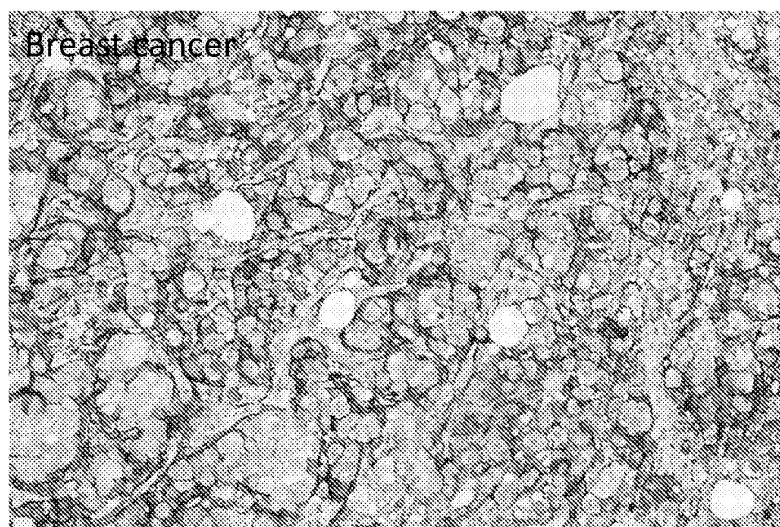


FIG. 3A

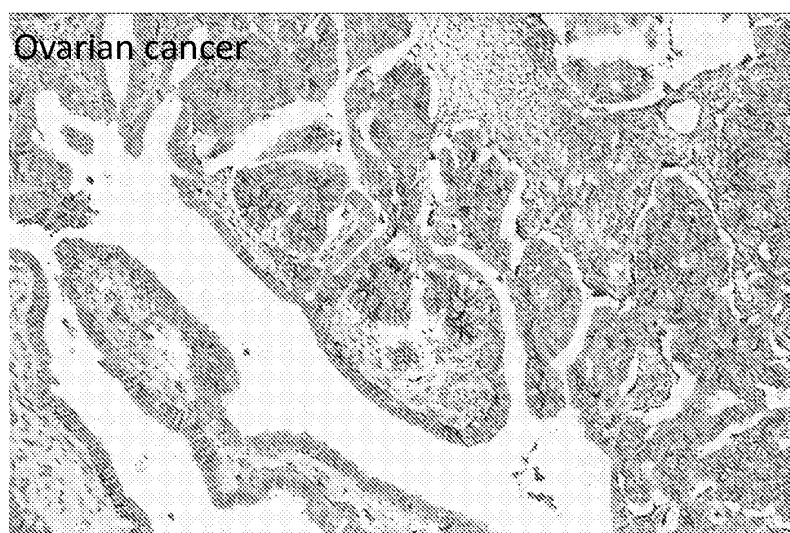


FIG. 3B

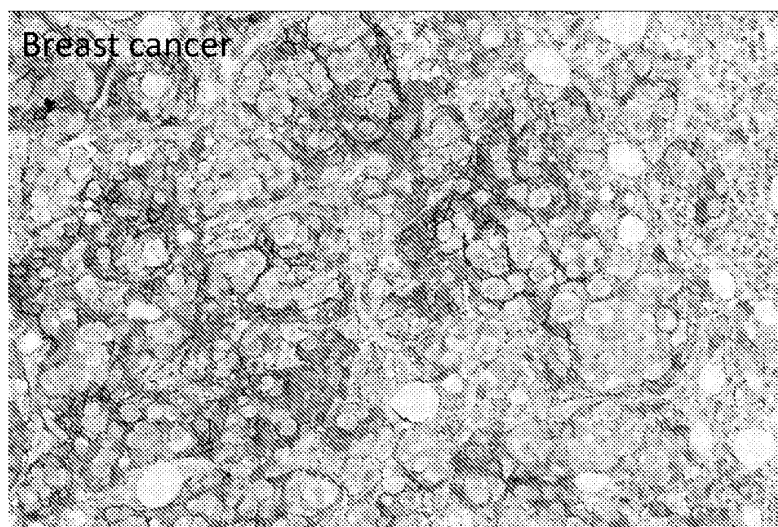


FIG. 4A

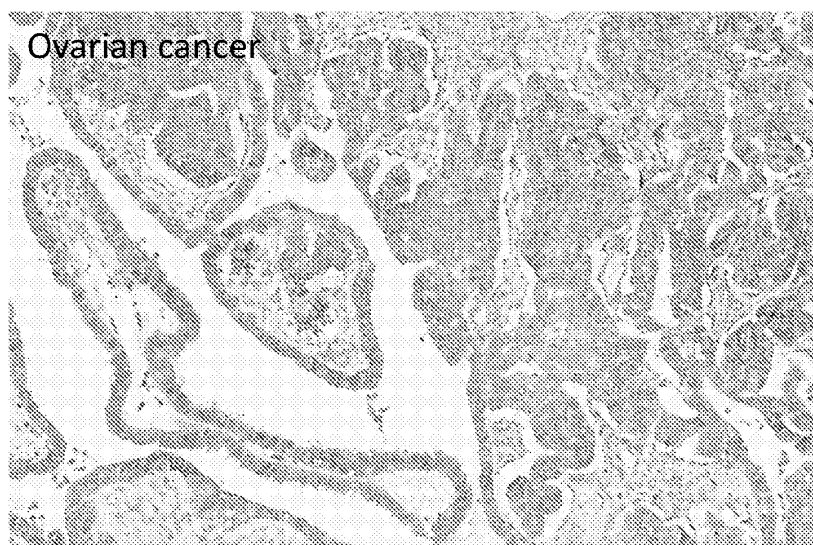


FIG. 4B

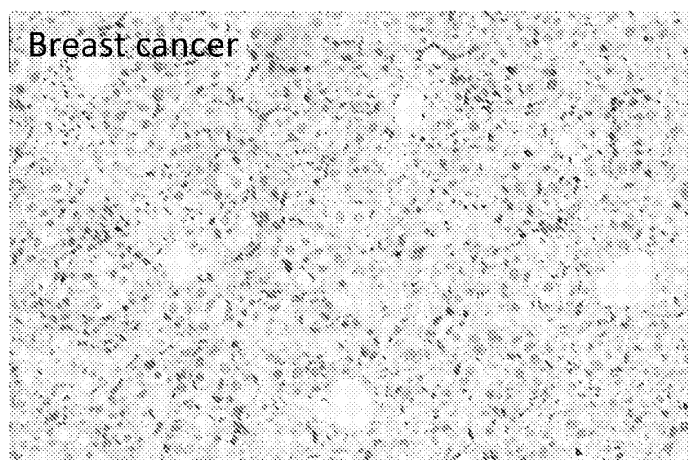


FIG. 5A

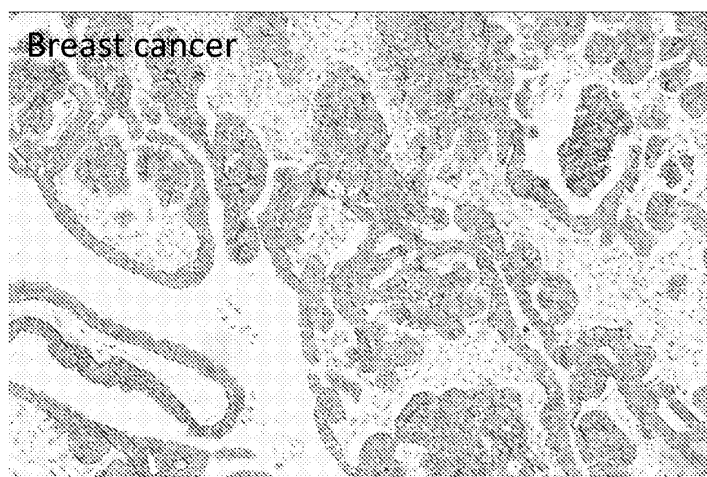


FIG. 5B

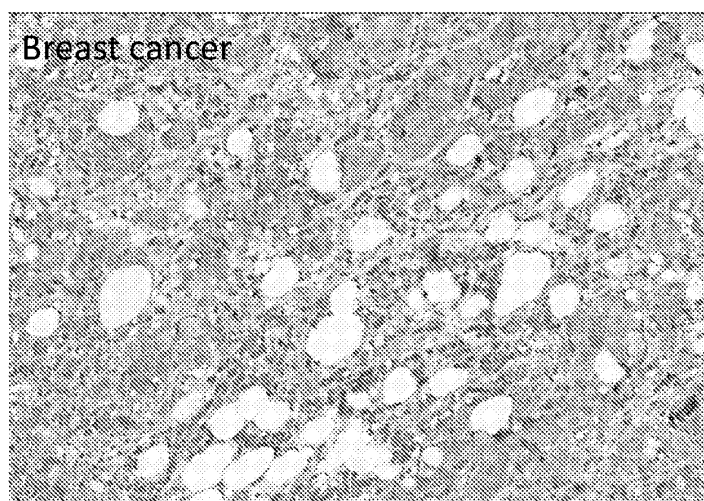


FIG. 6A

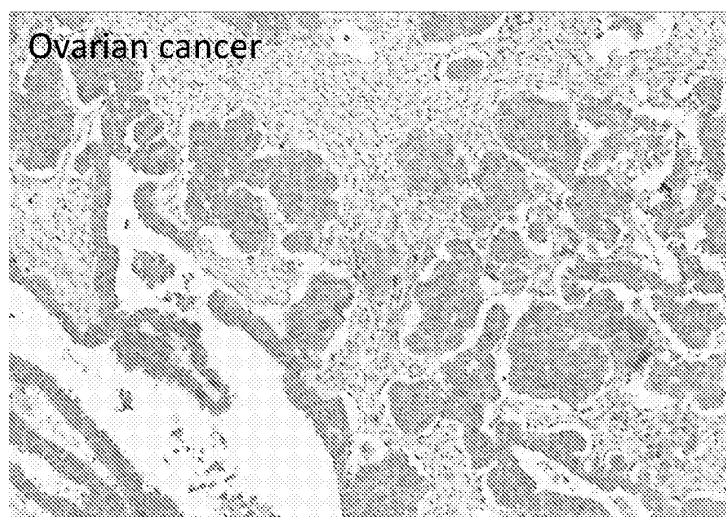


FIG. 6B

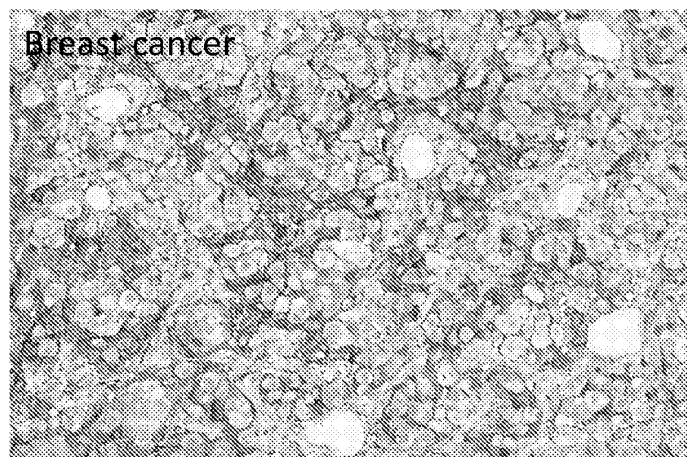


FIG.7A

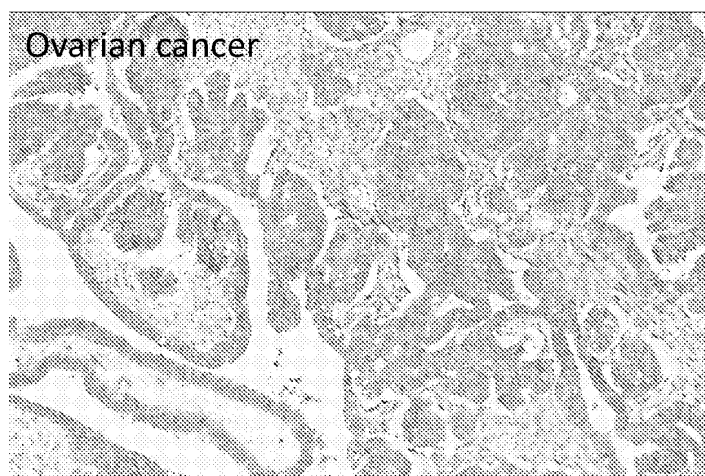


FIG. 7B

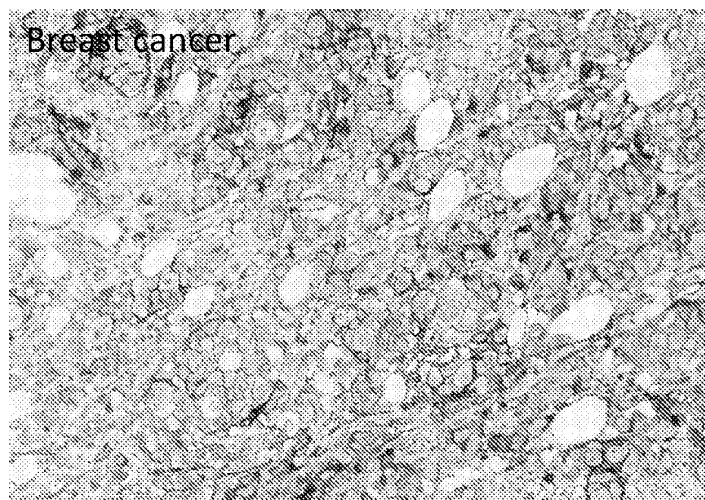


FIG. 8A

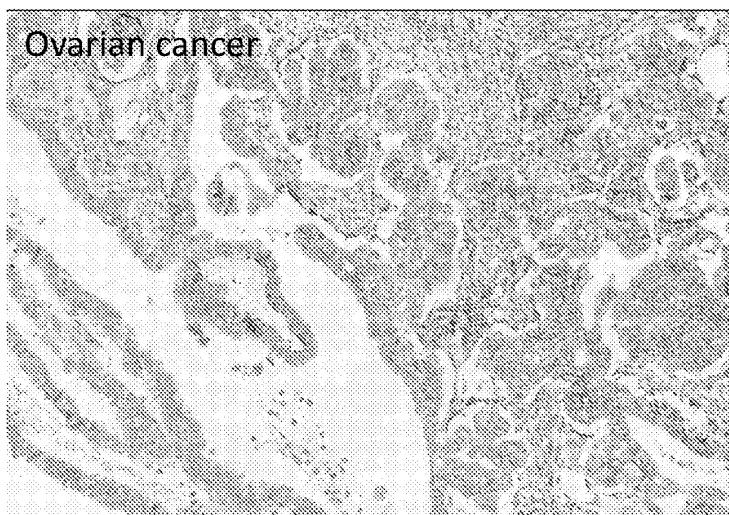


FIG. 8B

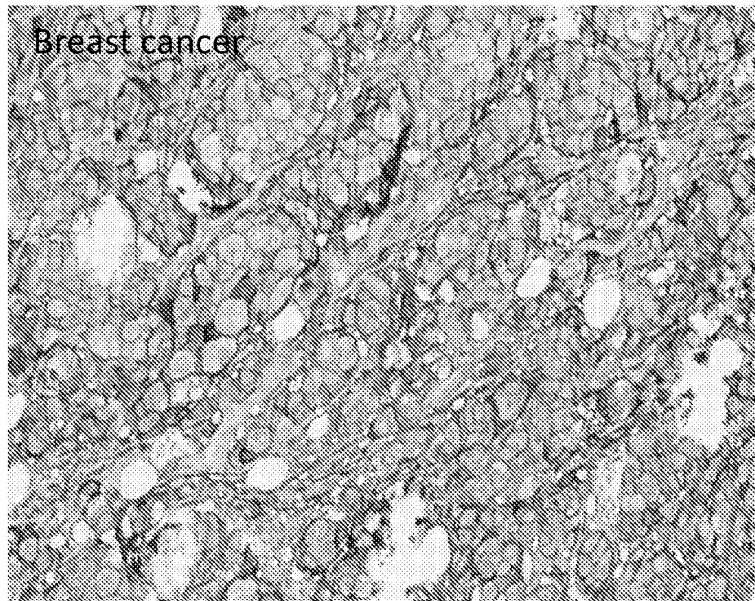


FIG. 9A

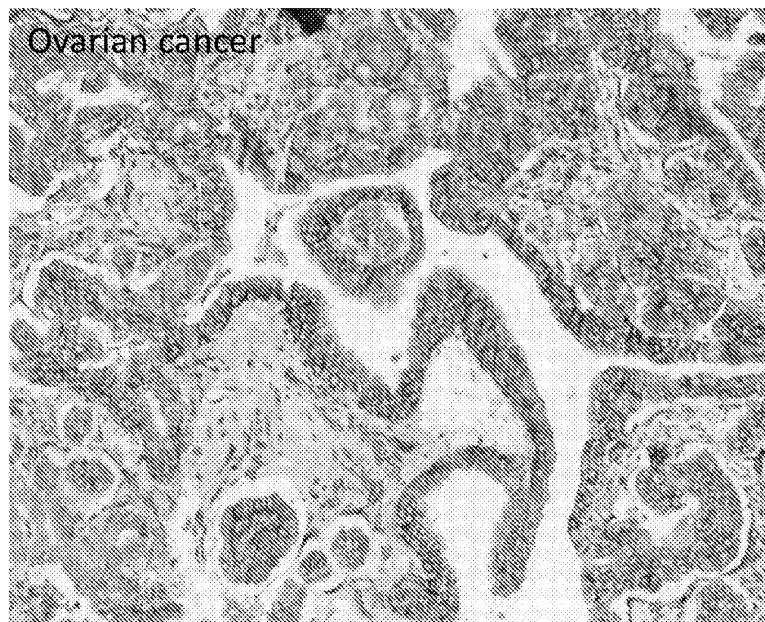


FIG. 9B

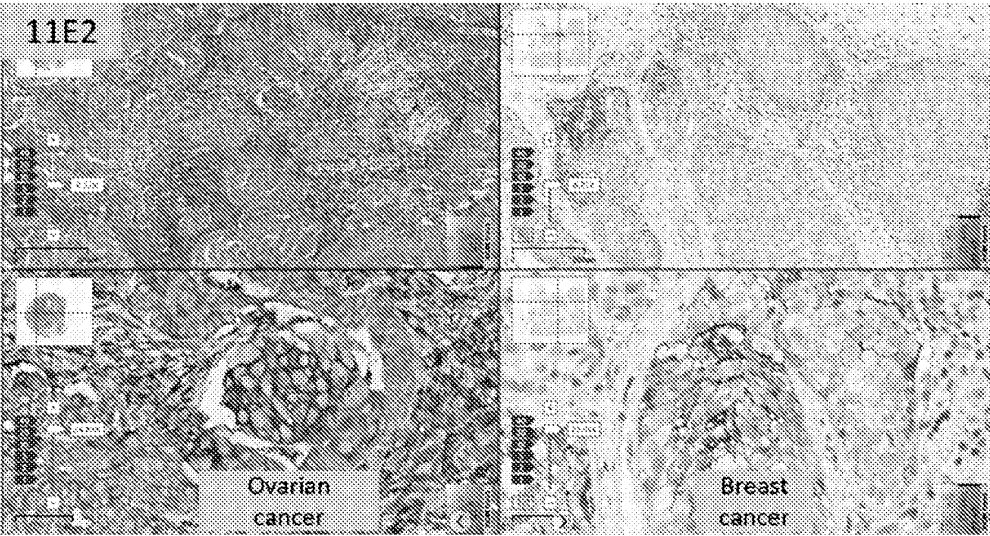


FIG. 10

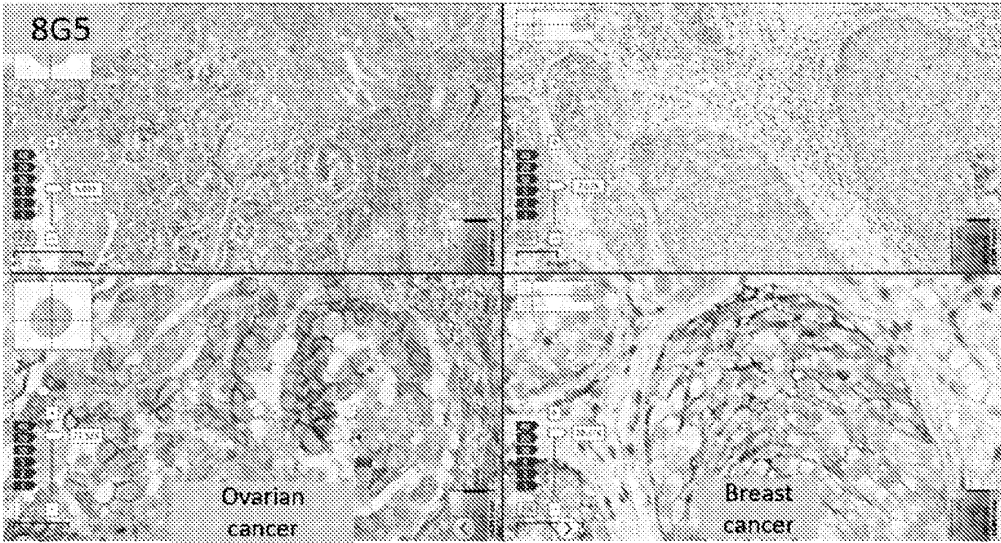


FIG. 11

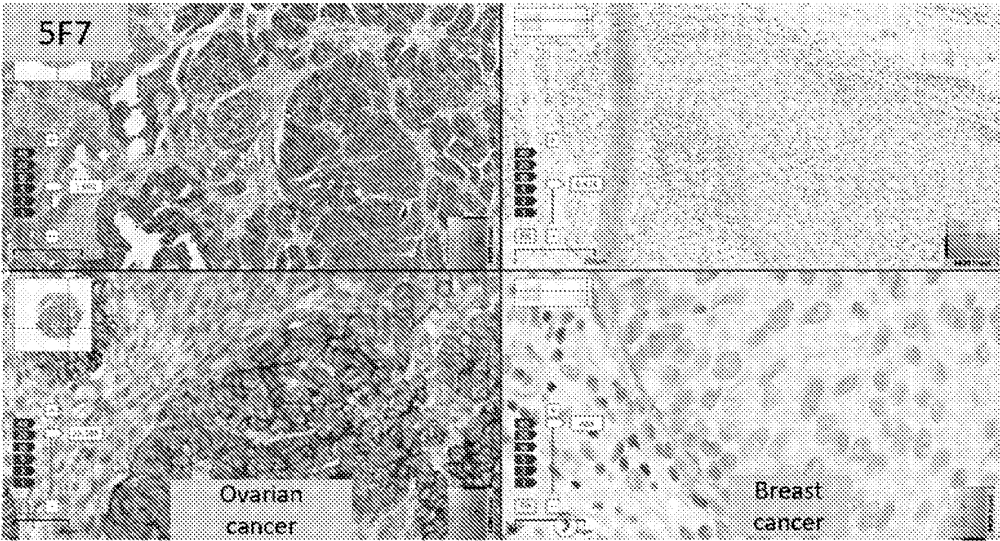


FIG. 12

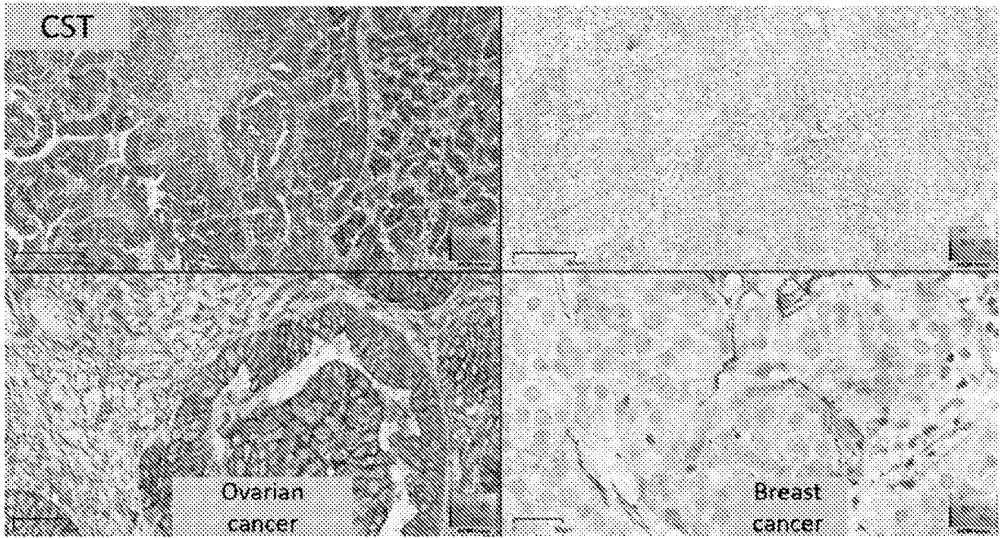


FIG. 13

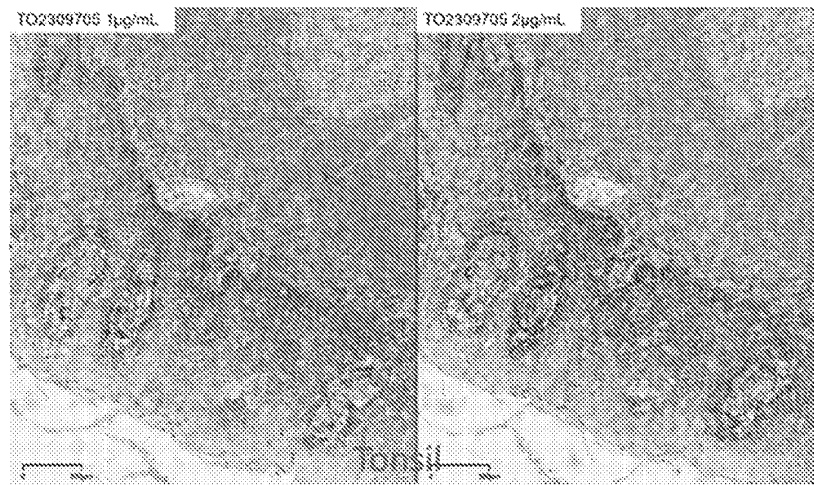


FIG. 14A

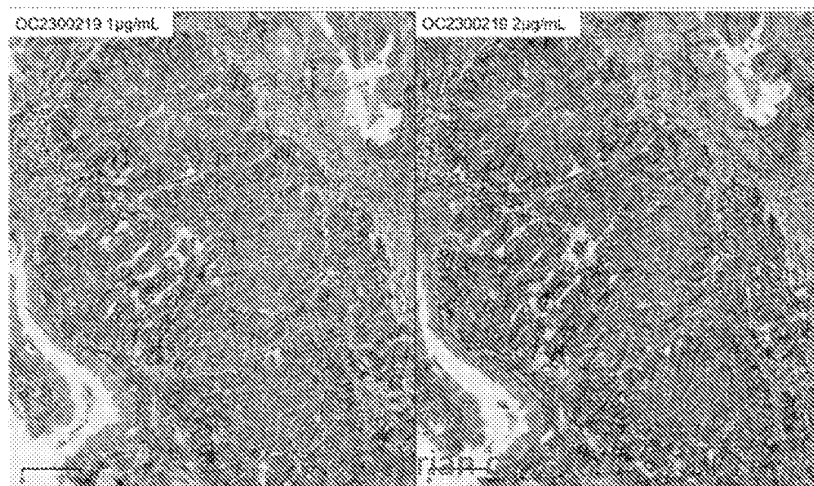


FIG. 14B

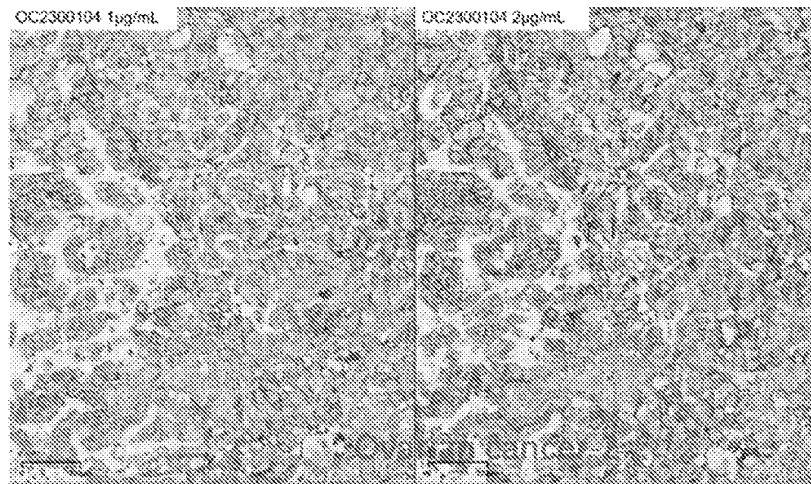


FIG.14C

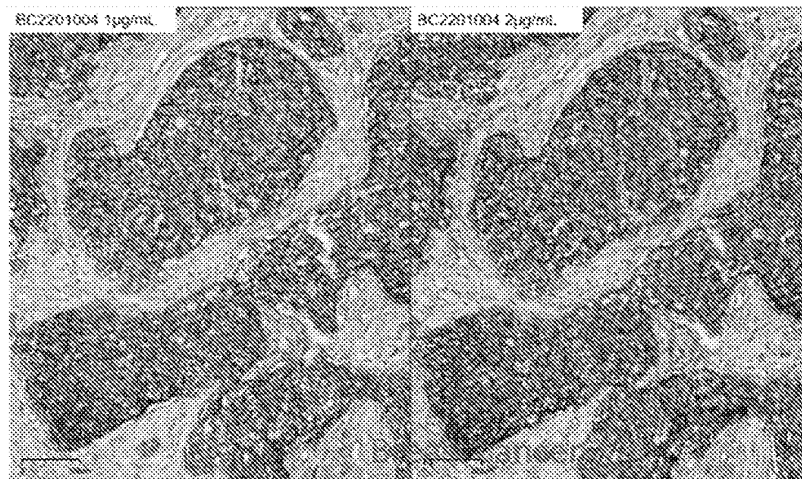


FIG.14D

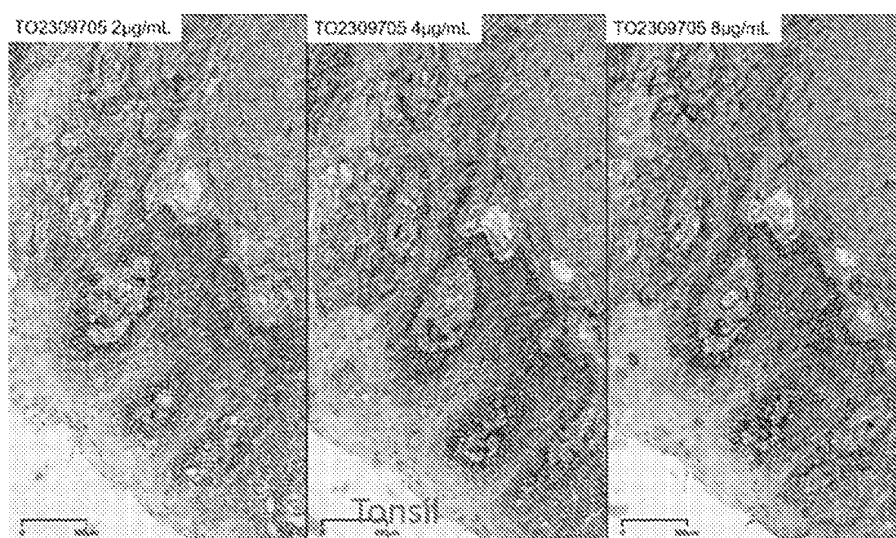


FIG.15A

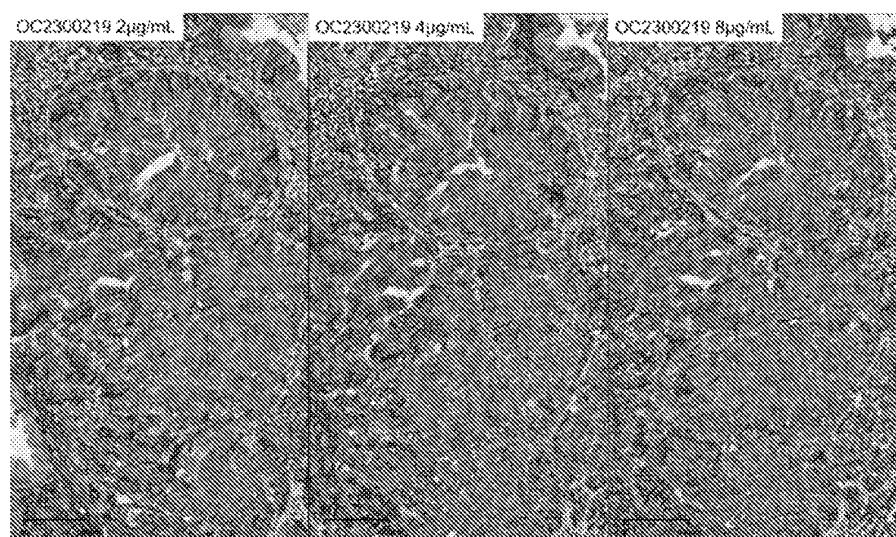


FIG.15B

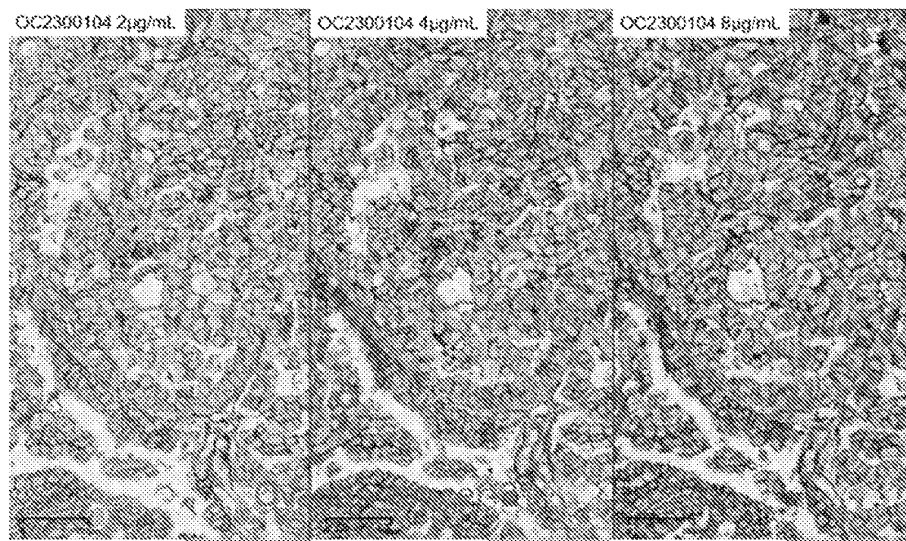


FIG.15C

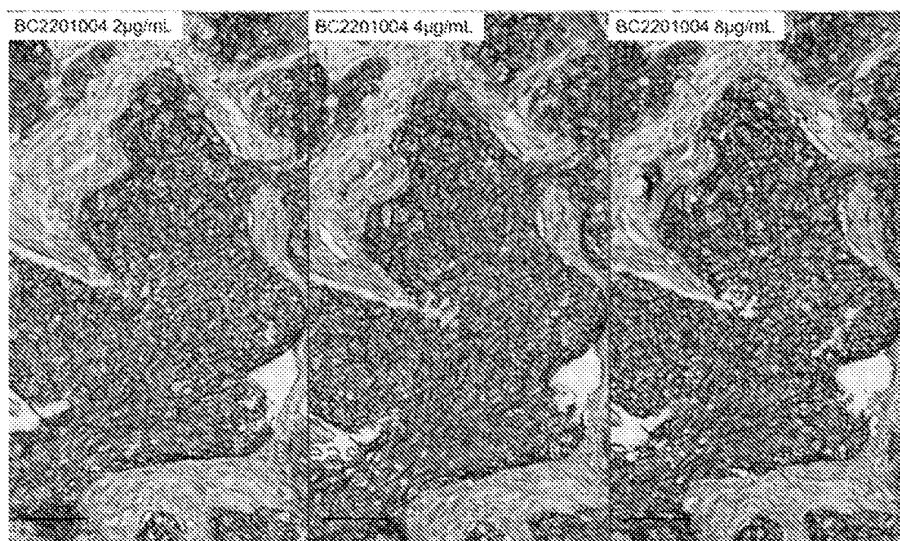


FIG.15D

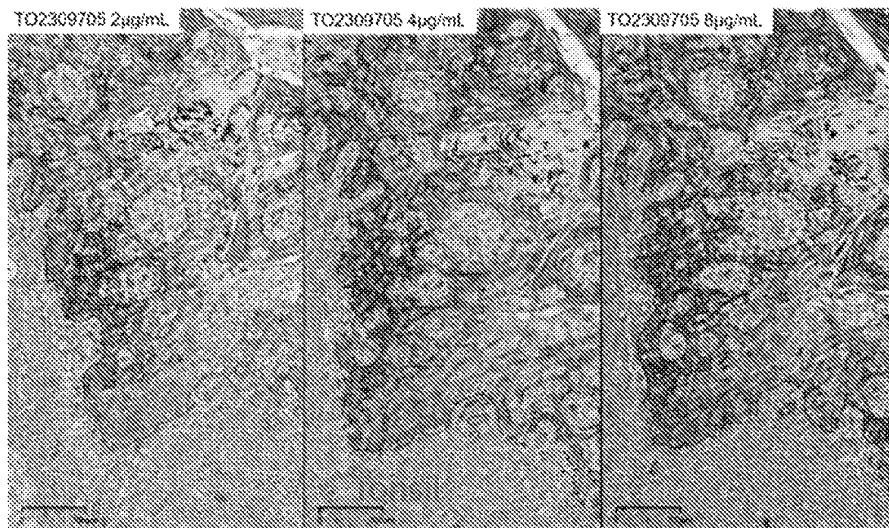


FIG. 16A

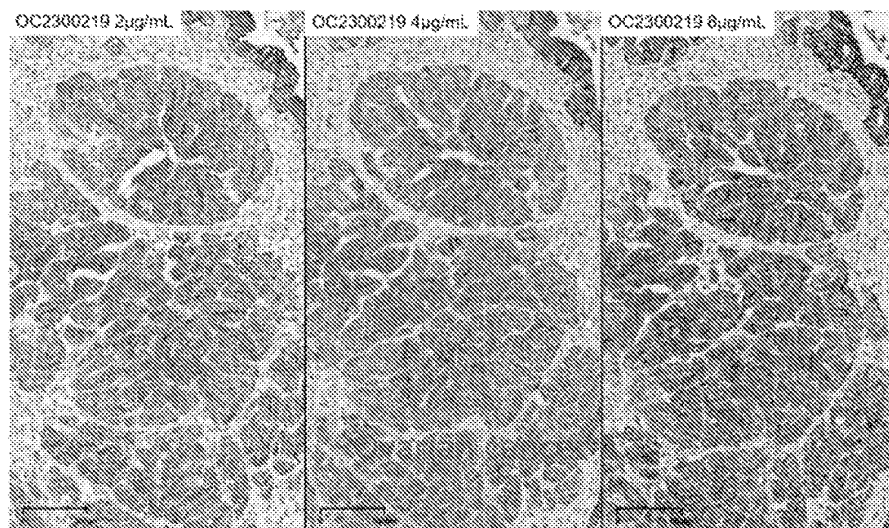


FIG. 16B

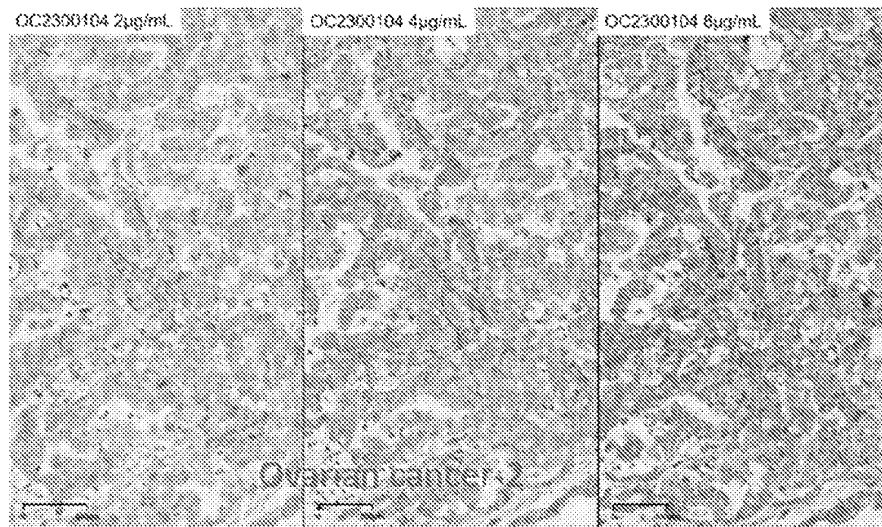


FIG. 16C

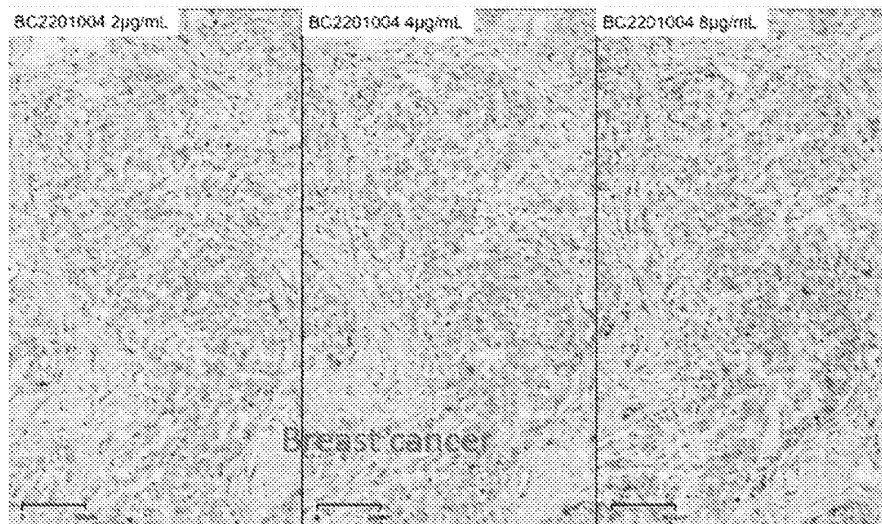


FIG. 16D

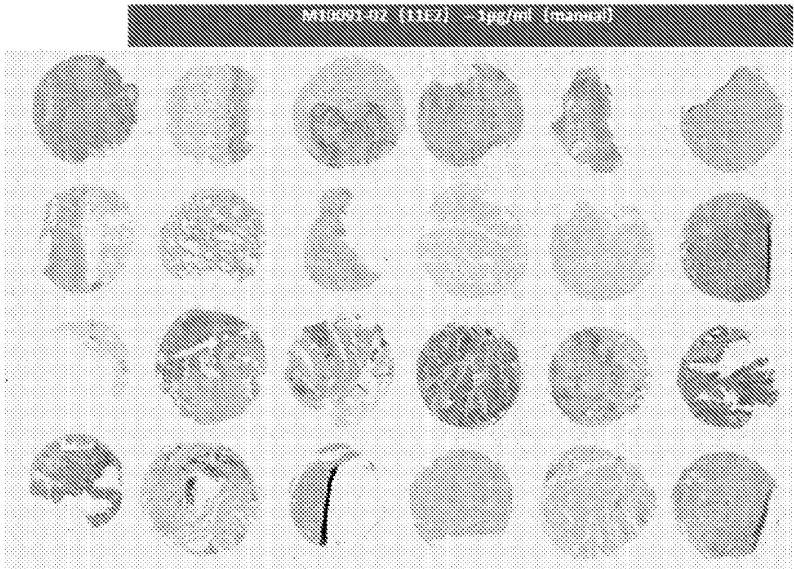


FIG. 17A

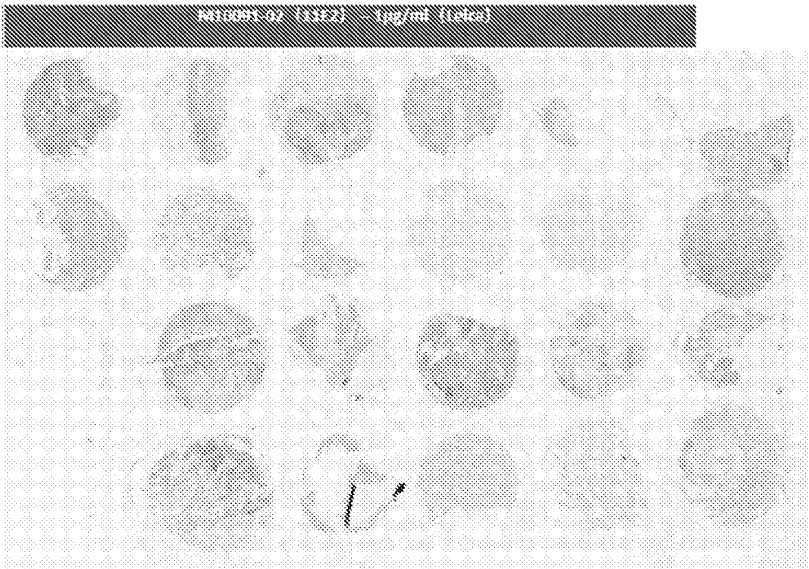


FIG. 17B

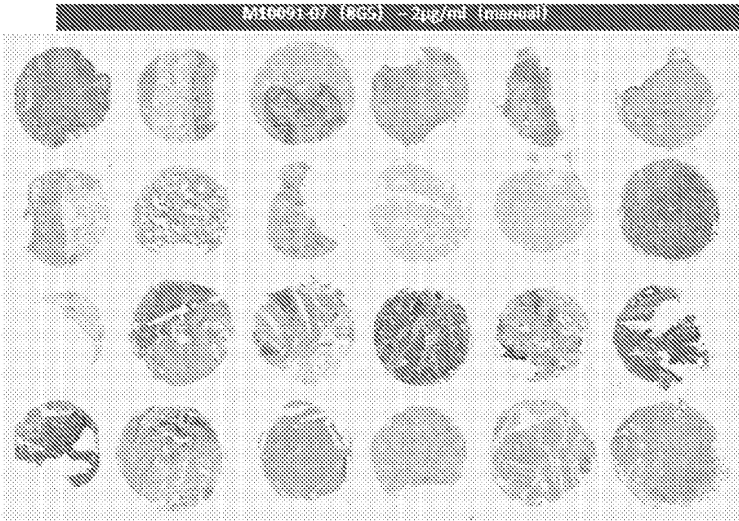


FIG. 18A

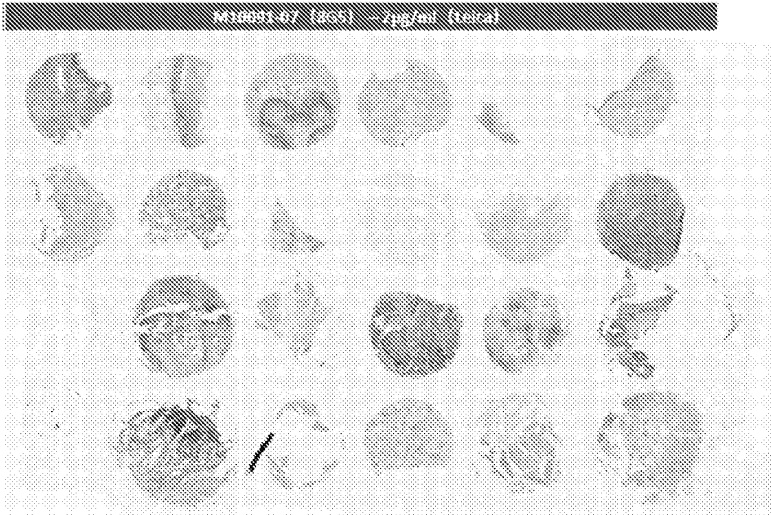


FIG. 18B

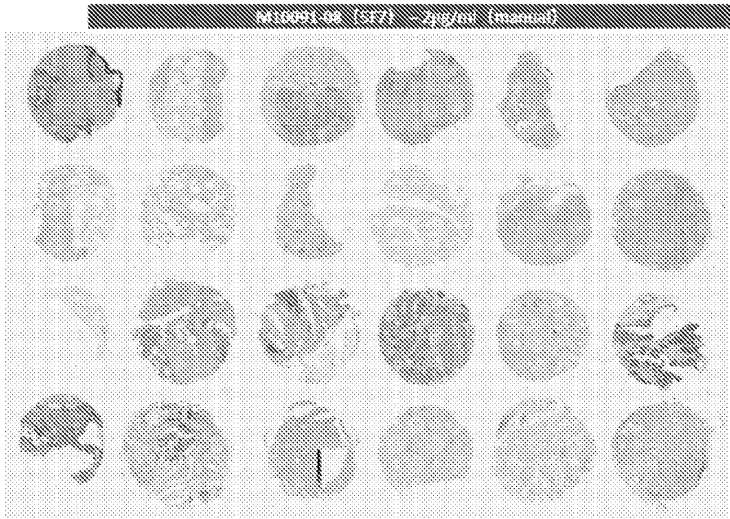


FIG. 19A

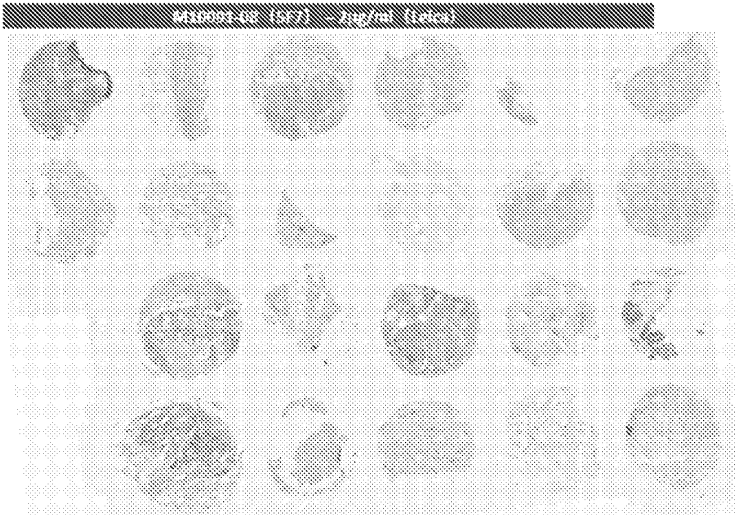


FIG. 19B

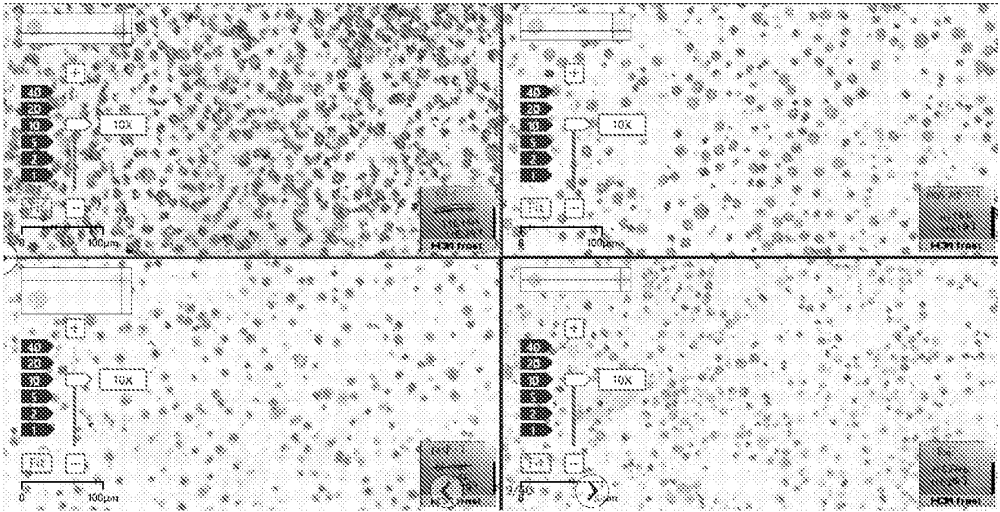


FIG. 20

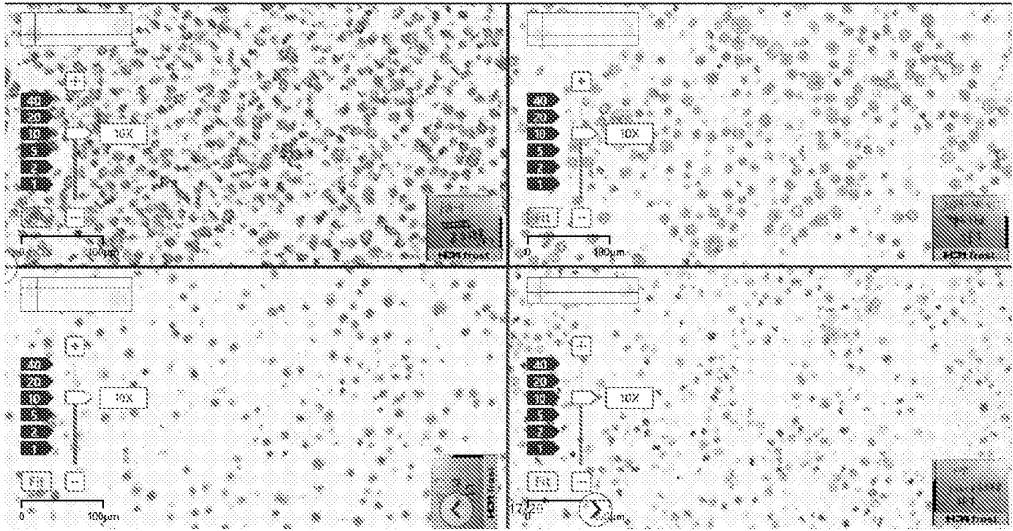


FIG. 21

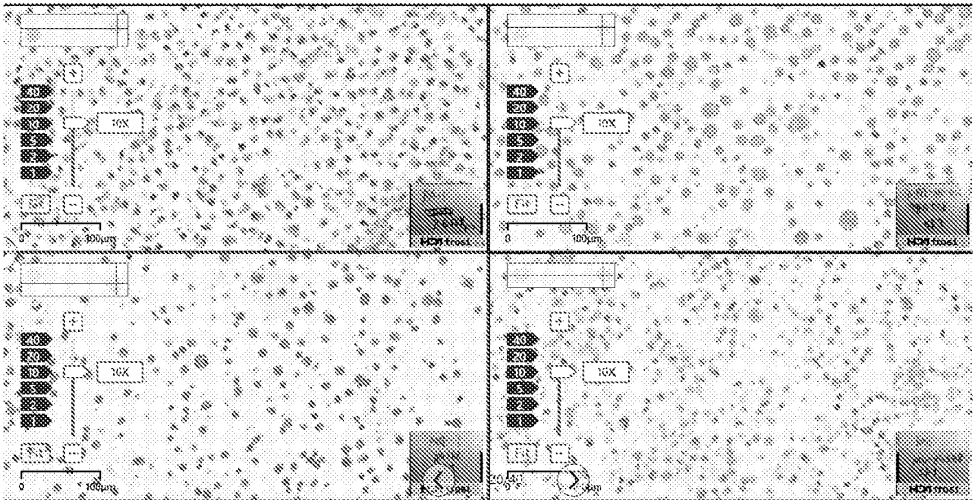


FIG. 22